Biochemical and Biophysical Research Communications 440 (2013) 105–111

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

ATP-citrate lyase is essential for macrophage inflammatory response

Vittoria Infantino [a,*], Vito Iacobazzi [b,c], Ferdinando Palmieri [b,c], Alessio Menga [b]

[a] Department of Science, University of Basilicata, 85100 Potenza, Italy
[b] Department of Biosciences, Biotechnology and Pharmacological Sciences, University of Bari, 70125 Bari, Italy
[c] CNR Institute of Biomembranes and Bioenergetics, 70125 Bari, Italy

ARTICLE INFO

Article history:
Received 3 September 2013
Available online 17 September 2013

Keywords:
ATP-citrate lyase
Nitric oxide
Reactive oxygen species
Prostaglandin E2
Inflammation
Immunometabolism

ABSTRACT

Growing evidence suggests that energy metabolism and inflammation are closely linked and that cross-talk between these processes is fundamental to the pathogenesis of many human diseases. However, the molecular mechanisms underlying these observations are still poorly understood. Here we describe the key role of ATP-citrate lyase (ACLY) in inflammation. We find that ACLY mRNA and protein levels markedly and quickly increase in activated macrophages. Importantly, ACLY activity inhibition as well as ACLY gene silencing lead to reduced nitric oxide, reactive oxygen species and prostaglandin E2 inflammatory mediators. In conclusion, we present a direct role for ACLY in macrophage inflammatory metabolism.

1. Introduction

Inflammation is the body's basic response to a variety of external or internal insults, such as infectious agents, physical injury, hypoxia, or disease processes [1]. Macrophages play a major role in the inflammatory process by detecting these insults and releasing various pro-inflammatory molecules including prostaglandins (PGs), reactive oxygen species (ROS), nitric oxide (NO) and cytokines. These factors promote inflammation by causing vasodilation and recruitment of neutrophils, monocytes and by altering the functionality of many tissues and organs. Depending on the inducer, the inflammatory response has a different physiological purpose and pathological consequences. For instance, during microbial infections one of the most potent macrophage activators is the gram-negative bacterial cell wall component lipopolysaccharide (LPS) which leads to the production of a variety of inflammatory mediators [2].

Tumour necrosis factor α (TNFα) and interferon γ (IFNγ) are the endogenous inducers produced in the tissues under stress, damage or otherwise malfunctioning. In this case the inflammation has the physiological purpose to adapt to stress, and restore a homeostatic state. However, a pathological consequence can be the development of inflammatory diseases. A comprehensive list of chronic inflammatory diseases would run to over 100, each of which shows high levels of inflammation. Among them are rheumatoid arthritis, systemic lupus erythematosus and Crohn's disease. Many of these pathological conditions are debilitating and are becoming increasingly common in our aging society. However, the number of safe and effective treatments is limited. To date, the major research effort has concentrated on those mediators responsible for initiation and maintenance of the pathological process. In contrast, little attention has been focused on metabolic signals which can be responsible for induction and/or control of the inflammatory response.

Here we investigate the role of ATP-citrate lyase (ACLY), a cross-link between glucose metabolism and fatty acid synthesis. In the cytoplasm, glucose-derived citrate is transformed, in the presence of ATP, into acetyl-CoA by ACLY [3,4]. Acetyl CoA is an essential substrate for cholesterol, isoprenoids and fatty acid synthesis pathways. Acetyl-CoA is also required for acetylation of nuclear histones in mammalian cells [5]. ACLY is most abundantly expressed in liver and white adipose tissue. Additionally, ACLY expression has been reported to be upregulated in many tumors, nonalcoholic fatty liver disease and other pathological conditions [6]. Surprisingly, we find that ACLY expression levels markedly and quickly increase in normal peripheral blood differentiated macrophages as well as in macrophage cell lines activated by exogenous and endogenous inducers. Furthermore, the specific ACLY activity inhibition or gene silencing is sufficient to reduce production of inflammatory mediators. Overall these results indicate a central role for ACLY in inflammation. In light of the evidence presented here, the ability of ACLY to integrate energy metabolism and inflammatory signaling makes it a particularly attractive target in human inflammatory diseases.

* Corresponding author.
E-mail address: vittoria.infantino@unibas.it (V. Infantino).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.09.037

2. Materials and methods

2.1. Cell culture

Mononuclear cells were isolated from heparinized blood of healthy adult volunteers and differentiated into macrophages as described previously [7]. Human monocytic/macrophage cells from hystiocytoma, U937 cells (HTL 94002, Interlab Cell Line Collection, Genoa, Italy), were cultured in Roswell Park Memorial Institute 1640 (RPMI 1640) medium supplemented with 10% (v/v) fetal bovine serum, 2 mM L-glutamine, 100 U penicillin and 100 μg/ml streptomycin at 37 °C in 5% CO_2 in a water-saturated atmosphere. U937 cells were differentiated with 10 ng/ml phorbol-12-myristate-13-acetate (PMA, Sigma–Aldrich, St Louis, MO, USA).

2.2. Activating stimuli

U937/PMA (differentiated U937) cells were treated for 16 h with 200 ng/ml *Salmonella typhimurium* LPS (Sigma–Aldrich), for 1 h with 5 ng/ml TNFα (Sigma–Aldrich), 10 ng/ml IFNγ (ImmunoTools GmbH, Friesoythe, Germany) or combined IFNγ and TNFα. Human macrophages differentiated from peripheral blood mononuclear cells (see Supplementary information) were treated for 1 h with 200 ng/ml LPS, 5 ng/ml TNFα, 10 ng/ml IFNγ or combined IFNγ and TNFα. Where indicated U937/PMA cells were treated with 20 μM TPCK (Sigma–Aldrich), 10 μM NIFU (Sigma–Aldrich), 500 μM HCA (Sigma–Aldrich), 5 μM SB-204990 ((+)-(3R*,5S*)-3-carboxy-11-(2,4-dichlorophenyl)-3,5-dihydroxyundecanoic acid, a gift from GlaxoSmithKline) or 250 nM RAD (Sigma–Aldrich) 1 h before stimulation with LPS, TNFα, IFNγ, or combined IFNγ and TNFα.

2.3. RNA interference

RNA interference experiments were performed as described previously [8] by using a specific pre-designed small interfering RNA (siRNA) targeting human ACLY (s917, Life Technologies, Paisley, UK). After 24 h, the medium was replaced with fresh medium and the siRNA-transfected U937/PMA cells were treated with LPS, TNFα, IFNγ, or combined IFNγ and TNFα. ROS and NO were measured 24 h after the addition of inducers. siRNA (C6A-0126, Life Technologies) with no significant similarity to human, mouse, or rat gene sequences was used as negative control [9].

2.4. Real-time PCR, SDS–PAGE and Western blotting

Total RNA was extracted and reverse transcripted as reported [10]. Real-time PCR was performed as previously described [11] by using human ACLY (Hs00982738_m1) and human β-actin (4326315E) taqMan® assays (Life Technologies). For immunoblot analysis, U937 cells were rinsed with ice-cold PBS and lysed using RIPA buffer. Thirty micrograms of total proteins were heated at 100 °C for 5 min, separated on 4–8% SDS polyacrylamide gels and transferred to nitrocellulose membranes. The membranes were then blocked for 1 h in a PBS solution containing 2% bovine serum albumin and 0.1% Tween 20, and then treated at room temperature with anti-ACLY (Aviva Systems Biology, San Diego, CA, USA) or anti-β-actin (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies. The immunoreaction was detected by Immobilon Western Chemiluminescent HRP Substrate (Millipore, Billerica, MA, USA).

2.5. NO, ROS and PGE_2 detection

Nitrite formation was detected by using 1H-naphthotriazole from 2,3-diaminonaphthalene (DAN, Life Technologies) [12]. For ROS analysis, U937/PMA activated cells were incubated with 10 μM DCFH2-DA (Life Technologies) for 30 min. The fluorescence was revealed by GloMax plate reader (Promega, Madison, WI, USA) [13]. PGE_2 was detected by the PGE_2 Enzyme Immunoassay Kit z(Arbor Assays, Ann Arbor, MI, USA) according to the manufacturer's instructions.

3. Results

3.1. Expression of ACLY in macrophages from peripheral blood

Inflammatory response is an energy-intensive process and metabolic changes occur in cells that participate in inflammation, such as activated macrophages [14]. In view of the central role of ACLY in energy metabolism, we tested whether inflammatory stimuli affected ACLY gene expression. First of all, macrophages from peripheral blood were treated with LPS, TNFα, IFNγ and by a combination of TNFα and IFNγ. Fig. 1A shows that both exogenous and endogenous inducers produced an increase in ACLY mRNA levels. Interestingly, this ACLY overexpression was observed at 1 h after all treatments, in agreement with the increase in protein levels. Among the different inducers, the combination of TNFα and IFNγ was more efficient in upregulating ACLY gene expression (Fig. 1A). These findings clearly show that multiple stimuli trigger ACLY overexpression in immune cells.

3.2. ACLY gene upregulation in LPS-activated macrophages

To further investigate the modulation of ACLY gene expression in inflammation, we used human differentiated U937 (U937/PMA) cells. We induced inflammation by LPS exposure and analyzed ACLY mRNA and protein levels. A markedly increase of about 2,5-fold ACLY activation was evident at 16 h after stimulation compared to untreated cells (Fig. 1B and S1A).

The main pathway reported for LPS-TLR4-induced signaling acts through nuclear factor κB (NF-kB) [15]. However, the signal transducer and activator of transcription (STAT) signaling can also be activated during LPS treatments [16]. To clarify the molecular mechanisms responsible for ACLY gene upregulation during LPS-activation we performed in silico analysis of the human ACLY gene promoter and we found two NF-kB and three STAT responsive elements. We tested the effect of the LPS-induced pathways on ACLY gene activation by using tosylphenylalanylchloromethane (TPCK) and nifuroxazide (NIFU), specific inhibitors of NF-kB [17] and STAT signaling [18], respectively. When U937/PMA cells were treated with LPS in the presence of TPCK or nifuroxazide, a reduction of ACLY mRNA and protein levels was observed as compared to cells treated with LPS alone (Fig. 1C and D). These results indicate that ACLY gene upregulation in microbial pathogen-induced macrophages is under control of both NF-kB and STAT transcription factors.

3.3. TNFα and IFNγ upregulate ACLY gene expression

To understand the effect of endogenous inducers on ACLY gene expression, we treated U937/PMA cells with TNFα and IFNγ alone or in combination. Surprisingly, at 1 h after stimulation, ACLY mRNA increased of about 50% when TNFα and IFNγ alone were used and even more in TNFα + IFNγ-activated U937/PMA as compared with untreated cells (Fig. 1E and S1B–D). A greater amount of ACLY protein was also detected after macrophage induction with respect to control cells (Fig. 1E).

It is known that TNFα acts by binding to its receptors TNFRs and leading to the activation of NF-kB [19]. As we found NF-kB responsive elements in ACLY gene promoter, we tested the involvement

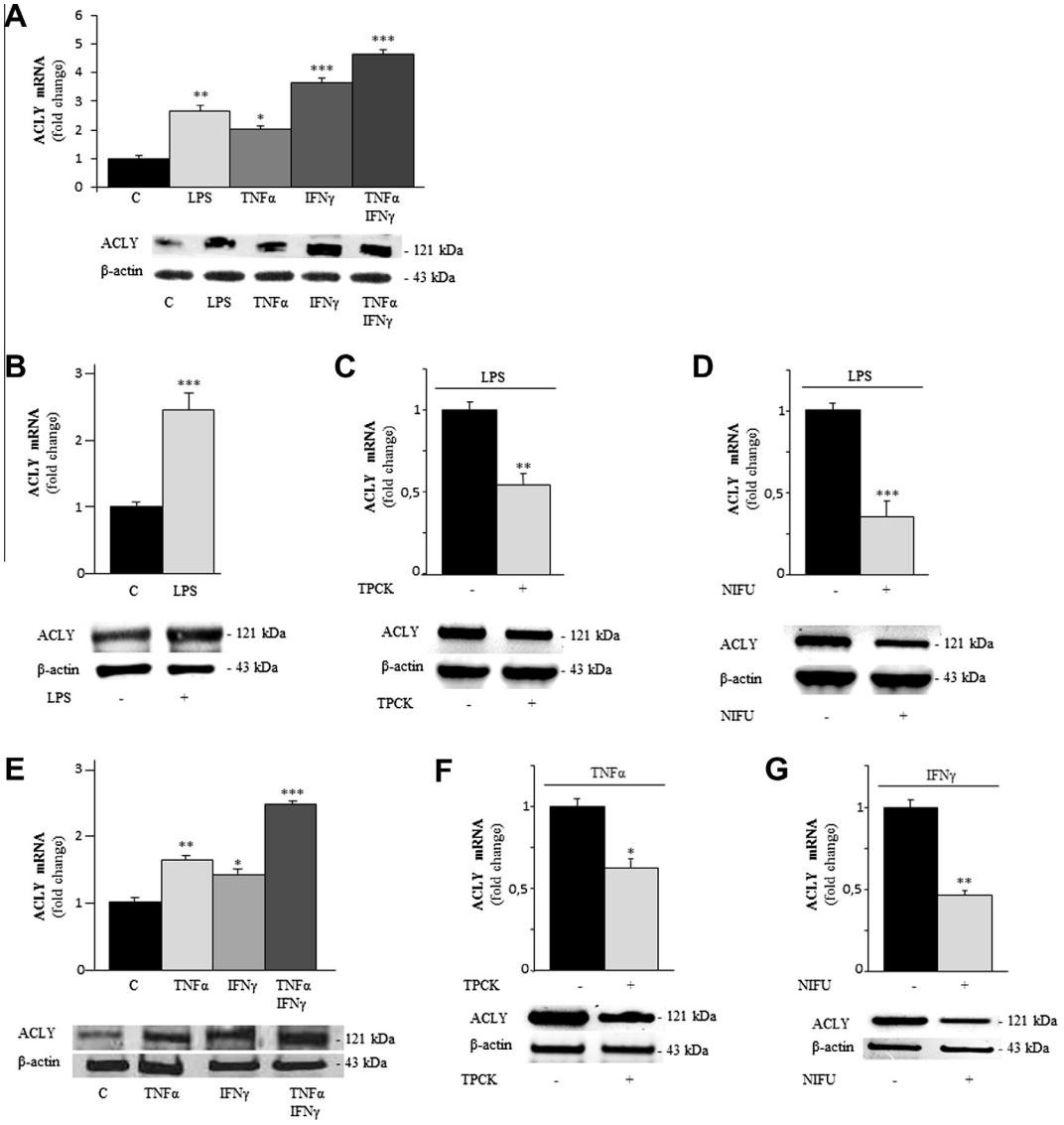

Fig. 1. ACLY expression in activated macrophages. Untreated monocyte-derived macrophages from peripheral blood (C) or treated with LPS, TNFα, IFNγ and IFNγ + TNFα were used to quantify ALCY mRNA (upper panel) and protein (lower panel) levels, respectively. (B) ALCY mRNA (upper panel) and protein (lower panel) levels from U937/PMA (C) and LPS-treated U937/PMA (LPS) cells were quantified. $^{***}P < 0.001$ (C) LPS-treated U937/PMA cells incubated with (+) or without (−) TPCK were used to quantify ALCY mRNA (upper panel) and protein (lower panel) levels, respectively. $^{**}P < 0.01$ (D) LPS-treated U937/PMA cells incubated with (+) or without (−) nifuroxazide (NIFU) were used to quantify ALCY mRNA (upper panel) and protein (lower panel) levels, respectively. $^{***}P < 0.001$ (E) ALCY mRNA (upper panel) and protein (lower panel) levels from U937/PMA cells untreated (C) or treated with TNFα, IFNγ and IFNγ + TNFα were quantified. (F) TNFα-treated U937/PMA cells incubated with (+) or without (−) TPCK were used to quantify ALCY mRNA (lower panel) and protein (upper panel) levels, respectively. $^{*}P < 0.05$ (G) IFNγ-treated U937/PMA cells incubated with (+) or without (−) nifuroxazide (NIFU) were used to quantify ALCY mRNA (lower panel) and protein (upper panel), respectively. $^{**}P < 0.01$. In (A) and (E) $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$ versus C. Real-time PCR values represent mean ± s.e.m. ($N = 6$).

of this pathway in TNFα activation. When TPCK was added to TNFα-activated U937/PMA cells, a reduction in both ACLY mRNA and protein levels was observed as compared with TNFα alone treated cells (Fig. 1F).

IFNγ signaling is triggered by the type-II IFN receptor activation, leading to downstream nuclear translocation of STAT transcription factors [20]. Given the presence of three STAT responsive elements in ACLY gene promoter, we verified the involvement of STAT-pathway in IFNγ-activated U937 cells by treating cells with IFNγ in the presence or absence of nifuroxazide. Real-time PCR and Western-blot experiments showed a strong reduction in both ACLY RNA and protein levels when nifuroxazide was added to cells with respect to IFNγ alone treated cells (Fig. 1G).

Our experiments clearly demonstrate an early ACLY activation in TNFα and/or IFN -stimulated macrophages. Thus, it is possible that ACLY gene upregulation in macrophages is necessary to drive the inflammatory response triggered by TNFα and IFNγ either alone or together.

3.4. Effect of the ACLY inhibition on PGE_2 production

As we found an ACLY upregulation in activated macrophages, we investigated the significance of these changes in inflammatory response. We tested the effect of ACLY specific inhibitors on the production of inflammatory mediators. Radicicol (RAD), which is a naturally occurring antifungal macrolide, noncompetitively inhibits ACLY activity [21]. However, it was much more widely studied for its ability to bind to heat shock protein 90. For this reason, we also tested the natural citrate analog hydroxycitrate (HCA) which is a potent ACLY inhibitor [22]. Finally, a more recent ACLY inhibitor, SB-204990, is effective in both in vivo and in vitro models [6]. Thus we tested the effect of all the above mentioned ACLY

inhibitors on inflammatory mediators in activated U937/PMA cells. First of all, we measured PGE_2, the main product of COX-2 pathway in inflammatory conditions, when ACLY activity was inhibited. The process of PGE_2 synthesis involves phospholipase A2 family members that mobilize arachidonic acid from cellular membranes, cyclooxygenases (constitutively active COX1 and inducible COX2), and finally PGE synthases [23].

The inflammatory response in U937/PMA cells was triggered by LPS, TNFα, IFNγ and by a combination of TNFα and IFNγ. As shown in Fig. 2 a marked reduction in PGE_2 production was found when ACLY activity was inhibited compared to untreated cells. All three inhibitors had no influence on cell viability at the tested concentrations (see Supplementary Fig. S2) and they were effective in reducing the PGE_2 levels upon macrophage activation with both exogenous and endogenous inducers. It is noteworthy that SB-204990 treatment restored the physiological amount of PGE_2 in the presence of endogenous inducers (Fig. 2B–D). All together, these results suggest that ACLY is necessary for PGE_2 synthesis in activated macrophages.

3.5. Effect of the ACLY inhibition on NO and ROS production

Synthesis of NO and ROS is closely related to cellular energy state. In fact, ROS are generated from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex in the presence of molecular oxygen and NADPH [24]. This reaction initiates a key step in immune defense, however, overproduction of ROS, most frequently due to excessive stimulation of NADPH oxidase by pro-inflammatory stimuli, results in oxidative stress [25]. NO, which is mediator and regulator of inflammatory response, is synthesized from L-arginine in a reaction catalyzed by inducible nitric oxide synthase (iNOS). Conversion of L-arginine to NO and L-citrulline also requires NADPH and O_2 as substrates.

We tested the effect of ACLY activity inhibitors on NO and ROS production in activated macrophages. We treated U937/PMA cells with TNFα and IFNγ alone or in combination and with LPS in the presence or absence of hydroxycitrate, radicicol and SB-20499. ACLY activity inhibition caused a great reduction in NO levels in immune cells induced with LPS, TNFα, IFNγ or a combination of IFNγ and TNFα (Fig. 3A–D). As shown for PGE_2, when SB-20499 was used, NO levels returned to near control values. On the other hand, hydroxycitrate and radicicol induced a strong decrease of NO levels than activated cells, especially in IFNγ treatment alone or in combination with TNFα (Fig. 3C and D). In the same experimental conditions, all three ACLY inhibitors reduced ROS levels (Fig. 3E–H). We did not observe significant differences among hydroxycitrate, radicicol and SB-20499 ACLY inhibitors in decreasing ROS production. In fact, all three inhibitors are able to restore ROS control levels when added to activated macrophages. Finally, we evaluated the effect of ACLY gene silencing on NO and ROS inflammatory mediators. ACLY silencing efficiency was confirmed by significantly decrease of ACLY mRNA and protein levels (data not shown). As shown in Supplementary Fig. S3, ACLY gene silencing in PMA/U937 cells induced with LPS, TNFα, IFNγ or a combination of IFNγ and TNFα led to a significant decrease in NO production. Additionally, ROS synthesis was significantly inhibited

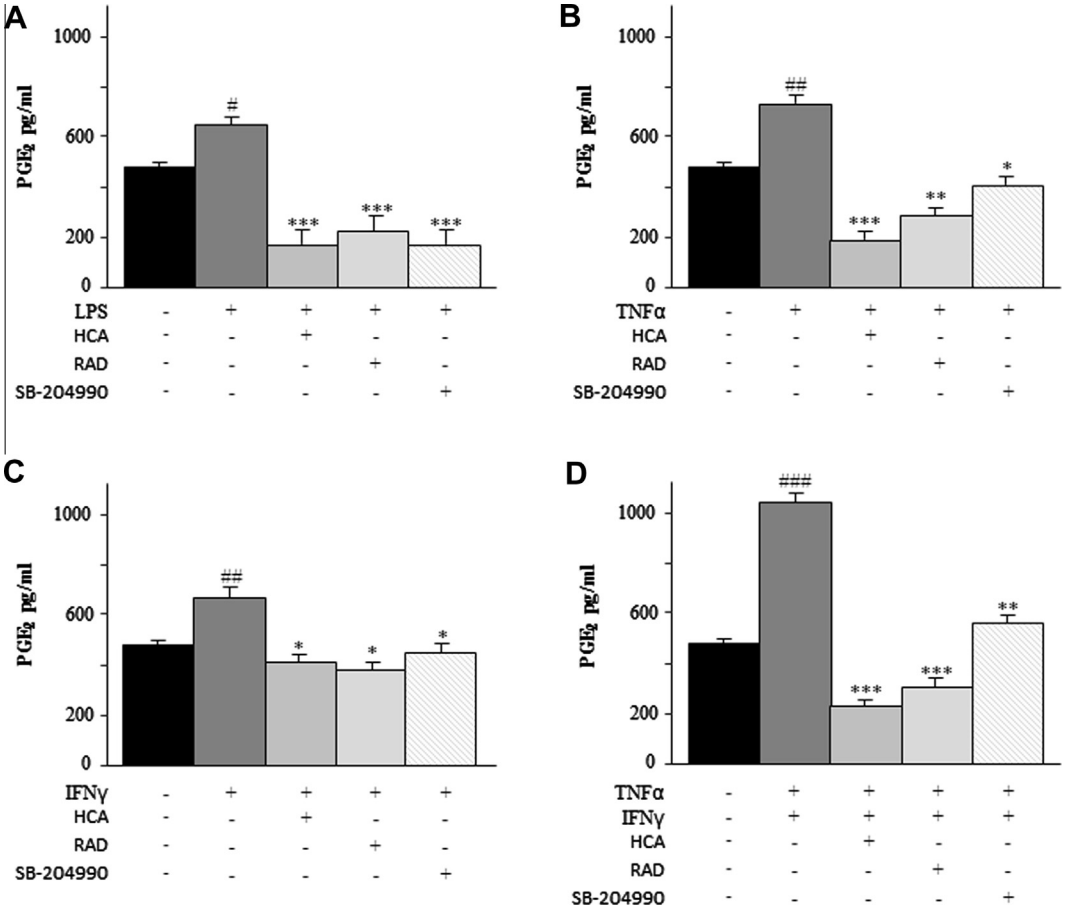

Fig. 2. Effect of ACLY activity inhibition on PGE_2 production. U937/PMA cells untreated (black bar) or treated with LPS (A), TNFα (B), IFNγ (C), a combination of TNFα and IFNγ (D) in the presence (+) or absence (−) of 500 μM hydroxycitrate (HCA), 250 nM radicicol (RAD) or 5 μM SB-204990 were assayed for PGE_2 secretion. Values represent mean ± s.e.m. ($N = 5$). $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$ versus induced and uninhibited macrophages (dark gray bars). $^{###}P < 0.001$, $^{##}P < 0.01$ and $^{#}P < 0.05$ versus uninduced macrophages (black bars).

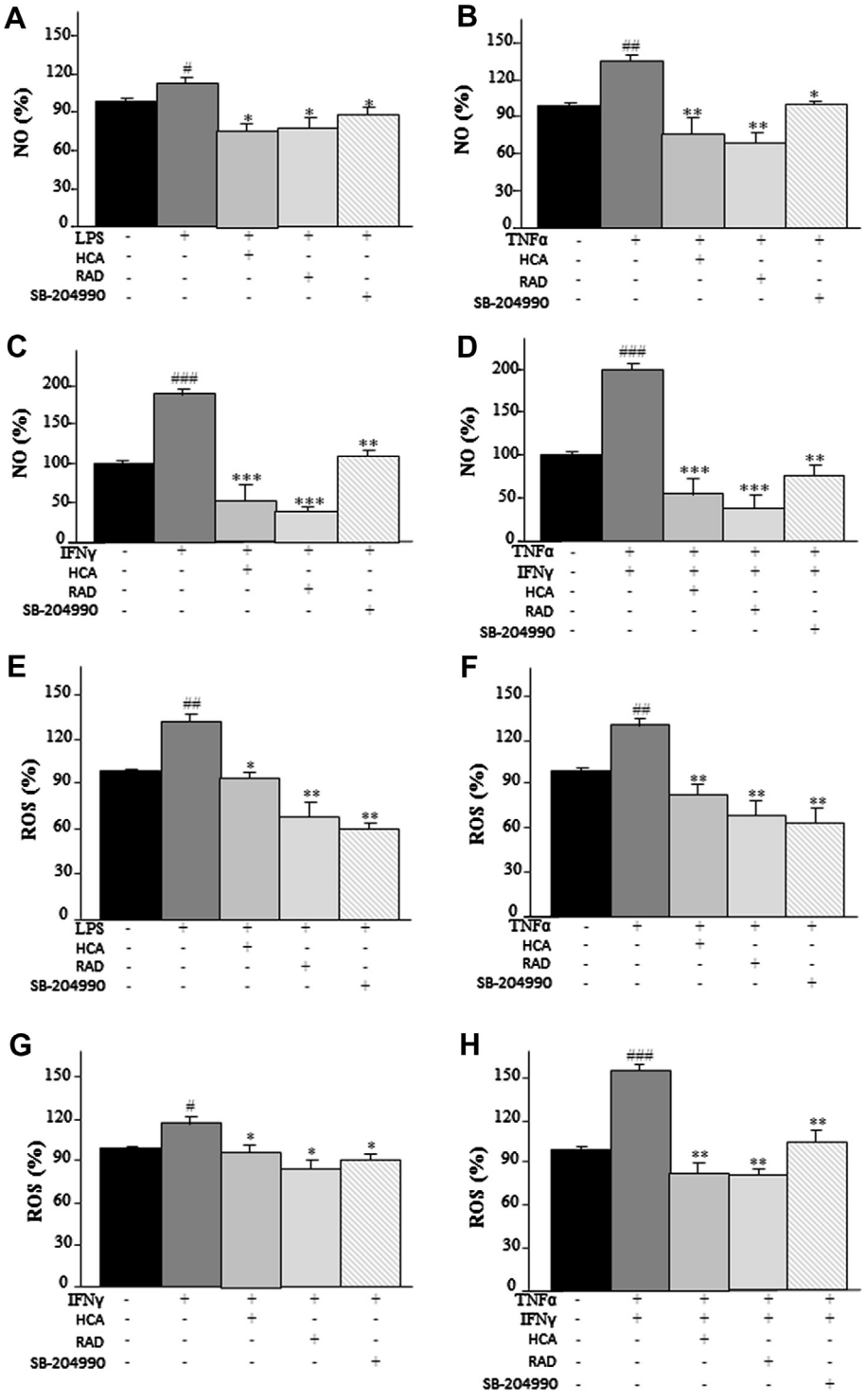

Fig. 3. Analysis of NO and ROS production in ACLY-inhibited macrophages. U937/PMA cells untreated (black bar) or treated with LPS (A), TNFα (B), IFNγ (C), a combination of TNFα and IFNγ (D) in the presence (+) or absence (−) of 500 μM hydroxycitrate (HCA), 250 nM radicicol (RAD) or 5 μM SB-204990 were assayed for NO production. U937/PMA cells untreated (black bar) or treated with LPS (E), TNFα (F), IFNγ (G), a combination of TNFα and IFNγ (H) in the presence (+) or absence (−) of ACLY inhibitors were assayed for ROS production. Values represent mean ± s.e.m. ($N = 5$). $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$ versus induced and uninhibited macrophages (dark gray bars). $^{###}P < 0.001$, $^{##}P < 0.01$ and $^{#}P < 0.05$ versus uninduced macrophages (black bars).

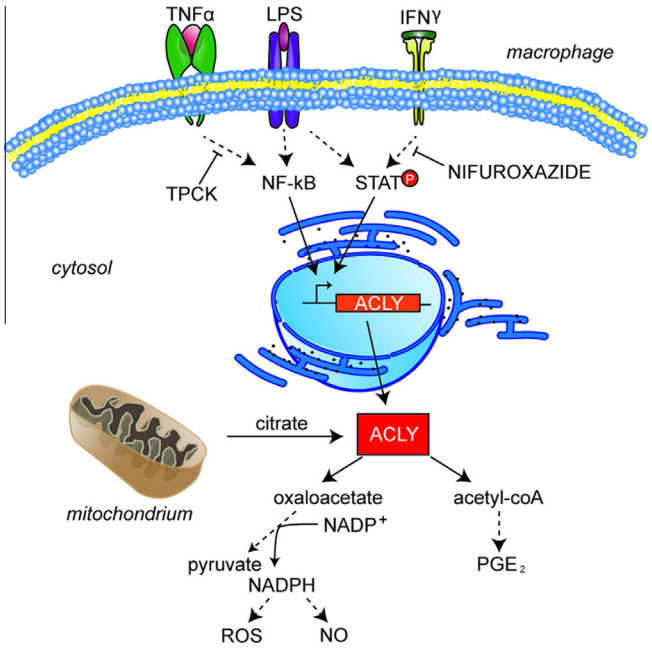

Fig. 4. Proposed mechanism for ACLY in inflammation. An inflammatory stimulus such as tumour necrosis factor α (TNFα), interferon γ (IFNγ) or lipopolysaccharide (LPS) induces ACLY gene activation through NF-kB and/or STAT signaling. Then, ACLY activity supplies acetyl-CoA for prostaglandins (PGs) synthesis and NADPH for reactive oxygen species (ROS) and nitric oxide (NO) production.

in silenced and induced when compared to non-silenced cells (Supplementary Fig. S3). These observations imply that ACLY is required for macrophage inflammatory activation triggered in response to various stimuli.

4. Discussion

Traditionally, immunity and energy metabolism have been considered as two distinct functions differentially regulated. More recently, increasing amount of evidence has demonstrated a strong interaction between them generating the new field "immunometabolism". The latest frontier of immunometabolism explores the molecular pathways linking metabolism and immune response.

In activated macrophages, the inflammatory response is characterized by the release of an array of pro-inflammatory mediators, which results in a signal transduction that activates the transcription of numerous pro-inflammatory genes. In these conditions, activated macrophages shift towards an increased glycolysis in concert with the attenuation of oxidative phosphorylation to maintain ATP levels [26]. It is likely that the altered metabolism parallels TCA cycle changes from being a purely catabolic pathway generating ATP to become, at least in part, an anabolic pathway. Recently we found an upregulation of the mitochondrial citrate carrier (CIC) gene expression in LPS-activated macrophages and a reduction of inflammatory mediators when CIC activity was inhibited [7]. To shed light on citrate metabolism and inflammation, the present study investigates the role of ACLY, the enzyme which acts immediately downstream to CIC, in activated macrophages. Very unexpectedly, the observed ACLY gene upregulation is earlier than CIC activation (24 h after LPS-stimulation) in macrophages from peripheral blood as well as in macrophage cell lines induced with LPS [7], even though CIC is essential for citrate export from mitochondria and therefore to provide the substrate for ACLY activity. In light of these observations, it can be assumed that CIC function needs only when the cytosolic citrate is depleted following the quick ACLY activation. This outcome suggests a primary role for cytosolic citrate, substrate of ACLY, which could be a signal molecule in inflammation. Importantly, our data also highlight an early gene upregulation of ACLY in IFNγ and/or TNFα-induced macrophages.

In agreement with the role of ACLY in induced macrophage, we observe a drastically reduction of PGE_2 levels when ACLY activity is inhibited. This is possible because PGE_2 production requires arachidonic acid, which in turn is synthesized by elongation of dietary linoleic acid with acetyl-CoA provided by ACLY activity (Fig. 4).

Finally, gene silencing as well as activity inhibition of ACLY decrease NO and ROS production. A possible explanation for these results comes from the biochemical function of ACLY. This enzyme, besides acetyl-coA, produces oxaloacetate first reduced to malate, which in turn is converted to pyruvate via malic enzyme with production of cytosolic NADPH plus H^+. Thus, ACLY can supply NADPH necessary for NO and ROS production during the inflammatory response of induced-macrophages (Fig. 4).

In conclusion, this study demonstrates that inflammatory response triggered by exogenous and endogenous inducers causes an early ACLY upregulation. ACLY activity is essential for the production of PGE_2, NO and ROS inflammatory mediators. Therefore, ACLY could be a new biomarker for predicting inflammation conditions and at the same time a potential target for inflammatory diseases.

Acknowledgments

This work was supported by grants from the Area Science Park [grant number J41H09000010007] to V. Infantino, the Ministero dell'Università e della Ricerca (MIUR) and the Universities of Basilicata and Bari "Aldo Moro". We thank GlaxoSmith-Kline for providing us with SB-20499 ACLY inhibitor.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.09.037.

References

[1] R. Medzhitov, Origin and physiological roles of inflammation, Nature 454 (2008) 428–435.
[2] G.M. Barton, A calculated response: control of inflammation by the innate immune system, J. Clin. Invest. 118 (2008) 413–420.
[3] J.A. Watson, M. Fang, J.M. Lowenstein, Tricarballylate and hydroxycitrate: substrate and inhibitor of ATP: citrate oxaloacetate lyase, Arch. Biochem. Biophys. 135 (1969) 209–217.
[4] V. Iacobazzi, V. Infantino, F. Palmieri, Epigenetic mechanisms and Sp1 regulate mitochondrial citrate carrier gene expression, Biochem. Biophys. Res. Commun. 376 (2008) 15–20.
[5] K.E. Wellen, G. Hatzivassiliou, U.M. Sachdeva, T.V. Bui, J.R. Cross, C.B. Thompson, ATP-citrate lyase links cellular metabolism to histone acetylation, Science 324 (2009) 1076–1080.
[6] G. Hatzivassiliou, F. Zhao, D.E. Bauer, C. Andreadis, A.N. Shaw, D. Dhanak, S.R. Hingorani, D.A. Tuveson, C.B. Thompson, ATP citrate lyase inhibition can suppress tumor cell growth, Cancer Cell 8 (2005) 311–321.
[7] V. Infantino, P. Convertini, L. Cucci, M.A. Panaro, M.A. Di Noia, R. Calvello, F. Palmieri, V. Iacobazzi, The mitochondrial citrate carrier: a new player in inflammation, Biochem. J. 438 (2011) 433–436.
[8] A. Menga, V. Infantino, F. Iacobazzi, P. Convertini, F. Palmieri, V. Iacobazzi, Insight into mechanism of in vitro insulin secretion increase induced by antipsychotic clozapine: role of FOXA1 and mitochondrial citrate carrier, Eur. Neuropsychopharmacol. 23 (2013) 978–987.
[9] V. Iacobazzi, V. Infantino, P. Convertini, A. Vozza, G. Agrimi, F. Palmieri, Transcription of the mitochondrial citrate carrier gene: identification of a silencer and its binding protein ZNF224, Biochem. Biophys. Res. Commun. 386 (2009) 186–191.
[10] V. Infantino, P. Convertini, F. Iacobazzi, I. Pisano, P. Scarcia, V. Iacobazzi, Identification of a novel Sp1 splice variant as a strong transcriptional activator, Biochem. Biophys. Res. Commun. 412 (2011) 86–91.
[11] V. Infantino, V. Iacobazzi, F. De Santis, M. Mastrapasqua, F. Palmieri, Transcription of the mitochondrial citrate carrier gene: role of SREBP-1,

upregulation by insulin and downregulation by PUFA, Biochem. Biophys. Res. Commun. 356 (2007) 249–254.
[12] R. Sala, B.M. Rotoli, E. Colla, R. Visigalli, A. Parolari, O. Bussolati, G.C. Gazzola, V. Dall'Asta, Two-way arginine transport in human endothelial cells: TNF-alpha stimulation is restricted to system y(+), Am. J. Physiol. Cell Physiol. 282 (2002) C134–143.
[13] H. Wang, J.A. Joseph, Quantifying cellular oxidative stress by dichlorofluorescein assay using microplate reader, Free Radic. Biol. Med. 27 (1999) 612–616.
[14] L.A. O'Neill, D.G. Hardie, Metabolism of inflammation limited by AMPK and pseudo-starvation, Nature 493 (2013) 346–355.
[15] S. Akira, K. Takeda, Toll-like receptor signalling, Nat. Rev. Immunol. 4 (2004) 499–511.
[16] P. Kovarik, D. Stoiber, M. Novy, T. Decker, Stat1 combines signals derived from IFN-gamma and LPS receptors during macrophage activation, EMBO J. 17 (1998) 3660–3668.
[17] A. Abate, H. Schroder, Protease inhibitors protect macrophages from lipopolysaccharide-induced cytotoxicity: possible role for NF-kappaB, Life Sci. 62 (1998) 1081–1088.
[18] E.A. Nelson, S.R. Walker, A. Kepich, L.B. Gashin, T. Hideshima, H. Ikeda, D. Chauhan, K.C. Anderson, D.A. Frank, Nifuroxazide inhibits survival of multiple myeloma cells by directly inhibiting STAT3, Blood 112 (2008) 5095–5102.
[19] A.A. Beg, T.S. Finco, P.V. Nantermet, A.S. Baldwin Jr., Tumor necrosis factor and interleukin-1 lead to phosphorylation and loss of I kappa B alpha: a mechanism for NF-kappa B activation, Mol. Cell Biol. 13 (1993) 3301–3310.
[20] K. Schroder, P.J. Hertzog, T. Ravasi, D.A. Hume, Interferon-gamma: an overview of signals, mechanisms and functions, J Leukoc Biol 75 (2004) 163–189.
[21] S.W. Ki, K. Ishigami, T. Kitahara, K. Kasahara, M. Yoshida, S. Horinouchi, Radicicol binds and inhibits mammalian ATP citrate lyase, J. Biol. Chem. 275 (2000) 39231–39236.
[22] H.N. Abramson, The lipogenesis pathway as a cancer target, J. Med. Chem. 54 (2011) 5615–5638.
[23] J.Y. Park, M.H. Pillinger, S.B. Abramson, Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases, Clin. Immunol. 119 (2006) 229–240.
[24] B.M. Babior, NADPH oxidase, Curr. Opin. Immunol. 16 (2004) 42–47.
[25] I.T. Lee, C.M. Yang, Role of NADPH oxidase/ROS in pro-inflammatory mediators-induced airway and pulmonary diseases, Biochem. Pharmacol. 84 (2012) 581–590.
[26] D. Arnoult, F. Soares, I. Tattoli, S.E. Girardin, Mitochondria in innate immunity, EMBO Rep. 12 (2011) 901–910.