Research Article

Sustained Immunoparalysis in Endotoxin-Tolerized Monocytic Cells

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Sepsis is associated with a strong inflammatory reaction triggering a complex and prolonged immune response. Septic patients have been shown to develop sustained immunosuppression due to a reduced responsiveness of leukocytes to pathogens. Changes in cellular metabolism of leukocytes have been linked to this phenomenon and contribute to the ongoing immunological derangement. However, the underlying mechanisms of these phenomena are incompletely understood. In cell culture models, we mimicked LPS tolerance conditions to provide evidence that epigenetic modifications account for monocyte metabolic changes which cause immune paralysis in restimulated septic monocytes. In detail, we observed differential methylation of CpG sites related to metabolic activity in human PBMCs 18 h after septic challenge. The examination of changes in immune function and metabolic pathways was performed in LPS-tolerized monocytic THP-1 cells. Passaged THP-1 cells, inheriting initial LPS challenge, presented with dysregulation of cytokine expression and oxygen consumption for up to 7 days after the initial LPS treatment. Proinflammatory cytokine concentrations of TNFα and IL1β were significantly suppressed following a second LPS challenge (p < 0.001) on day 7 after first LPS stimulation. However, the analysis of cellular metabolism did not reveal any noteworthy alterations between tolerant and nontolerant THP-1 monocytes. No quantitative differences in ATP and NADH synthesis or participating enzymes of energy metabolism occurred. Our data demonstrate that the function and epigenetic modifications of septic and tolerized monocytes can be examined in vitro with the help of our LPS model. Changes in CpG site methylation and monocyte function point to a correlation between epigenetic modification in metabolic pathways and reduced monocyte function under postseptic conditions.

1. Introduction

Sepsis is associated with an interplay of strong proinflammatory and anti-inflammatory responses. However, the latter may exacerbate host defense mechanisms and alter immune cell functions [1]. Prolonged immune dysfunction in patients coping with sepsis compromises their outcome upon secondary infections. These patients’ leukocytes displayed immunoparalysis, characterized by decreased inflammation and a generalized metabolic defect on the level of glycolysis and oxidative metabolism. Depending on the dose and type of a first trigger, a second stimulation can induce diverse responses [2, 3]. Epigenetic memory may provoke tolerance or training, i.e., to reduce or enhance the immune reaction [4, 5]. Endotoxin tolerance is a crucial homeostatic mechanism that prevents the excessive stimulation of the innate immune response upon sustained or recurrent toll-like receptor (TLR) stimulation [6]. Endotoxin tolerance is considered a type of innate immune memory, a condition describing tolerance towards pathogens, characterized by innate immune hyporesponsiveness or immunoparalysis [7, 8].

Prestimulation of monocytes or macrophages with high doses of lipopolysaccharide (LPS) can induce LPS tolerance [9]. Low doses of LPS, however, can augment the response
to the second LPS trigger, resulting in training instead of toler- 
ance, indicating that effects on memory are dose dependent 
[10, 11].

During infection, bacterial molecular patterns such as 
LPS affect signal transduction on various levels. Modifi-
cations of the TLR 4 signaling cascade, altered pro- and anti-
flammatory cytokine and chemokine levels, changes in the 
activation of transcription factors, and epigenomic mech-
isms may reprogram the host’s response [2]. Epigenetic 
effects including DNA methylation can be of transient or sus-
tained character [12]. Their influence on inflammatory and 
immunological processes is a matter of ongoing investiga-
tions. So far, little is known since the complexity of interac-
tions on clinical, cellular, and molecular levels impedes 
transfer and reproduction in cellular models. DNA methyla-
tion is the most stable mark, followed by shorter lived histone 
methylation, both enabling a preservation of alterations 
after cell division [13].

The vast majority of studies examining the mechanisms of 
inmate immunotolerance lacks long-term observations. 
Proliferation of cells is a prerequisite when investigating 
how parent cells pass on immunomodulatory effects, poten-
tially via epigenetic processes [14]. However, proliferation 
of PBMCs under culturing conditions is difficult to achieve. 
Stimulation with selective growth factors alters the metabolic 
response and cellular effector function after stimulation. 
Therefore, we developed a long-term model of LPS-
pretreated monocyctic THP-1 cells, undergoing continuous 
passaging in LPS-free media, and analyzed their phenotype.

Monocytes arise from myeloid progenitor cells in the 
bone marrow. Responding to differentiation stimuli and 
growth factors, monocytes enter the bloodstream. Upon 
inflammatory signals, monocytes migrate to infected tissues 
and start differentiation. Monocytes are central to the devel-
opment of both systemic inflammatory response syndrome 
and the compensatory anti-inflammatory response syn-
drome [15, 16]. Phagocytosis, antigen presentation, and cyto-
kine production of monocytes are influenced by metabolic 
modulation [17]. LPS induces reprogramming in macro-
phages and dendritic cells via TLR4 by suppression of oxida-
tive phosphorylation, increase in glucose uptake and 
glycolysis, and other metabolic pathways [18].

We intended to analyze how immunocompromisation is 
reflected on the level of CpG methylation. Furthermore, the 
progression of immune dysfunction and metabolic alteration 
on an extended period of time was our underlying question.

2. Materials and Methods

2.1. PBMC Isolation and LPS Stimulation. Fresh peripheral 
blood was drawn from four healthy volunteers. PBMC iso-
lation was performed by overlaying 6 ml of blood over each 
3 ml of Histopaque 1077 and 1119 (Sigma). 15 ml tubes were 
centrifuged at room temperature (RT) for 30 min at 700 × g. 
The resulting PBMC layer was retrieved and washed three 
times (10 min, 300 × g) with PBS at RT. Cells were resus-
pended at the indicated cell number in RPMI 1640 (Lonza, Basel, Switzerland) supplemented with 10% heat-
inactivated fetal bovine serum (FBS, Applichem, Darmstadt, 
Germany).

PBMCs of each subject were incubated with 10 ng LPS/ml 
(E. coli LPS 0111:B4, Sigma, St. Louis, MO, USA) or medium 
control (Figure 1). After 18 hours, samples were exposed to a 
higher dose of LPS (100 ng/ml media) or medium control. 
Three hours later, samples were centrifuged for 5 min at 
800 × g and 4°C. Supernatants and cell pellets were separated 
and kept at -80°C until further processing.

2.2. DNA Isolation, Bisulfite Conversion, and Infinium 
Methylation EPIC Bead Chip Array. PBMCs were harvested 
from four additional healthy volunteers. For each subject, 
107 PBMCs/ml were incubated with 10 ng LPS/ml or PBS 
for 18 hours and harvested by centrifugation. DNA was iso-
lated using a Genomic DNA Isolation Kit (Machery Nagel, 
Düren, Germany). The isolated DNA was stored at -20°C. 
Bisulfite conversion of DNA was performed utilizing Qiagen 
EpiTect Kit (Qiagen, Hilden, Germany) according to the 
manufacturer’s protocol. Infinium Methylation EPIC Bead 
Chip Array (Illumina, San Diego, USA) was run as published 
elsewhere [19].

2.3. THP-1 Culture and Passaging Experiment. THP-1 cells 
were obtained from ATCC (Manassas, VA, USA) and cul-
tured in RPMI 1640 (Lonza) supplemented with 10% heat-
inactivated FBS. For maintenance, THP-1 culture cells were 
seeded into T75 flasks and stimulated once with either LPS 
(1 μg/ml medium) or PBS as control (passage (P)0) 
(Figure 1). Before passaging, cells were counted, centrifuged 
(400 × g for 5 min), and resuspended at 108 cells/ml LPS-
free fresh media each time. In each passaging cycle, a portion 
of cells from both treatment groups was transferred to a new 
dish and received a second stimulation with LPS (1 μg/ml medium) or PBS.

2.4. RNA Isolation and cDNA Synthesis. RNA was isolated 
from 1 × 106 THP-1 cells or 6 × 106 PBMCs per sample. Cells 
were resuspended in 1 ml TRIzol (Thermo Fisher Scientific). 
After the addition of 200 μl chloroform (Applichem, 
Darmstadt, Germany), suspension was repeatedly inverted, 
followed by 10 min incubation at room temperature. Phases 
were separated by centrifugation (12,000 × g) at 4°C for 
15 min. The upper aqueous phase was transferred into a 
new RNase-free reaction tube. The RNA precipitated after 
addition of 500 μl isopropanol and 10 min incubation at 
room temperature. After centrifugation (12,000 × g, 10 min, 
4°C), the supernatant was discarded and the pellet was 
was washed twice using 1 ml 75% ethanol in DEPC-H2O, 
followed by centrifugation (10,000 × g, 5 min, 4°C). The air-
dried RNA pellet was resuspended in 20 μl DEPC-H2O. 
RNA concentration was determined spectrophotometrically 
(NanoDrop, Thermo Fisher Scientific). High-capacity cDNA 
Reverse Transcription Kit (Thermo Fisher Scientific) was
utilized to convert 2 μg total RNA to cDNA as described previously [20].

2.5. Quantitative Real-Time Polymerase Chain Reaction (qPCR). Expression levels of TNFα, IL-1β, IL-10, and 18S rRNA were determined using qPCR. Per reaction, 16 ng cDNA was mixed with 5 μl TaqMan Universal Master Mix (Thermo Fisher Scientific), 0.5 μl TaqMan Gene Expression Assay, and 2.3 μl nuclease-free water to a final volume of 10 μl in a 384-well optical reaction plate. Each sample was measured in triplicate wells and underwent 40 cycles of amplification on a ViiA7 real-time PCR system (Thermo Fisher Scientific). Ct values were determined with SDS Software 2.2 (Thermo Fisher Scientific). Target gene expression was normalized to an internal control and 18S RNA as non-inducible housekeeping gene.

2.6. Protein Expression (Enzyme-Linked Immunosorbent Assay). TNFα, IL-1β, IL-6, and IL-10 levels in cell culture supernatants were quantified using commercially available ELISA kits (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer’s protocol. Absorption was measured on a Cytation 3 multimode reader.

2.7. Oxygen Rate. 10⁵ THP-1 cells harvested from passage 3 ((P3), 7 days after initial LPS treatment) were reconstituted in fresh media and transferred to Transwell inserts with 0.4 μm pore size (Corning, NY, USA). Real-time O₂ measurements were performed using OxoDish® OD24 plates on an SDR reader (PreSens, Heidelberg, Germany) [21]. Plates with cells were equilibrated for 2 h in the incubator (37°C, 5% CO₂) before stimulation with PBS or LPS (1 μg/ml media). Afterwards, oxygen concentration in the media was measured.

2.8. MTT Assay. Reduction of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT, Sigma) to purple formazan was detected in a colorimetric assay. 100 μl MTT reagent (500 μg MTT/100 μl PBS) was added to 10⁵ cells taken from P3 (96-well plate). After a 2 h incubation period, 100 μl lysis reagent (100 ml N,N-dimethylformamide, 20 g SDS, 2.5 ml 80% acetic acid, and 2.5 ml 1 N HCl in 100 ml media).
ddH₂O) was supplemented. Plates were analyzed on a micro-titer plate reader (BioTek Instruments).

2.9. ATP, Lactate, NAD/NADH, and LDH Measurements. ATP content was determined using CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Madison, WI, USA). Lactate-Glo™ Assay (Promega) quantified extracellular lactate. NAD/NADH levels were measured with NAD/-NADH-Glo™ Assay (Promega). Lactate dehydrogenase (LDH) was detected with CytoTox96® NonRadioactive Cytotoxicity Assay (Promega). Relative luminescent units (RLU) or absorption was detected on a BioTek Cytation 3 multimode reader. RLU of NAD+ and NADH were normalized to levels measured in unstimulated THP-1 and are presented as arbitrary units (AU). All THP-1 cells utilized in this approach were harvested from P3.

2.10. Data Analysis and Statistical Evaluation. Infinium Methylation EPIC Bead Chip Array data were analyzed with GenomeStudio Methylation Module v1.8 and R bioconductor package “minfi” [22]. Differences in cytokine, NAD/-NADH, ATP, lactate, LDH, MTT, and oxygen assays were analyzed using one-way analysis of variance with Tukey’s multiple comparison test for \( n > 4 \) or nonparametric Kruskal-Wallis with Dunn’s multiple comparisons test for \( n \leq 4 \) (Prism 6.0c, GraphPad Software, La Jolla, CA, USA).

2.11. Ethical Approval. The study was approved by the local ethic board of the University Hospital Bonn (376/16). All donors provided informed consent.

3. Results

3.1. LPS Induces Innate Immunotolerance in a Human PBMC In Vitro Model. The first objective of our study was to prove the suitability of our in vitro PBMC model for the induction of long-term LPS tolerance. We aimed to unravel the link between the sustained dysregulated innate immune response as observed in septic patients and the modulation of CpG methylation.

To prove that 18 h of primary LPS stimulation is suitable for obtaining PBMCs with an LPS-tolerant phenotype, in vitro cytokine levels were determined in LPS-tolerized PBMCs vs. control PBMCs (see Figure 1). PBMCs responded to LPS with tolerance induction as reflected by quantification of TNFα gene and protein levels. Exposure to a second LPS stimulation did not further elevate TNFα transcription (Figure 2(a)) and cytokine secretion (Figure 2(b)) compared to cells treated with primary LPS stimulation alone \( (p < 0.05) \). IL-1β mRNA values demonstrated a similar pattern as TNFα with a low responsiveness of LPS-pretreated PBMCs towards a second LPS exposure (not significant). This observation was not reflected by IL-1β secretion. 18 h of initial LPS treatment elevated IL-10 gene expression and protein secretion. Restimulation with LPS for 3 h brought IL-10 mRNA transcription to a comparable level.

3.2. LPS Preincubation Causes Differential Methylation of Metabolic Pathway-Associated CpG Sites. 18 h of LPS incubation caused long-lasting LPS tolerance in PBMCs as proven by cytokine expression assays. Therefore, this point of time was also chosen for CpG methylation profiling. PBMCs donated by healthy volunteers were split, stimulated with either LPS or PBS for 18 h, and harvested for array analysis.

Processing the array data with Minfi Bioconductor package included quality assessment and correction for age, sex, and single-nucleotide polymorphisms. Afterwards, 1033 CpG sites demonstrated a significant LPS-induced reduction of methylation whereas 1972 CpG sites exhibited significantly enhanced methylation after LPS pretreatment (diff. score > +13 or < -13). Among the significantly modulated CpG sites (cutoff hypomethylated or hypermethylated sites: \(< -2.0 \) and \( > +2.0 \)) in the LPS-pretreated group, 37.63% of the alterations occurred in methylated CpG sites, 6.86% in unmethylated CpG sites, and 58.8% in marginally methylated CpG sites (Figure 3).

Differentially methylated CpG sites corresponding to pathophysiologically and physiologically relevant pathways were analyzed utilizing KEGG mapping. An accumulation of differentially methylated sites in metabolic pathway-related genes emerged (Table 1). Out of 1252 associated sites, 870 were differentially expressed. An enrichment of methylation changes in specific and uniform genomic regions in metabolism-correlated genes did not occur.

3.3. Sustained Immunoparalysis in LPS-Tolerant THP-1 Cells. Our array results highlight a correlation of functionally compromised immune cells and differentially methylated CpG sites in metabolic pathways. However, to exclude heterogeneity and evaluate functional relevance of our observations, we continued our studies in a single immortalized monocytic cell line.

First, we established a THP-1 in vitro model to induce long-term LPS tolerance. We investigated the sustainment of LPS tolerance up to three passing cycles after initial endotoxin challenge. Our model takes into account that the majority of cells in P2 and P3 are newly proliferated daughter cells, which possibly carry on LPS tolerance via epigenetic transmission (Supplement Figure 1). Compared to naïve THP-1 cells, restimulated immunoparalysed THP-1 cells responded with a significantly attenuated IL-1β and TNFα mRNA (Figure 4(a)) and protein expression (Figure 4(b)) upon second LPS treatment in P1, P2, and P3. IL-6 and IL-10 cytokine mRNA levels were rather increased upon another endotoxin challenge in passage 1 (P1) (not significant, Supplement Figure 2). Contrariwise, no considerable alteration of IL-6 and IL-10 was observed between treatment groups in passage 2 (P2). The kinetic of proinflammatory IL-1β and TNFα expression confirms long-term LPS tolerance in our in vitro model.

3.4. Alterations of Cellular Metabolism in Endotoxin Tolerance. After demonstrating an ongoing immune dysfunction in monocytes resting for 5 days after 48 h LPS challenge, the effects on cellular metabolism were assessed in cells of P3. First, oxygen consumption as a measurement of cellular respiration was quantified. Cells were stimulated with LPS or PBS at 0 h time point, and O₂ consumption was continuously measured. An exemplary measurement of nontolerized
THP-1 cells stimulated with PBS or LPS is given in Figure 5(a).

Comparing all four treatment groups, maximum oxygen consumption was observed in nontolerized THP-1 cells without additional LPS stimulation. Cells exposed to fresh LPS significantly reduced oxygen consumption compared to naïve cells, both 3 and 20 h after LPS stimulation (Figures 5(b) and 5(c)). LPS-pretreated cells taken from P3 without further LPS stimulation demonstrated intermediate O2 uptake not reaching the level of significance after 20 h of continuous measurement (Figures 5(b) and 5(c)). However, tolerized and nontolerized cells taken from P3 responded with a similar oxygen consumption towards a second LPS stimulus at 0 h.

We assumed that this observation came along with quantitative changes in the synthesis of ATP and NADH or enzymes participating in energy metabolism. However, ATP levels quantified in endotoxin pretreated and naïve THP-1 cells taken from P3 revealed no major differences between treatment groups (n = 6; Figure 5(d)).

MTT tests reflect the metabolic activity in cells, mainly dependent on NAD(P)H reduction equivalents and oxidoreductase activity. Results in treatment groups were expressed relative to values obtained from the PBS pretreated group. No difference between LPS-pretreated and naïve cells was observed (Figure 5(e)). Two hours of LPS stimulation slightly attenuated the rate of reduced MTT in naïve cells (not significant) and was unchanged in LPS-pretreated THP-1 cells from P3.

Intracellular LDH uses lactate and NAD+ to synthetize pyruvate and NADH. Upon cytotoxic stimuli, LDH is released from cells. In our model, LDH remained slightly increased (1.5-fold, n = 4, not significant) in LPS-pretreated THP-1 cells compared to PBS-pretreated cells (Figure 5(f)). Two hours of additional LPS treatment 7 d after pretreatment affected LDH activity in the same moderate way.

Lactate is an end product of glycolysis with regulatory properties of glucose homeostasis and energy production. To check for lactate, NAD+, and NADH accumulation in the media, we performed restimulation with LPS of tolerant and nontolerant THP-1 cells for 24 h. No differences in

![Figure 3: Methylation level of significant CpG sites. Methylome analysis of DNA was performed on PBMCs derived from healthy human donors (n = 4).](image-url)

![Figure 2: Induction of innate immunotolerance in human PBMCs. PBMCs (10^6 cells/ml) were isolated from whole blood (n = 4 individual donors) and stimulated for 18 h with 10 ng LPS/ml or PBS. Cytokine transcription (a) and secretion (b) were detected upon additional 3 h exposure to LPS or PBS (100 ng/ml). Mean ± SD; n = 4; *p < 0.05; **p < 0.01.](image-url)
lactate concentration in the media were detected 7 d after prestimulation, and 24 h of additional LPS stimulation did not cause any relevant increase of lactate level either (Figure 5(g)). LPS restimulation of both, tolerized and non-tolerized cells, significantly elevated the NAD+/NADH ratio, i.e., the reduction of NAD+ to NADH was decreased overall (Figure 5(j)).

Despite ongoing immune dysfunction in our model of LPS-tolerized THP-1 cells, the chosen readouts for cellular metabolism did not reveal any noteworthy alterations between tolerant and nontolerant THP-1 monocytes.

4. Discussion

Monocytes belong to the first line of defense against invading pathogens, by orchestrating the inflammation and contributing to sepsis outcome [23]. Monocytes from septic patients can exhibit endotoxin tolerance, compromising their function and paralyzing the immune response. Endotoxin tolerance and its influence on sepsis have already been studied [24]. However, the correlation of immunoparalysis, metabolic dysregulation, and epigenetic modifications in monocytes isolated from septic patients is a matter of ongoing investigation.

LPS can reprogram the inflammatory response toward a reduced inflammatory cytokine production (decreased TNFα production) in response to subsequent LPS challenge [25]. In our model of isolated human PBMCs, we detected attenuated TNFα and IL-1β cytokine mRNA expression and TNFα secretion upon LPS restimulation of tolerized PBMCs. The proinflammatory cytokines TNFα and IL-1β are accepted markers for demonstrating endotoxin tolerance [26, 27], while IL-10 reflects the anti-inflammatory cell response. Due to the specific properties of IL-1β processing and activation through the inflammasome [28], reduction of IL-1β protein as a result of attenuated mRNA transcription occurs temporally delayed.

The arising question was whether and how epigenetic mechanisms play a role in these observations. Epigenetic reprogramming may establish a form of innate immune memory against systemic infection or inflammation [29]. Histone and DNA methylation interact to silence TNFα expression [30]. Our results are in line with the observation that monocyte capacity to release anti-inflammatory mediators (e.g., IL-10) is neither impaired nor enhanced after sepsis and support the assumption that monocyte intracellular signaling is shifted from inflammatory response toward the production of anti-inflammatory molecules as a characteristic of tolerization [6, 31].

In order to link LPS tolerance with epigenetic modifications in PBMCs, we performed a CpG methylation array. DNA methylation is a stable epigenetic mark that is inheritable to daughter cells and changes DNA accessibility, thereby driving promoter activity. So far, methylation changes in human PBMCs manifested 18 h upon LPS challenge have
not been examined. Our methylation array revealed that differential methylation patterns occurred in metabolic pathway-associated genes in LPS-tolerized PBMCs. Metabolic pathway reprogramming and metabolic modulation of immune cell function have been of accelerating interest in the past few years [17, 32]. Defects in leukocyte energy metabolism are closely linked to the initial activation of the host defense and contribute to immunoparalysis in septic patients [15]. Due to interindividual variability in response to LPS stimulation as well as a low sample number, single CpG site methylation modifications did not reach significant values. This observation is reflected by Grondman et al., who also documented a heterogenic metabolic response in monocytes from healthy subjects rendered immunotolerant [33]. Tissue, age, and sex-specific DNA methylation changes have been reported. Nonetheless, KEGG pathway analysis revealed the highest differential methylation rate in CpG sites related to metabolic pathways.
Since DNA methylation is a stable epigenetic mark and can be passed on through multiple cell divisions, we suspected that LPS-induced alterations of metabolic activity may persist. Therefore, we aimed to develop a cell culture model that allows for long-term observations of LPS tolerance phenomena including metabolic activity in monocytic cells. Since vitality of PBMCs is known to rapidly decrease after 36 h, we established a model of extended LPS tolerance with repeatedly passaged monocytic THP-1 cells. First, we characterized the reliability of our model by detecting the standard parameters of endotoxin tolerance, observing that TNFα and IL-1β cytokine repression upon LPS restimulation was still measurable 7 days after initial LPS stimulation. Assuming a doubling of THP-1 cells within approximately 2 days, the observed variation in TNFα and IL-1β cytokine levels was not limited to parent cells with immediate contact to the stimulant but was also monitored in newly proliferated daughter cells.

In addition to the widely accepted response of monocytes to TLR4 stimulation by proinflammatory cytokine release, cells activated by LPS also undergo profound metabolic changes [34]. Activation of macrophages and dendritic cells by proinflammatory stimuli causes them to undergo a metabolic switch towards glycolysis [35]. Besides mere energy and biosynthetic precursor generation, there is growing evidence that metabolites also control immunological effector function [36]. With regard to our cytokine expression kinetics and based on our methylation array findings, we hypothesized that THP-1 cells tolerized with LPS demonstrate a profound metabolic switch after 7 d. Presumably, the significantly decreased O2 uptake of tolerized cells after LPS restimulation points towards an ongoing inflammatory phenotype with a reduced rate of oxidative phosphorylation and coverage of energy demand via glycolysis. However, our data highlight that in response to LPS pre-treatment key glycolytic enzymes, cofactors and molecules involved in energy transfer were not altered after 7 d, whereas LPS restimulation caused a reduction of NAD+ to NADH conversion. These observations are not congruent with our hypothesis, arising new questions. We are aware that assay results strongly depend on the time point of analysis chosen. As demonstrated by Zhu et al., metabolism and
bioenergetics of monocytes sequentially activate, deactivate, and ultimately resolve acute inflammation [34]. One additional aspect of interest, which has to be examined in future projects, is the host iron metabolism. By deregulating glucose metabolism, this mechanism has been shown to compromise the establishment of disease tolerance to sepsis [37, 38].

In vitro models often lack clinical relevance due to various methodological limitations. Most studies analyze short time intervals after LPS stimuli, which is barely avoidable when experimenting with PBMCs. On the other hand, immortalized cell lines do not share all physiologic properties of primary cells. THP-1 cells, which serve as a model for human monocytes, derived from a spontaneously immortalized monocyte-like cell line, developed in a case of acute monocytic leukemia [18]. They respond weakly to LPS due to low CD14 expression levels [39], which entails different LPS dosages to correct for the differences in LPS responsiveness. Thus, we increased the widely used LPS dosage of 10 ng/ml in PBMC experiments to 1 μg/ml in THP-1 experiments [40]. Furthermore, comparisons of THP-1 cells to freshly isolated PBMCs and monocytes revealed that these cells may release different cytokine quantities under identical experimental conditions [41]. Thus, clinical implications may better be inferred from cumulated experiments performed with primary, immortalized, and septic patient cell samples.

5. Conclusions

Our model reveals that LPS tolerance effects reflected on the level of cytokine regulation outlast metabolic alterations. Immune response and metabolic regulation are highly integrated and tend to reestablish homeostasis. Hence, the selected THP-1 model is appropriate when examining the termination of inflammatory processes in a single cell model under defined experimental conditions. Snapshot collections as well as large-scale array analysis of intertwined immunological reactions, epigenetic modifications, metabolic changes, and kinetics will steadily give further insight into sepsis pathophysiology.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

None is declared.

Authors’ Contributions

A.C., H.E., J.L.K., A.K., and L.W. performed the experiments. H.E., S.F.E., S.H.H., A.K., S.S., and L.W. evaluated the data. H.E. and C.K.W. designed the study with support from S.F. and A.H. H.E. and C.K.W. wrote the paper in consultation with S.F. and A.H.

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Supplementary Materials

Supplement Figure 1: THP-1 proliferation recovers within 5 days after 48 h of LPS stimulation. LPS or PBS were added to media in P0 for 48 h. Cell density was determined in all treatment groups at the end of P0 (48 h after treatment begin), P1 (96 h after treatment begin), and P2 (144 h after treatment begin) prior to resuspension in fresh media containing no LPS at a density of 106 cells/ml (A) (mean ± SEM, n = 8-12; *p < 0.05). At each time point, statistical differences between PBS and LPS treated cells were calculated with a t-test (*p < 0.05). Supplement Figure 2: LPS-tolerized (1 μg/ml) THP-1 monocytes demonstrate sustained IL-6 and IL-10 cytokine mRNA and protein alterations upon LPS restimulation (1 μg/ml) compared to previously untreated cells. mRNA transcription of IL-6 and IL-10 was measured in passage (P)1 and P2 after 48 h preincubation followed by resting durations of 0 h (P1) and 48 h (P2). Mean ± SD; n = 3. (Supplementary Materials)

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