Biochemical and Biophysical Research Communications xxx (2015) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Lactic acid delays the inflammatory response of human monocytes

Katrin Peter [a, *], Michael Rehli [a, b], Katrin Singer [a], Kathrin Renner-Sattler [a], Marina Kreutz [a, b]

[a] Department of Internal Medicine III, University Hospital Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany
[b] RCI Regensburg Center for Interventional Immunology, University Hospital Regensburg, Franz-Josef-Strauß-Allee 11, 93053 Regensburg, Germany

ARTICLE INFO

Article history:
Received 20 December 2014
Available online xxx

Keywords:
Tumor
Metabolism
Lactic acid
Monocyte
Inflammation
Signal transduction

ABSTRACT

Lactic acid (LA) accumulates under inflammatory conditions, e.g. in wounds or tumors, and influences local immune cell functions. We previously noted inhibitory effects of LA on glycolysis and TNF secretion of human LPS-stimulated monocytes. Here, we globally analyze the influence of LA on gene expression during monocyte activation. To separate LA-specific from lactate- or pH-effects, monocytes were treated for one or four hours with LPS in the presence of physiological concentrations of LA, sodium lactate (NaL) or acidic pH. Analyses of global gene expression profiles revealed striking effects of LA during the early stimulation phase. Up-regulation of most LPS-induced genes was significantly delayed in the presence of LA, while this inhibitory effect was attenuated in acidified samples and not detected after incubation with NaL. LA targets included genes encoding for important monocyte effector proteins like cytokines (e.g. TNF and IL-23) or chemokines (e.g. CCL2 and CCL7). LA effects were validated for several targets by quantitative RT-PCR and/or ELISA. Further analysis of LPS-signaling pathways revealed that LA delayed the phosphorylation of protein kinase B (AKT) as well as the degradation of IκBα. Consistently, the LPS-induced nuclear accumulation of NFκB was also diminished in response to LA. These results indicate that the broad effect of LA on gene expression and function of human monocytes is at least partially caused by its interference with immediate signal transduction events after activation. This mechanism might contribute to monocyte suppression in the tumor environment.

1. Introduction

Monocytes express a range of pattern-recognition receptors like toll-like receptors (TLRs), which are responsible for the activation of monocyte effector functions. Agonists for TLRs include microbial components, e.g. lipopolysaccharide (LPS) [1], and also endogenous danger signals which can be found amongst others in tumors [2]. Upon ligand binding, signaling cascades are initiated which eventually lead to the degradation of IκBα and to the nuclear transition of the transcription factor nuclear factor κ B (NFκB). In addition, several other signaling cascades such as MAPK signaling pathways and the PI3K/AKT signaling pathway are stimulated by TLRs [1].

Although the effector mechanisms of monocytes provide powerful means to fight tumors, tumor-infiltrating monocytes often exhibit protumoral characteristics including the decreased secretion of TNF or an increased production of IL-10 or IL-23. The altered phenotype of monocytes is thought to result from the presence of soluble factors like hyaluronan or lactic acid (LA) in the tumor milieu [3,4].

Since tumor cells largely limit their energy generation to glycolysis they produce lactate, secrete it together with protons into the microenvironment and acidify their stroma [5]. We demonstrated that LA and in part also the corresponding acidification inhibit TNF secretion and glycolysis of human monocytes [6]. In addition, high lactate concentrations in combination with low pH have been found to reduce the production of TNF and IL-1β by macrophages/monocytes [7]. We and others have reported an inhibition of the differentiation of monocytes to dendritic cells in the presence of LA [8,9]. In addition, we reported LA to strongly inhibit the activation of T cells [10]. However, LA or lactate have also been described to stimulate the expression of IL-23, IL-6 or IL-8 in mononuclear cells [4,11,12]. Furthermore, LA drives the polarization of tumor-associated macrophages [13].

The diverse effects of LA on various immune cells suggest that LA or lactate may influence widely used signaling pathways. Indeed both molecules have been demonstrated to influence several MAP kinases, NFκB signaling or the PI3K/AKT pathway [12,14,15].

Abbreviations: LA, L-lactic acid; NaL, sodium L-lactate; HCl, hydrochloric acid; GO, gene ontology; PTX3, pentraxin 3; GEM, GTP-binding protein overexpressed in skeletal muscle; EGR2, early growth response 2.

* Corresponding author. Fax: +49 941 9445593.
E-mail addresses: katrin.peter@ukr.de (K. Peter), michael.rehli@ukr.de (M. Rehli), katrin.singer@ukr.de (K. Singer), kathrin.renner-sattler@ukr.de (K. Renner-Sattler), marina.kreutz@ukr.de (M. Kreutz).

http://dx.doi.org/10.1016/j.bbrc.2015.01.005
0006-291X/

Based on our findings on the inhibition of TNF secretion and glycolysis by LA in human monocytes [6] we now aimed to globally characterize the effect of LA on monocytes. Using whole genome microarray analysis we observed a broad transient negative effect of LA and the associated acidification on the LPS-induced gene expression. Furthermore, the presence of LA delayed LPS-induced signaling pathways. These results indicate that LA profoundly influences the biology of human monocytes which might contribute to immune suppression in tumors.

2. Material and methods

2.1. Chemicals

Unless otherwise noted, chemicals were purchased from Sigma (Deisenhofen, Germany).

2.2. Isolation and culture of monocytes

Monocytes were obtained from healthy donors, as described previously [16], the isolation was approved by the local ethics committee. 1,66 × 10^6 monocytes/ml were cultured in RPMI-1640 supplemented with 2% human AB-serum (PAN Biotech, Aidenbach, Germany), L-glutamine (2 mmol/L), 50 U/mL penicillin and 50 μg/mL streptomycin (all from Gibco, Karlsruhe, Germany) in the presence of 10 and 20 mM L-lactic acid (LA) or 20 mM sodium L-lactate (NaL). LPS (from *Salmonella abortus equi* S-form, Enzo Life Sciences, Lörrach, Germany) was added to a final concentration of 100 ng/mL. Furthermore, monocytes were incubated with LPS in combination with 1% hydrochloric acid (HCl) to titrate the pH of the medium to ~7.1 or ~6.6, corresponding to the pH of media containing 10 or 20 mM LA, respectively.

2.3. Determination of cytokines

Detection of TNF and CCL2 in culture supernatants was performed using R&D systems (Minneapolis, USA) ELISA kits.

2.4. Preparation of RNA

Total cellular RNA was isolated using RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA concentration was measured with ND-1000 NanoDrop Spectrophotometer (Thermo Fisher Scientific, Schwerte, Germany) and quality was controlled on agarose gels or using Agilent Bioanalyzer (Böblingen, Germany).

2.5. Reverse transcription and quantitative real-time PCR (RT-qPCR)

Reverse transcription and RT-qPCR were performed as described previously [6]. Primer sequences (all purchased from Eurofins MWG Operon, Ebersberg, Germany, except primers for TNF (QuantiTect Primer Assay, Quiagen, Hilden, Germany)) are provided in Supplementary Table 1.

2.6. Whole genome expression analysis

Labeling, hybridization and scanning of high quality RNA was conducted as described previously [17]. Microarray analysis and GO term enrichment was performed using GeneSpring 10.2 software (Agilent Technologies, Böblingen, Germany). In case of multiple probes representing one gene the corresponding probes were averaged. Expression data were median-normalized and filtered as follows: genes considered undetectable (fluorescence intensity below 50 in less than three out of 32 samples) were excluded. The resulting gene list was reduced to ~2800 genes showing significantly different expression between relevant conditions (one-way ANOVA with asymptotic P-value calculation and Benjamini–Hochberg correction, 5% FDR).

2.7. Preparation of whole cell lysates and western blotting

Lysate preparation was performed as described previously [18]. In brief, 10 × 10^6 monocytes were washed with phosphate-buffered saline, pelleted, dissolved in 500 μl pre-equilibration buffer [18], centrifuged at 900 × g, dissolved in 150 μl lysis buffer [18] and incubated on ice for 10 min. Lysates were dissolved in 150 μl 2× SDS sample buffer and incubated at 95 °C for 10 min. After blotting membranes were incubated with antibodies against phospho-p38 MAP-Kinase (1:2000), IκBα (1:1000), phospho-AKT (1:1000) (all Cell Signaling, Danvers, USA) or β-actin (1:2000) (Sigma–Aldrich, Munich, Germany).

2.8. Preparation of nuclear lysates and detection of nuclear factor κ B (NFκB)

Nuclear lysates were prepared as described previously [19]. Briefly, 50 × 10^6 monocytes were washed with PBS, dissolved in 400 μl buffer A (Supplementary Table 2), incubated on ice for 3 min, centrifugated (3000 × g), resuspended in buffer A and lysed by passing through a 27G needle. After centrifugation (3000 × g) pelleted nuclei were washed in buffer A, lysed in buffer B (Supplementary Table 2), incubated on ice for 10 min and centrifuged (10,000 × g, 10 min). Protein concentration was determined by Pierce protein assay (Thermo Fisher Scientific, Schwerte, Germany). The amount of NFκB was measured using TransAM NFκB p65 ELISA kit (Active Motif, La Hulpe, Belgium).

3. Results

3.1. LA delays the LPS-induced transcriptional response

To analyze the impact of LA on activated monocytes we performed whole genome microarray expression analyses with monocytes incubated for 1 or 4 h with LPS in the presence or absence of 10 or 20 mM LA, NaL or in culture medium with pH 7.1 or 6.6 (corresponding to pH of 10 or 20 mM LA, respectively). As controls we used monocytes without incubation or monocytes incubated without LPS. To isolate LA-specific effects (relative to acidification), we focused on genes that were significantly regulated at least two-fold by LPS and at least two-fold different between the conditions 20 mM LA/LPS and LPS as well as 20 mM LA/LPS and pH 6.6/LPS. After 1 h incubation we found a predominantly negative impact of 20 mM LA and to a lesser extent of the corresponding acidification which had mostly disappeared after 4 h (Fig. 1 and Supplementary Fig. 1) indicating that 20 mM LA delayed LPS-induced gene activation. The incubation of monocytes with LPS and 20 mM NaL, 10 mM LA or medium with pH 7.1 only slightly affected the expression of few genes. In line with the focus on LPS-regulated genes, a number of gene ontology (GO) terms significantly enriched (FDR 5%) in the cluster were associated with an ongoing immune response (Supplementary Table 3).

3.2. LA affects gene expression with different pH-dependency and duration

For validation of array data we concentrated on genes showing interesting regulation patterns and/or coding for proteins with immunological function. To analyze the persistence of the LA effect we compared mRNA expression between monocytes incubated for 1 h and 17 h.

Please cite this article in press as: K. Peter, et al., Lactic acid delays the inflammatory response of human monocytes, Biochemical and Biophysical Research Communications (2015), http://dx.doi.org/10.1016/j.bbrc.2015.01.005