ELSEVIER

CYTOKINE

www.elsevier.com/locate/issn/10434666

Cytokine 37 (2007) 76–83

UVB-induced IL-18 production in human keratinocyte cell line NCTC 2544 through NF-κB activation

Alexia Grandjean-Laquerriere [a], Frank Antonicelli [b], Sophie C. Gangloff [a], Moncef Guenounou [a], Richard Le Naour [a,*]

[a] *Laboratoire d'Immunologie et de Microbiologie, IPCM, EA3796, IFR53, UFR de Pharmacie, 1 rue du Maréchal Juin, 51096 Reims Cedex, France*
[b] *Laboratoire de Biochimie, CNRS UMR 6198, IFR53, UFR de Médecine, 1 rue du Maréchal Juin, 51096 Reims Cedex, France*

Received 27 November 2006; received in revised form 8 February 2007; accepted 19 February 2007

Abstract

In the present study, we investigated the implication of NF-κB in the production of pro-inflammatory cytokine IL-18 by human keratinocytes stimulated by UVB. We demonstrated that NCTC 2544 keratinocyte cell line irradiated by UVB enhanced the IL-18 mRNA and protein secretion under its bioactive form. Overexpression of IL-18 by UVB irradiation was accompanied by NF-κB transcription factor activation using specific IL-18 gene sequence corresponding to NF-κB DNA binding site. The relationship between these transcription factors and IL-18 expression was confirmed using curcumin and PDTC, two inhibitors of NF-κB. Our results show that UVB and curcumin or PDTC co-treatment led to a down-regulation of IL-18 expression associated with an inhibition of NF-κB DNA binding. Hence, our results demonstrated that this transcription factor is implicated in biologically active IL-18 production by human keratinocytes irradiated by UVB.

Keywords: UVB; IL-18; NCTC 2544 keratinocyte cell line; NF-κB; Curcumin; PDTC

1. Introduction

Skin is one of the largest tissue of the body permanently aggressed by environmental stress such as sunlight, which has profound epidermis deleterious effects responsible for inflammation, immune depression, aging, and skin cancer. Keratinocytes are the major cell population of the epidermis involved in inflammatory process through cytokine production [1]. UVB-light irradiation of human keratinocytes induces the production of pro-inflammatory cytokines such as TNF-α, IL-1, IL-6, IL-8 and more recently IL-18 [2–6]. Of interest, it is thought that UVB-induced IL-18 overexpression by keratinocytes plays a critical role in many processes such as the malignancy of skin tumors through its effect on immune cells [7,8].

IL-18 is a pleiotropic pro-inflammatory cytokine [9] synthesized by various cell types [10–13]. Recent works have demonstrated that human keratinocytes are the major source of IL-18 and express significantly higher levels of IL-18 than normal human leucocytes, PBMC and monocytes [14]. In all cell types, IL-18 is always produced as a biologically inactive precursor whose activation requires an intracellular processing by the cysteine protease caspase-1 [15]. Under normal conditions, keratinocytes do not have caspase-1 [16]. Nevertheless, they secrete biologically active IL-18 after treatment with various pro-inflammatory mediators such as phorbol 12-myristate 13-acetate (PMA) and lipopolysaccharides (LPS) [17]. IL-18 is also regulated at the gene transcriptional level. The presence of NF-κB and AP-1 recognition sequences has been located in the upstream of the IL-18 promoter [18,19]. However, up to now only the AP-1 site has been involved in UVB-induced IL-18 production in a human keratinocyte cell line [6].

* Corresponding author. Fax: +33 3 26 91 37 20.
E-mail address: richard.lenaour@univ-reims.fr (R. Le Naour).

1043-4666/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2007.02.020

Owing that (i) NF-κB and AP-1 activation is the hallmark of keratinocyte stimulation upon UVB irradiation [20,21] and (ii) we reported that UVB irradiation of the human keratinocyte cell line NCTC 2544 led to NF-κB and AP-1 activation associated with the synthesis of pro-inflammatory cytokine TNFα, IL-6 and IL-8 [22], we wondered whether the transcription factor NF-κB was also involved in UVB-induced IL-18 production. The results presented here demonstrate that the human keratinocyte cell line NCTC 2544 express IL-18 upon UVB and LPS stimulation via NF-κB transcription factor activation.

2. Materials and methods

2.1. Reagents

Eagle's minimum essential medium (EMEN), RPMI 1640, fetal calf serum (FCS), Lymphoprep, L-glutamine, penicillin, streptomycin, phosphate-buffered saline (PBS), ethidium bromide, Trizol, oligo-dT primer, DTT, dNTP mix, Reverse transcriptase, *Taq* DNA polymerase and primers were obtained from Life Technologies (Cergy Pontoise, France). Curcumin, lipopolysaccharide (LPS), concanavalin A, poly(dI–dC), spermidine, PMSF, *O*-phenanthroline, leupeptin, glycerol, Hepes and pyrrolidine dithiocarbamate (PDTC) were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France). Aprotinin came from Bayer-AG (Leverkusen, Germany) and Nonidet P40 was obtained from Fluka Chemical LLC (Buchs, Switzerland). The double-stranded oligonucleotides (NF-κB sequence) and RNasine were obtained from Promega (Charbonnières, France). T4 polynucleotide kinase was from Ozyme (Saint-Quentin-En-Yvelines, France) and γ-^{32}P-ATP (3000 Ci mmol^{-1}) came from NEN Life Science Products (Le Blanc Mesnil, France). Monoclonal antibodies directed against human recombinant IL-18 (hrIL-18) were obtained from R&D systems (Minneapolis, USA).

2.2. Cell line and culture conditions

The human keratinocyte cell line NCTC 2544 (Biopredic, Rennes, France) was cultured in EMEN supplemented with 300 μg/ml L-glutamine, 100 U/ml penicillin, 50 μg/ml streptomycin and 10% heat-inactivated FCS (56 °C during 30 min). In each experiment, cells were seeded into 24-well plates (5×10^5 cells/well) and cultured to 90% confluence at 37 °C in a humidified atmosphere containing 5% CO_2. Cells were then irradiated with UVB light or treated with LPS (0.1 and/or 1 μg/ml) and incubated for 2–24 h at 37 °C in 5% CO_2. In some experiments, curcumin (10, 25 and 50 μM) or PDTC (50 and 100 μM) was added 1 h before irradiation or LPS treatment and maintained all along cell culture.

The human peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood of healthy donors by centrifugation over Lymphoprep gradients following the manufacturer's instructions. Isolated PBMC were washed in RPMI 1640 medium, suspended in RPMI 1640 medium supplemented with 10% heat-inactivated FCS, 300 μg/ml L-glutamine, 100 U/ml penicillin and 50 μg/ml streptomycin, seeded into 24-well plates (1×10^6 cells/well) and then used for the determination of IL-18 biological activity in conditioned keratinocyte media.

2.3. UVB irradiation

UVB irradiation (10–100 mJ/cm^2) was applied using a light source (0.98 mW/cm^2, 280–320 nm, maximum at 313 nm) supplied by a Bio-Sun fluorescent lamp (Vilbert Lourmat, Marne la Vallée, France). Control cells were mock irradiated. Two to 24 h after UVB-irradiation the viability of the UVB-treated cells was determined by trypan blue exclusion test. The viability was around 90% when cell culture supernatants were collected for cytokine determination or cells lysed in Trizol for cytokine mRNA analysis or nuclear proteins extracted for examination of transcription factor activation.

2.4. Analysis of cytokine steady-state mRNA level by reverse transcription-polymerase chain reaction (RT-PCR)

RNA extraction was performed using phenol/chloroform extraction followed by ethanol precipitation. RNA (0.4 μg) from each sample was reverse-transcript with oligo-dT as the first-strand cDNA primer and Moloney murine leukaemia virus reverse transcriptase σM-MLV RT superscript (Life Technologies, France) as described previously by Esnault et al. [23]. The sequences of the primers for IL-18, IFN-γ and internal control β-actin were (sense) IL-18 5′-GAA CCA GTA GAA GAC AAT TGC-3′, (antisense) IL-18 5′-GGT CTC TCT CTT TTT CAC AAG C-3′, (sense) IFN-γ 5′-AGT TAT ATC TTG GCT TTT CA-3′, (antisense) IFN-γ 5′-ACC GAA TAA TTA GTC AGC TT-3′, (sense) β-actin 5′-TGC TAT CCA GGC TGT GCT A-3′, (antisense) β-actin 5′-ATG GAG TTG AAG GTA GTT T-3′, respectively. Reverse transcribed RNA were subjected to PCR using following cycling conditions: 94 °C for 1 min, 62 °C (IL-18) or 58 °C (IFN-γ and β-actin) for 1 min, 72 °C for 1 min. Number of cycles for IL-18, IFN-γ and β-actin was 33, 35 and 25, respectively. The reagents and the classical PCR conditions have been previously optimized [23]. The amplification products were either separated electrophoretically on 2% agarose gels with ethidium bromide and analyzed by the imager analyzer Bio-Rad Fluor-S, or denatured and blotted onto Hybond–N$^+$ membrane (Amersham, Les Ulis, France). Following blotting, specific probes were 3′-end labelled with fluorescein-11-dUTP using the enhanced chemiluminescence (ECL) 3′-oligolabelling reagents (RPN 2130, Amersham, Les Ulis, France). Following hybridization and incubation with anti-fluorescein-horseradish peroxidase, detection was performed using hydrogen peroxide and luminol (RPN 2105, Amersham, Les Ulis, France). The luminescence was detected