Cytokine 63 (2013) 97–104

Contents lists available at SciVerse ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Interleukin-1beta stimulates platelet-activating factor production in U-937 cells modulating both its biosynthetic and catabolic enzymes

Ioanna C. Vlachogianni [a,b], Tzortzis Nomikos [b,*], Elizabeth Fragopoulou [b], George M. Stamatakis [a], Haralambos C. Karantonis [c], Smaragdi Antonopoulou [b], Constantinos A. Demopoulos [a]

[a] Faculty of Chemistry, National and Kapodistrian University, Athens, Greece
[b] Department of Nutrition and Dietetics, Harokopio University, Athens, Greece
[c] Department of Food Science and Nutrition, University of Aegean, Limnos, Greece

ARTICLE INFO

Article history:
Received 8 November 2012
Received in revised form 26 March 2013
Accepted 15 April 2013
Available online 11 May 2013

Keywords:
IL-1β
Platelet-activating factor
Monocytes
lyso-PAF acetyltransferase
PAF acetylhydrolase

ABSTRACT

Interleukin-1beta (IL-1β) is a potent agonist of platelet-activating factor (PAF) synthesis. The monocyte-derived PAF may amplify the inflammatory and thrombotic processes. The IL-1β-induced enzymatic alterations leading to increased PAF synthesis are ill-defined. In the present study the last enzymatic activities of the remodeling (acetyl-CoA:lyso-PAF acetyltransferase) and de novo (DTT-insensitive CDP-choline:1-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase) biosynthetic routes of PAF and its main catabolic enzyme, PAF acetylhydrolase, along with the intracellular and extracellular PAF levels were determined in homogenates and medium of U-937 after their stimulation with recombinant IL-1β.

IL-1β at 2.5 ng/mL induced an early (0.5–3 h) and a late (12 h) elevation of intracellular PAF levels (2-fold). Only a small portion of intracellular PAF (~10%) was released to the extracellular medium. IL-1β increased lyso-PAF acetyltrasnferase activity which was peaked at 3 h and kept elevated till 12 h. A rapid 1.5-fold increase of cholinephosphotransferase activity was observed in IL-1β stimulated cells. Finally, a transient stimulation of intracellular PAF-AH was induced by IL-1β at 3 h while incubation of U-937 with the PAF acetylhydrolase inhibitor pefabloc in the presence or absence of IL-1β led to a strong sustained increase of intracellular PAF levels.

In conclusion, both biosynthetic routes of PAF, along with its degradation can be modulated by IL-1β in a time-specific manner. The inhibition of PAF acetylhydrolase strongly augments PAF's intracellular levels implying its crucial role for the regulation of cellular PAF. The regulation of PAF's enzymatic machinery under inflammatory conditions is more complicated than we thought to be.

1. Introduction

Monocytes are circulating blood cells which represent a highly mobile component of the innate immune system involved in the nonspecific immunity of the human body and the inflammatory processes [1]. They have a crucial role in atherogenesis via their adhesion to the endothelium, their migration to the intima and their maturation into macrophages, initiating by this way the development of atherosclerosis [2]. The human histiocytic lymphoma cell line U-937 is one of the few that retain the characteristics of monocytes, making it an excellent model for laboratory research [3].

Inflammatory signals activate monocytes to produce a milieu of cytokines and chemokines which in turn initiate and propagate the inflammatory response of the host [4]. Among them, IL-1β has a pivotal role, since it is a key mediator in the cytokine network, controlling important metabolic pathways in the immune system [5]. IL-1β is not only produced by monocytes but it also activates them in a receptor-mediated fashion which indicates a possible autocrine mode of action. More specific, it induces the generation of reactive oxygen species and the activation of nuclear factor-kappa B (NF-κB) [6]. This autocrine loop of IL-1β may amplify the response of circulating monocytes to various inflammatory stimuli especially under conditions of subclinical inflammation.

Platelet Activating Factor (PAF) [7], one of the most potent, naturally produced, phospholipid mediators, may serve as a putative

Abbreviations: PAF, platelet-activating factor; lyso-PAF AT, acetyl-CoA:lyso-PAF acetyltransferase; PAF-CPT, DTT-insensitive CDP-choline:1-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase; PAF-AH, PAF-acetylhydrolase; IL-1β, interleukin-1β; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; DTT, dithiothreitol.

* Corresponding author. Address: Department of Nutrition and Dietetics, Harokopio University, 70 El. Venizelou Str., 17671 Athens, Greece. Tel.: +30 2109549305; fax: +30 2109577050.
E-mail address: tnomikos@hua.gr (T. Nomikos).
URL: http://www.ddns.hua.gr/en/content/home.htmL (T. Nomikos).

1043-4666/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cyto.2013.04.024

mediator of IL-1β actions on monocytes. Cellular experiments have previously demonstrated the ability of IL-1β to activate PAF synthesis in endothelial cells [8], mesangial cells [9] and human monocytes [10]. The monocyte-derived PAF may amplify the inflammatory and thrombotic processes by several mechanisms such as induction of von Willebrand factor release by endothelial cells [11], induction of IgG2 production by lymphocytes [12] and adhesion of monocytes to platelets [13]. The ability of PAF to serve as mediator of IL-1β actions has also been demonstrated in animal and cellular experiments with the use of PAF receptor antagonists. For example, WEB 2086 attenuated the TNFα- and IL-1α-induced cardiopulmonary alterations in anesthetized pigs [14], BN50730 suppressed NO production and sPLA2 release by astrocytes [15], while WEB-2170 and UK-74,505 inhibited leukocyte extravasation in rat mesenteric microvessels [16]. Apart from being a mediator of IL-1β actions, PAF is also an agonist or primer of IL-1 release by several cells such as monocytes [17], endothelial cells [18] and macrophages [19]. By this way, IL-1β and PAF create an autoregulatory loop which seems to amplify and ultimately regulate inflammatory processes in the vasculature.

The enzyme activities modulation and PAF synthesis increment into cells by IL-1β is ill-defined and not well-studied. PAF can be synthesized by two distinctly different enzymatic routes, known as the remodeling and the de novo pathways (see Supplementary material). The remodeling pathway involves a structural modification of pre-existing ether-linked choline-containing phospholipids that serve as structural components of membranes. The last step of PAF formation via the remodeling pathway is the acetylation of lyso-PAF catalyzed by at least two isoforms of acetyl-CoA:lyso-PAF acetyltransferases, namely LPCAT1 and LPCAT2 (lyso-PAF AT) [20,21]. It is believed that the remodeling route is activated by inflammatory stimuli leading to the rapid increase of PAF. On the other hand the de novo reaction pathway is thought to be responsible for the constitutive synthesis of PAF maintaining its resting levels in various tissues and blood [22]. The last step of this pathway is the conversion of 1-O-alkyl-2-acetyl-glycerol to PAF catalyzed by a specific dithiothreitol-insensitive CDP-choline:1-alkyl-2-acetyl-sn-glycerol cholinephosphotransferase (PAF-CPT) [23]. However, the regulation of the biosynthetic pathways of PAF seems to be more complicated than we thought to be since a recently cloned lyso-PAF AT isoform is Ca^{2+}-independent while our team recently has shown that, in heart failure patients, both PAF biosynthetic routes are correlated with well-established inflammatory markers [24]. Intracellular PAF is hydrolyzed and inactivated by PAF-acetylhydrolase, which is a Ca^{2+}-independent, phospholipase A_2 (PLA_2) [25]. This enzyme regulates intracellular PAF levels in monocytes and neutrophils counteracting PAF biosynthesis [26]. It is therefore obvious that intracellular PAF levels are determined by the relative activities of both its biosynthetic and catabolic enzymes. However, a simultaneous determination of the aforementioned enzymatic activities and an estimation of their contribution to PAF levels under inflammatory stimulation has never been made before. Under this perspective, aim of the present study is the simultaneous determination of lyso-PAF AT, PAF-CPT, PAF-AH and PAF in U-937 homogenates and medium after their stimulation with IL-1β.

2. Materials and methods

RPMI 1640 was purchased from Gibco BRL (Paisley, UK) and recombinant human interleukin-1β (IL-1β) from R&D Systems (Minneapolis, USA), while newborn calf serum (NCS), glutamine, penicillin, free fatty acid bovine serum albumin (BSA-FFA), 3-(4,5dimethylthiazol-2-yl-)5 diphenyltetrazolium bromide (MTT), trichloroacetic acid (TCA), Coomassie Brilliant Blue G-250, acetyl Coenzyme A and pefabloc, were obtained from Sigma (St. Louis, MO). Analytical grade solvents, liquid chromatography grade solvents and silica gel G, used for thin layer chromatography, were supplied from Merck (Darmstadt, Germany). 1-O hexadecyl-2-O-acetyl-sn-glycerol was purchased from Biomol International (Plymouth Meeting, PA, USA). 1-O-hexadecyl-2-[3H]acetyl-sn-glycerol-3-phosphocholine ([3H] PAF) with a specific activity of 10 Ci/mmol was obtained from New England Nuclear (Boston, Mass). The purification of PAF by HPLC was performed at room temperature on a HP HPLC Series 1100 liquid chromatography model (Hewlett Packard, Waldbronn, Germany) equipped with a cation exchange column, Partisil 10SCX 4.6 mm × 250 mm, from Whatman (England), a 100 μL loop Rheodyne (7725i) loop valve injector, a degasser G1322A, a quad gradient pump G1311A and a HP UV spectrophotometer G1314A as a detection system. The spectrophotometer was connected to a Hewlett–Packard (Hewlett Packard, Waldbronn, Germany) model HP-3395 integrator–plotter. PAF-induced platelet aggregation was measured in a Chrono-Log (Havertown, PA, USA) aggregometer (model 400-VS) coupled to a Chrono-Log recorder (Havertown, PA, USA) at 37 °C with constant stirring at 1200 rpm. Centrifugations were performed in a refrigerated superspeed Thermo Scientific Heraeus Labofuge 400R centrifuge (Heraeus). Homogenizations were carried out with a Vibra Cells Sonics Materials ultrasonic homogenizer (Danbury, USA). Scintillation liquid cocktail (dioxane base) was prepared by diluting 7 g PPO (2,5-diphenyloxazole), 0.3 g POPOP (1,4-bis(5-phenyloxazol-2-yl) benzene) and 100 g Napthalene in 1 L of dioxane containing 200 mL H_2O. The radioactivity was measured in a 1209 Rackbeta model scintillation counter (Pharmacia, Wallac, Finland). A U-937 human cell line was used in all experiments (kindly donated by Dr. Z. Varghese, Royal Free Hospital, Centre for Nephrology, University Collage Medical School, London, United Kingdom).

2.1. Culture and activation of U-937

Cells were routinely maintained in RPMI 1640 cell culture medium supplemented with 10% newborn calf serum (NCS), glutamine (2 mmol/L) and 0.01% (w/v) of penicillin and streptomycin. Cells were synchronized for 24 h in serum free medium (SFM) before use in all experiments and after that 2×10^6 cells in 2-mL SFM were cultured in the presence or absence (corresponding vehicle) of IL-1β (1–5 ng/mL, dissolved in PBS containing 0.1% BSA-FFA) for various time intervals (10 min–24 h). In some experiments U-937 were incubated with pefabloc (0.2 mM) or pefabloc (0.2 mM) and IL-1β (2.5 ng/mL). By the end of the incubation time cells were pelleted at 4 °C (500*g*, 10 min) and the supernatant was divided into two fractions. The first fraction, used for the determination of the released PAF-AH, was immediately stored at −80 °C while the second fraction was submitted to a Bligh–Dyer extraction [27] for PAF determination. The cells were washed twice with 1 mL PBS each time and the washed pellet was diluted in 1 mL of 50 mM Tris–HCl (pH 7.4). One-hundred microlitres of the cell suspension were used for protein determination, 450 μL were extracted by the Bligh–Dyer method for the determination of intracellular PAF and the rest was homogenized by sonication (4 times × 10 s, 35% max power with intervals of 1 min). The homogenate was centrifuged at 500*g* for 10 min (4 °C) and the supernatants were aliquoted and kept at −80 °C until the day used for PAF enzymes activity determination.

2.2. Determination of intacellular and extracellular PAF

The cell and medium lipid extracts were subjected to HPLC separation according to the method of Demopoulos et al. [28]. The PAF fraction, eluted between sphingomyelin and lyso-PC, was collected