Cytokine 64 (2013) 605–612

Contents lists available at ScienceDirect

Cytokine

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

Norepinephrine and adenosine-5′-triphosphate synergize in inducing IL-6 production by human dermal microvascular endothelial cells

Lori L. Stohl [a], Julie B. Zang [a], Wanhong Ding [a], Michela Manni [a], Xi K. Zhou [b], Richard D. Granstein [a,*]

[a] Department of Dermatology, Weill Cornell Medical College, 1305 York Avenue, 9th Floor, New York, NY 10021, United States
[b] Department of Public Health, Weill Cornell Medical College, 402 E. 67th Street, New York, NY 10065, United States

ARTICLE INFO

Article history:
Received 16 June 2013
Accepted 5 August 2013
Available online 8 September 2013

Keywords:
Endothelial cells
Norepinephrine
Adenosine-5′-triphosphate
IL-6

ABSTRACT

Endothelial cells (ECs) play important roles in cutaneous inflammation, in part, by release of inflammatory chemokines/cytokines. Because dermal blood vessels are innervated by sympathetic nerves, the sympathetic neurotransmitter norepinephrine (NE) and the co-transmitter adenosine-5′-triphosphate (ATP) may regulate expression of EC inflammatory factors. We focused on IL-6 regulation because it has many inflammatory and immune functions, including participation in Th17 cell differentiation. Strikingly, NE and ATP synergistically induced release of IL-6 by a human dermal microvascular endothelial cell line (HMEC-1). Adrenergic antagonist and agonist studies indicated that the effect of NE on induced IL-6 release is primarily mediated by β2-adrenergic receptors (ARs). By real-time PCR IL-6 mRNA was also synergistically induced in HMEC-1 cells. This synergistic effect of NE and ATP was reproduced in primary human dermal endothelial cells (pHDMECs) and is also primarily mediated by β2-ARs. Under conditions of stress, activation of the symphathetic nervous system may lead to release of ATP and NE by sympathetic nerves surrounding dermal blood vessels with induction of IL-6 production by ECs. IL-6 may then participate in immune and inflammatory processes including generation of Th17 cells. Production of IL-6 in this manner might explain stress-induced exacerbation of psoriasis, and perhaps, other skin disorders involving Th17-type immunity.

1. Introduction

Endothelial cells (ECs) are strategically located between the blood and tissue compartments and, therefore, are in a position to play important roles in the initiation and regulation of inflammation [1]. In part, this is through the release of inflammatory chemokines/chemokines which allow them to communicate with other cells and organs and thus modulate immune activities [2–4]. They also express adhesion molecules that mediate rolling, adhesion and transmigration of leukocytes out of the vasculature and into tissues such as the skin [5,6]. Endothelial cells produce a number of chemokines that bind to and signal through specific receptors on leukocytes, ultimately attracting them to areas of inflammation [3,7], as well as cytokines including IL-6.

The last several decades have provided strong evidence that the nervous system and immune system are involved in functional cross talk. Interactions between the nervous, immune and endocrine systems are mediated by numerous molecules including cytokines, neurotransmitters, neuropeptides, hormones and their respective receptors. These interactions play an important role in many immune responses including inflammatory diseases and host susceptibility [8–11].

Stress has complex effects on the immune system and can affect both innate and acquired immunity. Stressors may be physical or psychological and can be acute or chronic. The stress response is controlled by elements of the central and peripheral nervous systems. Stress has been shown to have stimulative or inhibitory effects on the immune system depending on the type, duration and intensity of the stressor applied [12–14].

Under conditions of stress, two main neurological pathways are activated, the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system (SNS). Activation of these two pathways results in the release of several types of stress hormones including glucocorticoids, and catecholamines from the adrenal medulla and, especially, norepinephrine by sympathetic nerve termini. These two pathways play major roles in integrating and regulating different immune responses [15,16]. A third axis, the neurotrophin neuropeptide axis also plays a role [17]. Recent evidence suggests a link between stress and disease susceptibility, especially chronic

Abbreviations: EC, endothelial cell; ATP, adenosine-5′-triphosphate; NE, norepinephrine; SNS, sympathetic nervous system; AR, adrenergic receptor; pHDMEC, primary human dermal endothelial cells; ICAM-1, intercellular adhesion molecule 1; Prop, propranolol; Sal, salbutamol; DM, depleted medium.

* Corresponding author. Tel.: +1 646 962 7546; fax: +1 646 962 0040.
E-mail address: rdgranst@med.cornell.edu (R.D. Granstein).

1043-4666/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.cyto.2013.08.005

inflammatory diseases including rheumatoid arthritis, asthma, atherosclerosis and irritable bowel disease as well as psoriasis and certain other skin diseases [16,18–20].

The SNS innervates both primary (bone marrow and thymus) and secondary (spleen and lymph nodes) immune organs, as well as the skin and other organs and tissues. [15,21–25]. The SNS also innervates the vasculature allowing it to regulate vasomotor functions and release of blood cells from the blood marrow. Recent evidence indicates the SNS is important in regulation of proinflammatory conditions [11,26] and that sympathetic neurotransmitters have an important role in regulating immune and inflammatory responses [10,15,26].

It has long been hypothesized that stress can influence certain skin conditions such as rosacea, psoriasis and atopic dermatitis [18,27–31]. Accumulating experimental evidence indicates that the neuroendocrine system plays a key role in cutaneous inflammation [20,32–34]. The SNS within skin is supplied by postganglionic fibers of the paravertebral chain ganglia [35,36]. NE released from sympathetic varicose axon terminals diffuses from the release site; thus, NE transmits its signals nonsynaptically to immune cells and the endothelium. Circulating NE, as well as that released from SNS peripheral nerves locally, may modulate immune function by binding to ARs expressed on immune cells, often resulting in changes in cytokine/chemokine production. ARs are heteromeric 7-transmembrane spanning G-coupled-proteins and are subdivided into 3 classes, each of which contains three members; α1 (α1A, α1B, α1D), a2 (α2A, α2B, α2C); and β (β1, β2, and β3). ARs are also present on endothelial cells [37,38]. In human and mouse skin the β2-AR appears to be the most abundant AR. It is the subtype of β-ARs expressed by the major cell types found in skin including keratinocytes [39,40], fibroblasts [41] and melanocytes [42]. However, α-ARs are also present in the skin [43,44]. It has previously been shown that NE enhances lipopolysaccharide-induced IL-6 release from cells of the human dermal microvascular endothelial cell line HMEC-1 [45].

Adenosine-5′-triphosphate (ATP) participates in many intra- and extracellular functions [46,47]. Extracellular ATP may act in an autocrine or paracrine manner and exerts important effects on many cell types. ATP can also be released from many cell types and is a sympathetic co-transmitter along with NE and neuropeptide Y [48–50]. ATP binds to purinergic P2 receptors, which belong to either the ionotropic P2X receptor family (ligand gated channels) or the metabotropic P2Y receptor family (G protein-coupled receptors), with activation of downstream signaling pathways [46,51,52]. Purinergic receptors are also expressed by macro- and micro-vascular endothelial cells [53]. We have demonstrated that HMEC-1 cells express mRNA for several P2 receptors [54]. We have also previously shown that ATP (as well as ATPγS, a hydrolysis-resistant long-lived analog of ATP) increases the secretion of IL-6 and the chemokines CXCL8 (interleukin-8), CCL2 (monocyte chemoattractant protein-1) and CXCL1 (growth related oncogene-α) by HMEC-1 cells as well as by primary human dermal microvascular endothelial cells (pHDMECs) [54,55]. ATPγS also upregulates expression of intercellular adhesion molecule 1 (ICAM-1) by HMEC-1 cells [54]. We hypothesized that under conditions of stress, activation of symphathetic nerves may lead to release of ATP by nerves associated with dermal vessels followed by release of cytokines/chemokines by endothelial cells and upregulation of ICAM-1 leading to enhanced recruitment of inflammatory cells into skin interstitium.

Under conditions of stress, activation of symphathetic nerves may lead to release of both ATP and NE in the vicinity of dermal blood vessels. Complex interactions between the effect of ATP and that of NE may regulate the ability of endothelial cells to release certain types of cytokines/chemokines. In this regard, it has been reported that exposure of rat thymic epithelial cells to both NE and ATP resulted in an additive effect on IL-6 synthesis [56]. However, the influence of co-transmitters on immune responses and cutaneous inflammation, particularly at the endothelial level is poorly understood. In this study we have examined the effect of the sympathetic co-transmitters NE and ATP on IL-6 release by the dermal microvascular endothelial cell line HMEC-1 and pHDMECs. We focused on the cytokine IL-6 because it is involved in differentiation of Th17 cells [57–65], which are now believed to be key in the pathogenesis of psoriasis [63–72].

2. Materials and methods

2.1. Reagents

Norepinephrine was purchased from EMD Biosciences, Inc. (La Jolla, CA). ATP (cell culture grade) and phentolamine (Phent) were from Sigma–Aldrich (St. Louis, MO). Propranolol (Prop), ICI 118,151 (ICI), isoproterenol (Iso) and salbutamol (Sal) were from Tocris (Ellisville, MO). The high capacity cDNA kit and Power SYBER Green Master Mix were obtained from Applied Biosystems (Foster City, CA).

2.2. Cell culture and media

HMEC-1 cells were a gift from T.J. Lawley (Emory University, Atlanta, GA). This cell line was created by immortalizing HDMECs via simian virus 40 transformation and retains many properties of native dermal microvascular endothelial cells including cell adhesion molecule expression and cytokine/chemokine production [2,73]. HMEC-1 cells were maintained in endothelial cell basal media (EBM; Lonza, Walkersville, MD), supplemented with 10% heat inactivated fetal bovine serum (FBS; Gemini, Bio-Products, Sacramento, CA), 100 U/ml penicillin, 100 μg/ml streptomycin, (Mediatech, Manassas, VA), 10 ng/ml epidermal growth factor (BD Biosciences, Bedford, MA) and 1 μg/ml hydrocortisone (Sigma–Aldrich, St. Louis, MO). Cells were maintained at 37 °C in a humidified atmosphere with 5% CO_2. In experiments that examined the effect of drugs on cytokine production or RNA transcription, cells were incubated in EMB supplemented with 2% FBS and penicillin/streptomycin only and referred to as depleted media (DM). Primary neonatal foreskin human dermal microvascular endothelial cells (neonatal pHDMECs) were from pooled donors and were obtained commericially (Lonza, Walkersville, MD). Primary endothelial cells were grown in Endothelial Basal Medium-2 (EBM-2) supplemented with EGM-2-MV SingleQuots (Lonza), containing supplements and growth factors (hydrocortisone, hEGF, FBS, VEGF, hFGF-B, R3-IGF-1, ascorbic acid and gentamicin/amphotericin-B).

2.3. Cytokine ELISAs

HMEC-1 cells were plated and adhered in 12-well plates at 2×10^5 cells/well in complete media. After approximately 4 h cells were switched to depleted media and incubated overnight. Sixteen hours later the media was replaced with fresh depleted media and cells were treated with various concentrations of NE and/or ATP for the times indicated and supernatants were harvested. For neonatal pHDMECs, 0.15×10^5 cells/well were plated in 12-well plates in CM media and incubated overnight. Medium was replaced with fresh CM and cells were treated with NE and/or ATP as indicated and supernatants were harvested 8 h later. IL-6 quantitation was performed by sandwich enzyme-linked immunosorbent assay (ELISA) with matched antibody pairs and standards from BD Biosciences (San Jose, CA). Optical density was determined using a Versamax microplate reader (Molecular Devices, Sunnyvale, CA) and analyzed with Softmax software.