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Original Contribution

Oxidative stress and 8-iso-prostaglandin $F_{2\alpha}$ induce ectodomain shedding of CD163 and release of tumor necrosis factor-α from human monocytes

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Abstract

CD163 is a membrane glycoprotein of the cysteine-rich scavenger receptor superfamily. Upon an inflammatory stimulus CD163 undergoes ectodomain shedding and the soluble protein has been shown to play a role in downregulation of inflammation. The purpose of the present study was to identify a physiological activator of CD163 shedding that is consistently present under inflammatory conditions. Therefore, we elucidated whether oxidative stress or 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) is involved in shedding of CD163. Oxidative stress induced by H_2O_2 or a NO donor as well as 8-iso-$PGF_{2\alpha}$ induced significant shedding of CD163. In contrast, release of CD163 was not stimulated by $PGF_{2\alpha}$. We identified both calcium and reactive oxygen species as common cellular mediators of CD163 release. Since shedding of both CD163 and tumor necrosis factor-α (TNFα) is known to be mediated by a TIMP-3-sensitive metalloproteinase we examined whether release of TNFα was induced by the same mediators that trigger shedding of CD163. Only oxidative stress generated by H_2O_2 as well as 8-iso-$PGF_{2\alpha}$ and $PGF_{2\alpha}$ enhanced TNFα secretion. Thus, we identified novel common and divergent activators of shedding of CD163 and TNFα. These inducers of shedding are present in inflammation and might play an important role in membrane protein cleavage.

Keywords: Ectodomain shedding; CD163; TNFα; Reactive oxygen species; Calcium; Oxidative stress; 8-iso-prostaglandin $F_{2\alpha}$; Prostaglandin $F_{2\alpha}$; Cyclosporine A; Inflammation

Introduction

CD163 is a membrane glycoprotein of the cysteine-rich scavenger receptor (SRCR) superfamily. Its expression is restricted to human monocytes and macrophages [1,2]. It has been suggested early that CD163—previously known as RM3/1 antigen—is involved in anti-inflammatory functions of monocytes/macrophages in vivo [3,4]. Investigations of the regulation of CD163 membrane expression revealed an upregulation by anti-inflammatory compounds or mediators such as natural and synthetic glucocorticoids or interleukin 10 (IL-10) [2,4,5]. In contrast, downregulation of CD163 was observed after exposure to inflammatory mediators tumor necrosis factor-α (TNFα) or lipopolysaccharide (LPS) [5,6]. The function of monocytes/macrophages expressing CD163 and their role in the inflammatory process remained elusive until CD163 was identified as a scavenger receptor for hemoglobin–haptoglobin [7]. It was suggested that this function might be related to attenuation of inflammation [7–9].

Abbreviations: SRCR, cysteine-rich scavenger receptor; IL, interleukin; TNFα, tumor necrosis factor-α; LPS, lipopolysaccharide; PKC, protein kinase C; sCD163, soluble CD163; MMP-9, matrix metalloproteinase 9; ROS, reactive oxygen species; TACE, tumor necrosis factor-α-converting enzyme; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; FP, fluticasone-17-propionate; PMA, phorbol 12-myristate 13-acetate; CsA, cyclosporine A; SNAP, *S*-nitroso-*N*-acetylpenicillamine; NALC, *N*-acetyl-L-cysteine; BAPTA-AM, 1,2-bis-(2-aminophenoxy)-ethane-*N,N,N′,N′*-tetraacetic acid, tetraacetoxy-methyl ester; FITC, fluorescein isothiocyanate; PBS, phosphate-buffered saline; FACS, flow cytometric analysis; BSA, bovine serum albumin; CL, chemiluminescence; RLU, relative light units; AUC, area under the curve.

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This, however, is not the only link between the CD163 and the inflammatory process. We demonstrated that CD163 undergoes protein kinase C (PKC)-dependent ectodomain shedding upon phorbol ester stimulation in vitro and it also exists as a soluble protein [10]. This proteinase-mediated shedding can be inhibited by a tissue inhibitor of matrix metalloproteinases, TIMP-3 [11]. Soluble CD163 (sCD163) is also generated regularly in vivo as it was discovered in plasma samples of healthy volunteers. In plasma of patients with inflammatory diseases, significantly elevated levels of sCD163 were found [11–13]. Obviously, basal shedding can be augmented under inflammatory conditions.

Purified sCD163 actively inhibits phorbol ester-induced proliferation of lymphocytes in vitro [14]. Additionally, sCD163 decreases expression of CD69 and mRNA expression of matrix metalloproteinase 9 (MMP-9) in lymphocytes [15]. The inhibition of T-lymphocyte activation is only mediated by sCD163, not by the membrane-bound protein [15]. These in vitro observations were recently confirmed by in vivo data. In patients with synovitis elevated local concentrations of sCD163 were associated with decreased T-cell activation [16]. These in vitro and in vivo data suggest that sCD163 is a biologically active molecule that plays a functional role in inflammatory processes.

Shedding of sCD163 is rapidly initiated by exposure of monocytes to phorbol esters [10]. Phorbol esters or calcium ionophores are well known to induce ectodomain shedding of various transmembrane proteins molecules [17]. Though these reagents are effective they are also nonphysiological compounds that cannot account for the activation of shedding in vivo. Bacterial endotoxin (LPS) and immune complexes have been recently suggested as natural stimuli for CD163 shedding in vivo [18,19]. Most probably, however, these are not the only pathophysiological activators. Since elevated levels of sCD163 are frequently observed in the inflammatory context, an activator of CD163 shedding should be consistently present under this condition as well.

In inflammation, reactive oxygen species (ROS) play a special role as signaling molecules which contribute to cell injury and degenerative processes such as cartilage degradation in rheumatic diseases [20,21]. In this context, matrix degrading enzymes (MMPs) significantly contribute to the pathogenesis of various chronic inflammatory diseases such as asthma or rheumatoid arthritis [22,23]. Notably, ectodomain shedding of proteins is mediated by transmembrane metalloproteinases. Two types of proteinases are known to be involved in this process: metalloproteinase disintegrins (also known as ADAMs: a metalloproteinase and desintegrin) and MMPs [24]. MMPs can be activated by ROS via transcriptional induction or directly by posttranslational activation [25–27]. The activation of one prominent member of the ADAM family, ADAM 17 or tumor necrosis factor-α converting enzyme (TACE), has been also shown to be mediated by ROS [28]. Interestingly, ROS have been also described to downregulate proinflammatory genes under certain circumstances [29].

Reactive oxygen species additionally generate molecules that might serve as markers or mediators of oxidative stress. Examples are various lipids produced by free radical oxidation of polyunsaturated fatty acids such as F_2-isoprostanes [30]. Considerable research interest focused on 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) that was identified as a valuable marker of oxidative stress in various clinical settings such as cardiovascular or neurological diseases [30]. Since oxidative stress and inflammation are closely related, 8-iso-$PGF_{2\alpha}$ might also serve as a marker of inflammatory conditions [31]. In addition to its potential as a biomarker several pathophysiological effects of 8-iso-$PGF_{2\alpha}$ have been identified. This isoprostane was reported to serve as potent bronchoconstrictor and to modulate platelet aggregation and adhesion [30]. It must be assumed that the entire spectrum of biological effects of 8-iso-$PGF_{2\alpha}$ is not fully uncovered yet.

The purpose of the present study was to identify an inflammation-related activator of CD163 ectodomain shedding. Therefore, we elucidated whether oxidative stress or the lipid peroxidation product 8-iso-$PGF_{2\alpha}$ induces shedding of CD163. Since the shedding of CD163 obviously involves an enzyme of the ADAM family, possibly TACE [11,18], and the time course of LPS-induced shedding was similar for CD163 and TNFα [18] we additionally determined the influence of oxidative stress and 8-iso-$PGF_{2\alpha}$ on TNFα release from human monocytes.

Methods and materials

Reagents

Fluticasone-17-propionate (FP) was a generous gift from GlaxoSmithKline (Greenford, UK). Phorbol 12-myristate 13-acetate (PMA), cyclosporine A (CsA), *S*-nitroso-*N*-acetylpenicillamine (SNAP), penicillamine, bacterial lipopolysaccharide (LPS), propidium iodide, and luminol were from Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Hydrogen peroxide (H_2O_2) 30% was purchased from Merck (Darmstadt, Germany). 8-Iso-prostaglandin $F_{2\alpha}$ and prostaglandin $F_{2\alpha}$ were obtained from Cayman Chemical (Ann Arbor, MI).

Inhibitors

TAPI-1 was purchased from Merck Biosciences GmbH (Bad Soden, Germany), *N*-acetyl-L-cysteine (NALC) and 1,2-bis-(2-aminophenoxy)-ethane-*N,N,N′,N′*-tetraacetic acid, tetraacetoxy-methyl ester (BAPTA-AM) were from Sigma-Aldrich.

Antibodies

The monoclonal antibody RM3/1 mouse IgG_1 was purchased from BMA Biomedical AG (Augst, Switzerland), and the isotype control mouse IgG_1 and fluorescein (FITC)-