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Lactoferrin modified by hypohalous acids: Partial loss in activation of human neutrophils

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ABSTRACT

Previously we have shown that lactoferrin (LTF), a protein of secondary neutrophilic granules, can be efficiently modified by hypohalous acids (HOCl and HOBr), which are produced at high concentrations during inflammation and oxidative/halogenative stress by myeloperoxidase, an enzyme of azurophilic neutrophilic granules. Here we compared the effects of recombinant human lactoferrin (rhLTF) and its halogenated derivatives (rhLTF-Cl and rhLTF-Br) on functional responses of neutrophils. Our results demonstrated that after halogenative modification, rhLTF lost its ability to induce mobilization of intracellular calcium, actin cytoskeleton reorganization, and morphological changes in human neutrophils. Moreover, both forms of the halogenated rhLTF prevented binding of *N*-acetylglucosamine-specific plant lectin *Triticum vulgaris* agglutinin (WGA) to neutrophils and, in contrast to native rhLTF, inhibited respiratory burst of neutrophils induced by N-formyl-L-methionyl-L-leucyl-L-phenylalanine and by two plant lectins (WGA and PHA-L). However, we observed no differences between the effects of rhLTF, rhLTF-Cl, and rhLTF-Br on respiratory burst of neutrophils induced by phorbol 12-myristate 13-acetate (PMA), digitonin, and number of plant lectins with different glycan-binding specificity. Furthermore, all rhLTF forms interfered with PMA- and ionomycin-induced formation of neutrophil extracellular traps. Thus, halogenative modification of LTF is one of the mechanisms involved in modulating a variety of signaling pathways in neutrophils to control their pro-inflammatory activity.

1. Introduction

Oxidative modification of proteins is a well-known phenomenon, which is driven by multiple mechanisms mediated by reactive oxygen, nitrogen and halogen species (ROS, RNS, and RHS, respectively), products of lipid peroxidation, metal ions with variable valency, and reducing sugars [1–4]. These modifications may not only be destructive for relevant proteins, but also play an essential role in regulating functional responses of cells associated for instance with redox processes and transcriptional/translational machinery [2,3,5]. Redox regulation is especially important for native immunity because neutrophils produce variety of highly reactive species, hydrolyzing enzymes, and regulatory factors aiming to inactivate invading pathogens [6–8].

It is well established that myeloperoxidase (MPO) plays a key defensive role in innate immunity by facilitating the destruction of a wide range of pathogens, including bacteria, fungi, viruses and parasites [9,10]. Given their high reactivity, neutrophil-generated hypohalous acids (HOCl, HOBr) are able to interact with almost all biological

Abbreviations: LTF, lactoferrin; rhLTF, recombinant human lactoferrin; HRP, horseradish peroxidase; fMLP, N-formyl-L-methionyl-L-leucyl-L-phenylalanine; PMA, phorbol 12-myristate 13-acetate; PNA, *Arachis hypogaea* agglutinin; Con A, *Canavalia ensiformis* agglutinin; SBA, *Glycine hispida* agglutinin; PHA-L, *Phaseolus vulgaris* agglutinin; VSA, *Vicia sativa* agglutinin; WGA, *Triticum vulgaris* agglutinin; PBS, phosphate-buffered saline; MPO, myeloperoxidase; $[Ca^{2+}]_i$, cytoplasmic Ca^{2+}; ROS, reactive oxygen species; RHS, reactive halogen species; RNS, reactive nitrogen species; HOCl, hypochlorous acid; HOBr, hypobromous acid; rhLTF-Cl, recombinant human lactoferrin modified by hypochlorous acid; rhLTF-Br, recombinant human lactoferrin modified by hypobromous acid; NETs, neutrophil extracellular traps; NETosis, formation of neutrophil extracellular traps.

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