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The expression of human resistin in different leucocyte lineages is modulated by LPS and TNFα

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ABSTRACT

Objective: Human resistin has been linked to several inflammatory diseases such as atherosclerosis. This study aimed to clarify the expression of resistin in different inflammatory cells and its effect on endothelial cells.
Results: In this study, RNA and protein expression of resistin were detected in human primary neutrophils, monocytes, and T cells as well as in human Jurkat T cells, RPMI-8226 B cells, monocytic U937, and myeloblastic HL-60 cell lines. The highest resistin protein and mRNA level were detected in neutrophils, primary monocytes, and monocytic U937 cells. The RNA expression of resistin was upregulated both in neutrophils and in U937 cells after exposure to LPS. Also TNFα induced resistin RNA expression in neutrophils, U937, T-lymphocytic Jurkat cells, and B-lymphocytic RPMI-8226 cells. The RNA and protein expression of resistin decreased as the monocytic U937 cells differentiated into macrophage-like cells. In endothelial EA.hy 926 cells, resistin increased the expression of MCP-1 and PECAM-1 and adhesion of monocytes to endothelial cells.
Conclusions: The wide-ranging expression of resistin in white blood cells and the upregulation of its expression by inflammatory reagents LPS and TNFα support the fact that increased resistin could be involved in several inflammatory diseases.

1. Introduction

Atherosclerosis is considered to be a complex and chronic inflammatory disease. The development of atherosclerotic plaque within the arterial wall involves several cell types including endothelial, smooth muscle and inflammatory cells (reviewed in [1]).

Human resistin (*RETN*) has been thought to have a role in atherosclerosis since it has been detected in the monocytes/macrophages inside the plaques [2,3] and it can promote changes towards atherosclerosis in endothelial and smooth muscle cells. It has been reported that resistin induces VCAM-1 both at the RNA [4] and the protein levels [2,5,6] and furthermore it activates MCP-1 secretion [2,6] in endothelial cells. Even though it can increase the levels of these adhesion molecules Skilton and co-workers reported that resistin did not affect monocyte adhesion to endothelial cells [5]. In smooth muscle cells, resistin induces proliferation [7] and migration [3].

In addition to atherosclerosis, human resistin has been linked to several other chronic and acute inflammatory states such as rheumatoid arthritis [8–11], inflammatory bowel disease [12], asthma [13], inflammatory disease of respiratory tract [14], and sepsis [15]. Several studies have shown that peripheral blood mononuclear cells (PBMC) and some immortalized blood cell lines express resistin [16–20].

The recently discovered fourth member of the resistin gene family in mouse, resistin-like gamma (*Retnlg*), has been proposed to have functional homology to human resistin [20,21]. The expression of resistin-like gamma has been detected in mouse granulocytes [22] whereas human resistin is expressed both in acute myelo-monocytic and lymphoblastic leukemia cell lines [16].

Endotoxin infusion has been reported to increase plasma resistin levels [19,23]. In support of that observation, *in vitro* experiments have detected an increase in resistin in primary PBMC [9,15,24,25] and PBMC originated macrophages [19] after LPS exposure. Proinflammatory cytokine TNFα induces expression of resistin in these cells as well [9,19,24]. These expression studies as well as the connection to a wide range of inflammatory diseases emphasize the importance of resistin in the immune system.

We have previously reported that the number of blood leucocytes is an independent explanatory factor for plasma resistin concentration [26]. However, the expression of resistin in different primary human white blood cell populations has not been studied in detail. So far the focus has been only in monocytes/macrophages.

In this study we evaluated resistin expression in several primary human white blood cells and in the corresponding immortal cell lines. Since the expression of resistin in monocytes is induced by LPS and

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TNFα, we studied their effects to resistin production in other leucocytes, a topic which has not been explored previously.

2. Materials and methods

2.1. Cell culture

Human monocytic U937, B-lymphocytic RPMI 8226, T-lymphocytic Jurkat (clone E6-1), myeloblastic HL-60 cell lines, and primary neutrophils + eosinophils (NE) were grown in RPMI 1640 supplemented with 2 mM L-glutamine, 10 mM HEPES, 1 mM Na-pyruvate, and 4.5 g/L glucose. The same medium supplemented with 0.05 mM β-mercaptoethanol was used for the monocytic cell line, THP-1. African green monkey kidney cells COS-7 were cultivated in DMEM. Human endothelial cell line EA.hy 926 was grown in DMEM with addition of HAT (hypoxanthine, aminopterin, thymidine) and 10 mM HEPES. All growth mediums were supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin, and 10% FBS. Cell cultures were maintained in 5% CO_2 at 37 °C.

NK-92 cell pellet was a kind gift from Dr. Mari Strengell, Department of Viral Diseases and Immunology, National Institute for Health and Welfare, Helsinki, Finland.

2.2. Isolation of human white blood cells

For separation of human white blood cell populations, blood was collected altogether from 10 healthy adult volunteers in 3–5 isolations into Vacutainer™ CTP™ tubes (BD) and treated according to the manufacturer's instructions for separations of blood cell fractions. Approval for the collection of the samples was obtained from the Ethical Committee of University of Oulu.

Red blood cells were degraded with lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 2 mM EDTA). MACS® Technology (Miltenyi Biotec) was used to separate neutrophils + eosinophils (NE), T cells, B cells, and monocytes with anti-CD15, -CD3, and -CD19 Microbeads and Monocyte Isolation Kit II, respectively. The purity of separated cell fractions was evaluated with flow cytometry (FACSort, BD) using fluorochrome-conjugated antibodies (BD). CD45 was used as universal white cell marker, whereas NE, T cells, B cells, and monocytes were detected with CD13 + CD33, CD2 + CD7, CD20, and CD64 + CD14 antibodies, respectively. In the results, purity is presented as mean ± standard deviation. Cells were either used immediately for cell culture experiments or frozen in liquid nitrogen for RNA and protein isolations.

2.3. Exposure of white blood cells

After separation, human primary NE cells were seeded onto 24-well plates in growth medium with 10% FBS and allowed to stabilize for 1 h prior to exposure to 10 ng/mL LPS (Sigma-Aldrich), TNFα (Bender MedSystems) or PBS. Cells were collected after 2, 5 and 24 h for RNA extraction. U937, RPMI-8226 and Jurkat cells were plated in serum free growth medium and after 1 h were exposed to 10 ng/mL LPS, TNFα or PBS. Cells were collected after 2, 5 and 24 h for RNA extraction. Growth medium for resistin protein measurement was collected from RPMI-8226 and Jurkat cell exposures after 24 hour incubations. In the U937 cell cultures, 2 and 5 hour medium samples were also collected.

2.4. Differentiation of THP-1, U937, and HL-60 cells

To differentiate THP-1 and U937 monocytes into macrophages, the cells were seeded onto 6-well plates (5×10^5 cells/well) and treated with 400 and 62 ng/mL of PMA (Sigma-Aldrich), respectively. After 3 days, the mediums were collected and total RNA and cellular proteins of the cells were isolated.

HL-60 cells were differentiated into neutrophils with 1.25% DMSO in growth medium. After 3 and 5 days of differentiation, total RNA, medium, and cellular proteins of the cells were isolated.

2.5. DNA construct and transfection

The full length human resistin cDNA was amplified from monocytic THP-1 mRNA by three rounds of PCR using oligonucleotides designed to create KpnI (5′) and XhoI (3′) cutting sites. The digested fragment was cloned into the pcDNA3.1(+) mammalian expression vector (Invitrogen). No tags were included. The integrity of the RETNpcDNA construct was verified by sequencing.

In the transient transfection, COS-7 cells were seeded onto a 10 cm culture dish (1.3×10^6 cells) in DMEM supplemented with 10% FBS and allowed to attach overnight. Cells were transfected with 10 μg either RETNpcDNA or empty pcDNA3.1(+) vector using Fugene® HD transfection reagent (Roche) for 48 h in the presence of 10% FBS. The expression level of resistin from the conditioned medium was analysed and the medium was used for the adhesion assay.

2.6. Adhesion assay and expression of adhesion molecules

Adhesion assay was modified from the protocol of Akeson and Woods [27] and it was validated with TNFα (Bender MedSystems). Briefly, EA.hy 926 endothelial cells were seeded onto a black 96-well Nunclon™Δ Optical Bottom plate (Nunc) in the growth medium and let to attach before overnight starvation. EA.hy 926 cells were exposed for 24 h to human recombinant resistin (Alexis Biochemicals), boiled (95 °C for 10 min) recombinant resistin, COS-7 transfection conditioned mediums (described above) or PBS. To rule out the possible effect of any minor endotoxin remnant in the recombinant resistin, adhesion assay was also conducted in the presence of 10 μg/mL polymyxin B (Sigma-Aldrich). All exposures were done in FBS free conditions except the experiments with transfection conditioned medium which contained 10% FBS. After the exposures, Calcein AM (Molecular Probes) labelled monocytic THP-1 cells were co-incubated with treated EA.hy 926 cells at 37 °C for 30 min. Non-adherent THP-1 cells were removed

Table 1
Oligonucleotide primers used in quantitative real-time PCR.

Gene	Accession number	Primer
β-actin	NM_001101	Forward: 5′ AGAGCTACGAGCTGCCTGAC
		Reverse: 5′ AGCACTGTGTTGGCGTACAG
E-selectin	NM_000450	Forward: 5′ GGACACAGCAAATCCCAGTT
		Reverse: 5′ CACATTGGAGCCTTTTGGAT
GAPDH[a]	P04406	Forward: 5′ GAGTCAACGGATTTGGTCGT
		Reverse: 5′ GACAAGCTTCCCGTTCTCAG
ICAM-1[b]	X06990	Forward: 5′ CAGAGGTTGAACCCCACAGT
		Reverse: 5′ CCTCTGGCTTCGTCAGAATC
MCP-1[c]	X14768	Forward: 5′ CCCCAGTCACCTGCTGTTAT
		Reverse: 5′ TGGAATCCTGAACCCACTTC
PECAM-1[d]	M28526	Forward: 5′ AGACAACCCCACTGAAGACG
		Reverse: 5′ TTGCAGCACAATGTCCTCTC
P-selectin	NM_003005	Forward: 5′ CATGGATTGCTCTCCATCCT
		Reverse: 5′ CCTCATTTGGAACTGGGAGA
Resistin	NM_020415	Forward: 5′ TAGGGCAATAAGCAGCATTG
		Reverse: 5′ ACTGGCAGTGACATGTGGTC
TNFα[e]	M10988	Forward: 5′ CCCGAGTGACAAGCCTGTAG
		Reverse: 5′ GAGGTACAGGCCCTCTGATG
VCAM-1[f]	BC085003	Forward: 5′ TAAAATGCCTGGGAAGATGG
		Reverse: 5′ GGTGCTGCAAGTCAATGAGA

[a] Glyceraldehyde-3-phosphate dehydrogenase.
[b] Intercellular adhesion molecule-1.
[c] Monocyte chemotactic protein-1.
[d] Platelet/endothelial cell adhesion molecule-1.
[e] Tumor necrosis factor α.
[f] Vascular cell adhesion molecule-1.