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Pentraxin-3 regulates the inflammatory activity of macrophages

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ABSTRACT

Background and aims: Pentraxin-3 (PTX3) reportedly has protective roles in atherosclerosis and myocardial infarction, and is a useful biomarker of vascular inflammation. However, the detailed functions of PTX3 in inflammation are yet to be elucidated. This study aimed to investigate the function of PTX3 in macrophages.
Methods: PMA-treated THP-1 cell line (THP-1 macrophage) and monocyte-derived human primary macrophages were treated with recombinant PTX3. Cytokine and chemokine levels in the THP-1 culture medium were measured as well as monocyte chemoattractant protein (MCP-1) concentrations in the Raw 264.7 cell culture medium. PTX3-silenced apoptotic macrophages (THP-1 cell line) were generated to investigate the roles of PTX3 in phagocytosis.
Results: In the presence of PTX3, macrophage interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α) and MCP-1 levels were reduced significantly (−39%, $P=0.007$; −21%, $P=0.008$; and −67%, $P=0.0003$, respectively), whilst activated transforming growth factor-β (TGF−β) was detected in the THP-1 macrophages ($P=0.0004$). Additionally, PTX3 induced Akt phosphorylation and reduced nuclear factor-kappa B (NF-κB) activation by 35% ($P=0.002$), which was induced by TNF-α in THP-1 macrophages. Furthermore, silencing of PTX3 in apoptotic cells resulted in increased macrophage binding, elevated expression rate of HLA-DR (+30%, $P=0.015$) and CD86 (+204%, $P=0.004$) positive cells, and induction of IL-1β (+36%, $P=0.024$) production. Conversely, adding recombinant PTX3 to macrophages reduced CD86 and HLA-DR expression in a dose-dependent manner.
Conclusions: We identified PTX3 as a novel regulator of macrophage activity, and this function suggests that PTX3 acts to resolve inflammation.

1. Introduction

Inflammation, resolution of inflammatory processes and tissue repair, are pivotal factors in many diseases. When inflammation occurs, many cytokines and mediators, including pentraxins, are produced as needed for the immune response. During healing, positive processes are evoked to resolve the inflammation and activate multiple inhibitory pathways, including local transforming growth factor-beta (TGF-β) activation, recruitment of suppressive monocytes and upregulation of M2 macrophages and regulatory T cells, which can produce and secrete inhibitory mediators such as TGF-β and interleukin (IL)-10 [1].

Pentraxins are a superfamily of pattern recognition proteins that constitute the prototypic components of the humoral arm of the innate immune system. C-reactive protein (CRP) and serum amyloid P component (SAP) are short pentraxins produced by the liver. In contrast, pentraxin-3 (PTX3) was the first long pentraxin identified as an IL-1-inducible protein in endothelial cells and a tumor necrosis factor (TNF)-stimulated protein in fibroblasts [2,3].

PTX3 assembles into a four protein tetramer, with two links together forming an octameric structure [4]. PTX3 recognizes pathogen-associated molecular patterns. Moreover, it interacts with C1q, ficolin-1, ficolin-2 and mannose-binding lectin, all of which are recognition molecules in the classical and lectin complement pathways [3]. Furthermore, PTX3 has been found to have significant interaction with fibroblast growth factor (FGF)-2 [5], Fcγ receptor [6] and P-selectin [7].

PTX3-deficient mice exhibit exacerbated myocardial damage following coronary artery ligation and reperfusion associated with greater no-reflow areas, increased neutrophil infiltration, decreased numbers of capillaries and increased numbers of apoptotic cardiomyocytes [8]. Moreover, double-knockout mice, lacking PTX3 and apolipoprotein E, exhibit increased atherosclerosis and macrophage accumulation in atherosclerotic lesions compared

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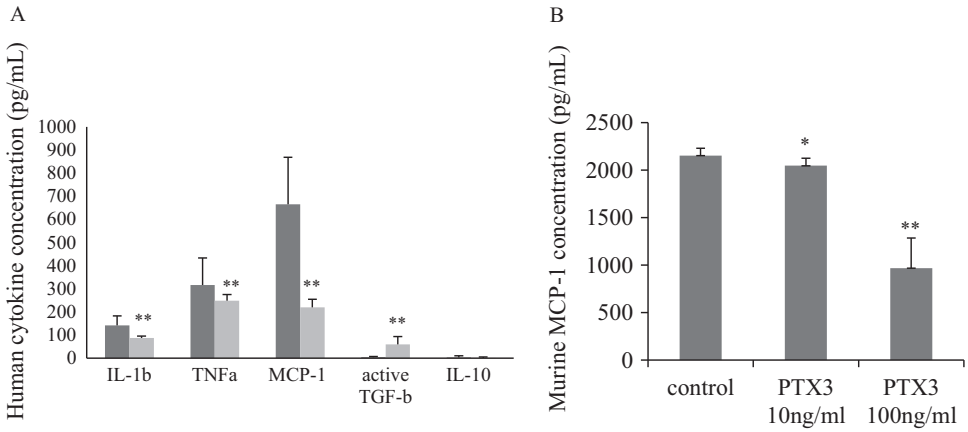

Fig. 1. rPTX3 reduced inflammatory cytokines and induced active TGF-β (A) THP-1 macrophage-conditioned culture medium was assessed for cytokines using the Luminex system. The culture medium was serum-depleted and incubated with THP-1 cells for 24 h with (grey columns) or without 10 ng/mL rPTX3 (black columns), (means ± SD, $n=8$; $^{**}p<0.01$); (B) rPTX3 reduced MCP-1 production by murine Raw 264.7 macrophage cells, activated by 20 nM of PMA and cultured with or without human rPTX3 for 24 h. The cultured medium was collected and measured using ELISA (means ± SD, $n=8$; $^{*}p<0.05$, $^{**}p<0.001$). PMA: phorbol-myristate-acetate.

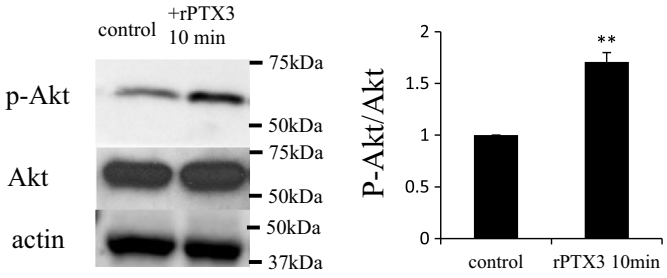

Fig. 2. rPTX3 modulated intracellular signaling. Western blotting displayed increased phosphorylation of Akt in THP-1 macrophages. The graphs show the densitometry value analysis of western blots. The western blots were performed at least three times ($^{**}p<0.001$).

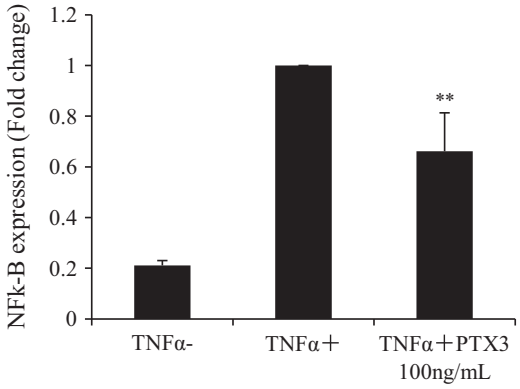

Fig. 3. rPTX3 reduced TNFα-induced NFκB activation. HUVECs were transfected with pGL4.32[luc2P/NFκB-RE/Hygro] reporter vector and TNF-α was added. Pretreatment with rPTX3 reduced NF-κB activation (means ± SD, $n=5$; $^{**}p<0.001$ vs. TNF-α(+)).

with that observed in apolipoprotein E only knockout mice, suggesting that PTX3 possesses a cardiovascular protective function [9]. Furthermore, PTX3 is also reported to be expressed in atheromas [10,11]. However, the detailed mechanisms underlying these functions of PTX3 remain to be elucidated.

In clinical studies, the usefulness of PTX3 as a biomarker has emerged in many diseases, including unstable angina pectoris [12], acute coronary syndrome [13], chronic heart failure [14], heart failure with a normal ejection fraction [15], and Takayasu arteritis [16]. Upregulation of PTX3 in the serum is considered to be an indicator of vascular inflammation [17]; however, little evidence is available regarding the role and results of PTX3 upregulation.

Although some studies have been carried out to investigate the relationship between PTX3 and macrophages, the function of PTX3 in macrophages has not been studied to date. In the present study, we investigated the influence of PTX3 in macrophages in vitro, and found that it regulated inflammatory activities of these cells.

2. Materials and methods

2.1. Ethics statement

The research met all applicable standards for the ethics of experimentation and was approved by the Ethics Committee of the Faculty of Medicine of Saga University (No. 26-56). Participants provided written informed consent prior to the experiment.

2.2. Cell culture and materials

Cells were cultured at 37 °C under 5% CO_2. The human THP-1 cell line was purchased from Riken Bioresource Center (RCB1189). THP-1 cells were cultured in RPMI medium with 10% fetal bovine serum (FBS). THP-1 macrophages were generated by incubating the cells with 100 nmol/L of phorbol-myristate-acetate (PMA) for 24 h. Raw 264.7 cells were purchased from ATCC and cultured in DMEM with 2 mM Glutamine and 10% Foetal Bovine Serum (FBS). Recombinant PTX3 (rPTX3) was provided by Perseus Proteomics Inc. (Tokyo, Japan). It was made by the procedure that was written in the article [12]. Human umbilical vein endothelial cells (HUVECs) and specific medium for HUVEC culture were obtained from Lonza (Basel, Swiss). Experiments using HUVECs were conducted with cells between passage numbers three and five (P3–P5). Human monocyte-derived macrophages were generated from healthy volunteers' blood.

Peripheral blood mononuclear cells (PBMC) were separated using Ficoll-Paque PLUS solution and lymphocytes were washed away after monocyte adhesion on tissue culture-treated culture plates. Cells were cultured with 20 ng/mL of granulocyte macrophage-colony stimulating factor (GM-CSF, Pepro Tech, Rocky Hill, NJ) in RPMI medium containing 10% FBS and were incubated for 6 days prior to assaying. TGF-β neutralizing antibody was obtained from R&D Systems (Minneapolis, MN. Ab-100-NA rabbit polyclonal, 10 μg/mL).