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Complement MASP-1 enhances adhesion between endothelial cells and neutrophils by up-regulating E-selectin expression

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ABSTRACT

The complement system and neutrophil granulocytes are indispensable in the immune response against extracellular pathogens such as bacteria and fungi. Endothelial cells also participate in antimicrobial immunity largely by regulating the homing of leukocytes through their cytokine production and their pattern of cell surface adhesion molecules. We have previously shown that mannan-binding lectin-associated serine protease-1 (MASP-1), a complement lectin pathway enzyme, is able to activate endothelial cells by cleaving protease activated receptors, which leads to cytokine production and enables neutrophil chemotaxis. Therefore, we aimed to investigate how recombinant MASP-1 (rMASP-1) can modify the pattern of P-selectin, E-selectin, ICAM-1, ICAM-2, and VCAM-1 adhesion molecules in human umbilical vein endothelial cells (HUVEC), and whether these changes can enhance the adherence between endothelial cells and neutrophil granulocyte model cells (differentiated PLB-985). We found that HUVECs activated by rMASP-1 decreased the expression of ICAM-2 and increased that of E-selectin, whereas ICAM-1, VCAM-1 and P-selectin expression remained unchanged. Furthermore, these changes resulted in increased adherence between differentiated PLB-985 cells and endothelial cells. Our finding suggests that complement MASP-1 can increase adhesion between neutrophils and endothelial cells in a direct fashion. This is in agreement with our previous finding that MASP-1 increases the production of pro-inflammatory cytokines (such as IL-6 and IL-8) and chemotaxis, and may thereby boost neutrophil functions. This newly described cooperation between complement lectin pathway and neutrophils via endothelial cells may be an effective tool to enhance the antimicrobial immune response.

1. Introduction

The effectiveness of the immune system depends on the prompt and specific response against pathogens. Therefore, it is not surprising that immunological subsystems cooperate with one another for the quick recognition and elimination of invaders or altered host cells. A good example of this cooperation is the teamwork of the complement system and neutrophil granulocytes against bacteria and fungi.

The complement system can be activated through different routes: the classical, the alternative, and the lectin pathways. After recognition of the target structure by any of the three pathways, a cascade of serine proteases is activated, and this leads to the cleavage of C3, the central molecule of the complement system. C3b, C4b and their degradation fragments act as opsonins enhancing the phagocytic activity of neutrophils (and other cell types).

Abbreviation: HUVEC, human umbilical vein endothelial cell; MBL, mannan-binding lectin; MASP, mannan-binding lectin-associated serine protease; PAR, protease-activated receptor.

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C3a and C5a are amongst the most potent chemotactic factors for neutrophils attracting them to the site of the infection.

Mannan-binding lectin (MBL)-associated serine protease-1 (MASP-1) is the primary enzyme of the lectin pathway (Heja et al., 2012a; Heja et al., 2012b; Megyeri et al., 2013). MASP-1 is activated upon the recognition of special pathogen/danger-associated motifs by MBL, collectin-11 (CL-K1), or ficolins (1-, 2-, and 3-ficolin) (Kjaer et al., 2013; Matsushita et al., 2013). Following its autoactivation, MASP-1 cleaves MASP-2 and C2, thereby ensuring further activation of the lectin pathway. However, we and other groups have described several other functions of MASP-1 beyond complement activation (Dobo et al., 2014). MASP-1 can cleave high-molecular-weight kininogen to bradykinin, a vasoactive and nociceptive peptide (Dobo et al., 2011). It can also cleave prothrombin, FXIII and TAFI, thus MASP-1 can modulate the function of the clotting and fibrinolytic systems (Jenny et al., 2015; Krarup et al., 2008; Hess et al., 2012). Moreover, active MASP-1 (both in native form complexed with MBL and as recombinant, rMASP-1) is able to cleave protease activated receptors (PAR1, 2 and 4), and this generates a pro-inflammatory signal in endothelial cells (Megyeri et al., 2009; Megyeri et al., 2014). Upon this stimulus, the endothelial cells produce IL-6 and IL-8 and thus, recruit neutrophil granulocytes (Jani et al., 2014).

Endothelial cells are vascular site specific regulators of several physiological processes (Aird, 2007a; Aird, 2007b). To interact with circulating blood cells, endothelial cells must utilize a wide range of adhesion molecules. These adhesion molecules can be classified according to their structure, subcellular localization, and function. Upon traumatic vascular injury, endothelial cells translocate their pre-formed P-selectin molecules onto the cell surface by degranulation occurring within a few minutes (Hattori et al., 1989; Rondaij et al., 2006). P-selectin is the major lectin-type adhesion molecule of endothelial cells that interacts with platelets to ensure their adherence to the injured vessel wall. Inflammation induced by invading microorganisms or necrotic debris stimulates endothelial cells to modify the pattern of other adhesion molecules. Sialyl-Lewis X recognizing E-selectin is *de novo* synthesized by endothelial cells within hours of induction to enhance the initial adhesion and rolling of leukocytes on the endothelium (Abbassi et al., 1993; Walz et al., 1990). ICAM-1, a receptor of beta-2 integrins, is expressed on the surface of most endothelial cells (and of some leukocytes) at a low level; however, its expression is up-regulated within 12–24 h in response to pro-inflammatory stimuli (Tonnesen, 1989; Dustin et al., 1986; Pober et al., 1987). It participates in the transmigration of all types of leukocytes. VCAM-1 is another inflammatory adhesion molecule synthesized *de novo,* but only later (24 h after) an appropriate stimulus. As a partner of VLA-4, it has a major role in the homing of T-cells and monocytes, as well as subsets of neutrophils also use this adhesion molecule (Carlos and Harlan, 1994; Elices et al., 1990). ICAM-2 is an endothelial cell specific adhesion molecule. Although it is constitutively expressed on non-activated endothelial cells, its exact role is still not fully understood. ICAM-1 and ICAM-2 share their integrin ligands. However, the affinity spectrum of ICAMs towards different beta-2 integrins differ, and ICAM-2 does not induce outside-in signaling of integrin partner molecules (Shimaoka et al., 2001; Thompson et al., 2002). ICAM-2 expression decreases upon pro-inflammatory stimuli (McLaughlin et al., 1998).

To control leukocyte homing during inflammation, the production of cytokines and the expression of adhesion molecules are usually co-regulated in endothelial cells. We have previously described that rMASP-1 can induce IL-6 and IL-8 production in HUVECs, which is regulated predominantly by the p38-MAPK pathway (Jani et al., 2014). Therefore, in this study we investigated how rMASP-1 can modify the pattern of P-selectin, E-selectin, ICAM-1, ICAM-2, and VCAM-1 adhesion molecules in endothelial cells. We also explored whether these changes can enhance the adherence between endothelial cells and neutrophil granulocytes.

2. Material and methods

2.1. Reagents

We used recombinant catalytic fragment of human MASP-1 (CCP1-CCP2-SP, hereinafter: rMASP-1). rMASP-1 was expressed in *E. coli* as described by Ambrus et al. (Ambrus et al., 2003), and prepared according to Dobó et al. (Dobo et al., 2008). Briefly, after dissolving inclusion bodies and refolding in glutathion/oxidized glutathion containing buffer for one month, rMASP-1 autoactivated during dialysis. Then sequential ion-exchange chromatographies of rMASP-1 were performed using anion-exchange (Source 30Q, GE Healthcare) and cation-exchange (SP Sepharose High Performance, GE Healthcare) columns. Purity was checked by PAGE, whereas enzymatic activity was measured by SLGR-AMC substrate cleaving assay. The rMASP-1 preparations were free of bacterial contaminations, since its NFκB nuclear translocating activity could not be blocked with Polymyxin B or DNase, unlike control LPS and bacterial DNA. Furthermore, it could be inhibited by C1-Inhibitor as described previously (Megyeri et al., 2009; Megyeri et al., 2014; Jani et al., 2014). The mouse anti-human P- and E-selectin, ICAM-1, ICAM-2 and VCAM-1 antibodies were purchased from Bender MedSystems (Affimetrix, Inc., San Diego). The FITC-conjugated mouse anti-human LFA-1 (CD11a/CD18), MAC-1 (CD11b/CD18), CD15 and CD49d antibodies were purchased from ImmunoTools (ImmunoTools GmbH, Germany). The Alexa Fluor® 568-conjugated goat anti-mouse secondary antibody was purchased from Invitrogen (Invitrogen Co., Oregon, USA) and the horseradish-peroxidase (HRP) conjugated secondary antibody was obtained from Southern Biotech (SouthernBiotech, Birmingham, USA). The recombinant human E-selectin was supplied by Sino Biological (Sino Bilogical Inc., Bejing, P.R. China). All other reagents were purchased from Sigma-Aldrich, unless otherwise stated.

2.2. Preparation and culturing of human umbilical vein endothelial cells (HUVECs)

Cells were harvested from fresh umbilical cords obtained during normal deliveries of healthy neonates by collagenase digestion as described earlier (Jani et al., 2014; Oroszlan et al., 2006). HUVECs were kept in gelatin-precoated flasks (Corning® Costar®) in MCDB131 medium (Life Technologies) completed with 5% heat–inactivated fetal calf serum (FCS), 2 ng/mL human recombinant epidermal growth factor (R&D Systems), 1 ng/mL human recombinant basic fibroblast growth factor (Sigma), 0.3% Insulin Transferrin Selenium (Life Technologies), 1% Chemically Defined Lipid Concentrate (Life Technologies), 1% Glutamax (Life Technologies), 1% Penicillin-Streptomycin antibiotics (Sigma), 5 μg/mL Ascorbic acid (Sigma), 250 nM Hydrocortisone (Sigma), 10 mM Hepes (Sigma), and 7.5 U/mL Heparin (this completed medium is hereinafter: Comp-MCDB). Each experiment was performed on at least three independent primary HUVEC cultures from different individuals before the 4th passage. The study was conducted in conformity with the WMA Declaration of Helsinki; its protocol was approved by the Semmelweis University Institutional Review Board (permission number: TUKEB64/2008), and all participants provided their written informed consent before inclusion.

2.3. Culturing of the PLB-985 cell line, as a model for neutrophils

PLB-985 cells were grown in RPMI-1640 medium (Life Technologies) supplemented with 10% FCS, 1% PEST, and 1% Glutamax. Then, the cells were differentiated into neutrophil-like cells for 6 days in