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Trypsin differentially modulates the surface expression and function of channel catfish leukocyte immune-type receptors

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ABSTRACT

Channel catfish (*Ictalurus punctatus*) leukocyte immune-type receptors (IpLITRs) are immunoregulatory proteins that control innate immune cellular responses. Previously, we demonstrated that two representative IpLITR forms, IpLITR 2.6b and IpLITR 1.1b, engage distinct components of the phagocytic machinery resulting in unique target capture and engulfment phenotypes. IpLITR-induced phagocytic mechanisms were also differentially susceptible to temperature and pharmacological inhibitors of canonical signaling mediators. In the present study, we examined the sensitivity of IpLITR-mediated phagocytosis to the endogenous serine-protease trypsin, a well-known mediator of immunoregulatory receptor functions. Trypsin selectively reduced IpLITR 1.1b cell surface expression and phagocytic activity in a dose-dependent manner. We also observed a significant alteration of the IpLITR 1.1b phagocytic phenotype post-trypsin exposure; whereas, the IpLITR 2.6b-mediated target engulfment phenotype was unchanged. Recovery experiments suggested that trypsin-induced inhibition of IpLITR 1.1b-dependent phagocytosis was reversible and that the re-establishment of phagocytic function was associated with a recovery of receptor surface expression. Cell-surface biotinylation and immunoprecipitation studies demonstrated that IpLITR 1.1b normally exists as a mature (~70 kDa) protein on the cell surface. However, trypsin treatment reduced expression of the mature receptor and processed IpLITR 1.1b into an ~60 kDa form. The trypsin-generated and putative immature IpLITR 1.1b form was not present on the cell surface; suggesting that the cleaved receptor may have been internalized, post-processing, by regulated endocytosis. Taken together, these results reveal a unique role for trypsin as a selective modulator of IpLITR-mediated phagocytosis and highlight a conserved role for serine proteases as potent immunomodulatory factors.

1. Introduction

When pathogens circumvent anatomical barriers, neutrophilic granulocytes are rapidly recruited to the site of infection where they release a variety of pro-inflammatory mediators and antimicrobial factors (Kobayashi et al., 2005). In particular, neutrophil-derived serine proteases (NSPs) function as potent antimicrobial agents that also have additional immunomodulatory roles (Losso, 2008; Segal, 2005; Stoller and Aboussouan, 2005). NSPs are stored in azurophilic granules and consist predominantly of elastase, cathepsin G, and proteinase 3; each of which display unique substrate specificity (Cheronis and Repine, 1993; Korkmaz et al., 2010). NSP-mediated proteolysis participates in complex physiological functions including the destruction of foreign microbes, tissue remodeling, and enzyme-mediated processing of immune proteins (Losso, 2008; Segal, 2005; Stroller and Aboussouan, 2005). For example, these enzymes target receptor proteins including toll-like receptor (TLR)-4 (Devaney et al., 2003), CD14 (Le-Barillec et al., 1999; 2000; Nemoto et al., 2000), CD43 (Remold-O'Donnell and Parent, 1995), CD23 (Brignone et al., 2001), complement receptor 1 (Hamacher et al., 1998; Sadallah et al., 1999), β_3-integrins (Bank et al., 1999), intercellular adhesion molecule 1, vascular cell-adhesion molecule 1 (Champagne et al., 1998; Levesque et al., 2001), and the cell surface-bound ligand binding chains of the IL-6 receptor (Bank et al., 2000). As a result, NSP-dependent processing contributes to numerous immune cell effector functions such as regulating cytokine secretion, cellular migration, adhesion, complement activation, respiratory burst, and clearance of apoptotic cells during inflammation.

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Abbreviations

LITR	leukocyte immune-type receptor
NSP	neutrophil serine proteases
TLR	toll-like receptor
Ig	immunoglobulin
MR	mannose receptor
ITAM	immunoreceptor tyrosine-based activation motif
IgSF	immunoglobulin superfamily
FcR	Fc receptor
D	domains
RBL	rat basophilic leukemia
FBS	fetal bovine serum
HA	hemagglutinin
mAb	monoclonal antibody
YG	yellow green
PBS	phosphate buffered saline
BSA	bovine serum albumin
MFI	mean fluorescent intensity
ASB	antibody staining buffer
PFA	paraformaldehyde
HRP	horseradish peroxidase
SA	streptavidin
PAR	protease activated receptor
EGFR	epidermal growth factor receptor
MMPs	matrix metalloproteinases

Proteolysis also selectively influences the phagocytic activity of innate immune cells through direct targeting of phagocytic receptors. In particular, the high-affinity immunoglobulin (Ig)G receptor (FcγRI; CD64) is cleaved from the surface of macrophages by trypsin, whereas the low-affinity IgG receptor (FcγRII; CD32) is insensitive to trypsin-mediated processing (Cooney et al., 2001; Unkeless, 1977). FcγRIIb is also shed from the surface of activated human neutrophils (Huizinga et al., 1988; Dransfield et al., 1994; Homburg et al., 1994), although the enzymes required for these cleavage events can vary and involve both metalloproteinases as well as serine proteases (Middelhoven et al., 2001). Trypsin can also inhibit mannose receptor (MR)-mediated phagocytosis due to cleavage of its ectodomains, resulting in rapid internalization and degradation. This process is suggested to down-regulate receptor activation in the protease-rich environment produced at inflammatory sites (Shepherd et al., 1990). During chronic inflammation associated with cytstic fibrosis and bronchiectasis, neutrophil-derived elastases selectively cleave phosphatidylserine receptors, but not CD32, to significantly reduce the ability of macrophages to clear apoptotic bodies (Vandivier et al., 2002). Reduced clearance of apoptotic cells also occurs in damaged heart tissue due to the unique ability of human cardiomyocytes to directly influence macrophage phagocytic activities via the secretion of proteases (Zhang et al., 2015). Altogether, these examples illustrate the important role that endogenous proteases play in the regulation of receptor-mediated phagocytosis.

Exogenously-derived proteases from pathogens also serve important immunomodulatory roles during immune-system evasion. Specifically, bacterial enzymes cleave antibody and complement proteins, which block the opsonization of microbes to inhibit engulfment by phagocytes (reviewed in Sarantis and Grinstein, 2012; Potempa and Potempa, 2012), target intracellular signaling molecules belonging to the TLR pathway (Li et al., 2005), and can also digest surfactant protein-A (Kuang et al., 2011). Collectively, these examples indicate that host and pathogen proteases play vital roles in tissue homeostasis, inflammation, infection, and disease. However, the cellular mechanisms underlying the potent immunomodulatory properties of proteases, and in particular their selectivity for immunoregulatory receptor subtypes, are not well understood.

In the present study, we examined the effects of the serine protease trypsin on channel catfish (*Ictalurus punctatus*) leukocyte immune-type receptor (IpLITR)-mediated phagocytosis. IpLITRs activate phagocytosis by utilizing receptor-specific sets of intracellular signaling proteins that control discrete cellular morphologies during target capture and engulfment (Lillico et al., 2015). Specifically, IpLITR 2.6b activates phagocytosis using a classical immunoreceptor tyrosine-based activation motif (ITAM)-mediated pathway, whereas the functionally versatile receptor IpLITR 1.1b controls a unique ITAM-independent phagocytic mode (Lillico et al., 2015). Interestingly, the ectodomains of IpLITRs are distantly related to a variety of mammalian Ig superfamily members, including those that belong to the human Fc receptor (FcR) family (Stafford et al., 2006, 2007; Montgomery et al., 2011; Fei et al., 2016); however, it is not currently known if any IpLITRs directly bind antibodies (Taylor et al., 2016). Like mammalian FcR proteins, IpLITR 2.6b and IpLITR 1.1b also vary in their ectodomain compositions: IpLITR 2.6b has two Ig-like domains (D1 and D2), while IpLITR1.1b contains four Ig-like domains (D1, D2, D3, and D4). Early studies of mammalian FcR subtypes determined that protease sensitivities varied considerably depending on FcR ectodomain compositions (Unkeless and Eisen, 1975). FcγRI is cleaved by trypsin due to its unique membrane proximal D3 region, whereas the low affinity FcγRII contains only D1 and D2 and is not sensitive to trypsin activity (Unkeless and Eisen, 1975). Our findings also demonstrate that IpLITRs are differentially sensitive to trypsin-mediated processing and that ectodomain composition, likely within the membrane proximal Ig-like domains of IpLITR 1.1b, appears to directly contribute to trypsin sensitivity. Taken together, these results highlight a unique role for trypsin-dependent receptor modifications during IpLITR-mediated phagocytosis and also demonstrate how receptor-specific regulation of immunological responses can be differentially controlled by extracellular proteases in a basal vertebrate immunoregulatory receptor model.

2. Materials and methods

2.1. Expression of IpLITRs in the rat basophilic Leukemia-2H3 (RBL-2H3) cell line

Transfection, selection, sorting, and stable expression of N-terminal hemagglutinin (HA) epitope-tagged pDISPLAY IpLITR 2.6b/IpFcRγ-L and pDISPLAY IpLITR 1.1b in RBL-2H3 (ATCC® CRL-2256™) cells has already been described in detail elsewhere (Cortes et al., 2014). In this study, IpLITR 2.6b refers to the IpLITR 2.6b/IpFcRγ-L chimeric construct that encodes two extracellular Ig-like domains (IpLITR 2.6b; GenBank Accession: ABI23577) fused with the transmembrane and ITAM-encoding cytoplasmic tail of the catfish signaling adaptor IpFcRγ-L (GenBank Accession: AF543420). IpLITR 1.1b encodes the full-length TS32.17 L1.1b sequence (GenBank Accession: ABI16050). RBL-2H3 cells were transfected and grown at 37 °C and 5% CO_2 in complete culture media (minimal essential media (MEM)/Earl's balanced salt solution (GE Healthcare, Baie d'Urfe, QC, CAN) supplemented with 2 mM L-Glutamine (Life