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Tollip-induced down-regulation of MARCH1

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ABSTRACT

In addition to their classical antigen presenting functions, MHC class II molecules potentiate the TLR-triggered production of pro-inflammatory cytokines. Here, we have addressed the effect of Tollip and MARCH1 on the regulation of MHC II trafficking and TLR signaling. Our results show that MARCH1-deficient mice splenocytes are impaired in their capacity to produce pro-inflammatory cytokines in response to poly(I:C) and that TLR3 and MHC II molecules interact in the endocytic pathway. Knocking down Tollip expression in human CIITA$^+$ HeLa cells increased expression of HLA-DR but reduced the proportion of MHC II molecules associated with the CLIP peptide. Truncation of the HLA-DR cytoplasmic tails abrogated the effect of Tollip on MHC class II expression. While overexpression of Tollip did not affect HLA-DR levels, it antagonized the function of co-transfected MARCH1. We found that Tollip strongly reduced MARCH1 protein levels and that the two molecules appear to compete for binding to MHC II molecules. Altogether, our results demonstrate that Tollip regulates MHC class II trafficking and that MARCH1 may represent a new Tollip target.

1. Introduction

In humans, 10 members of the toll-like receptor (TLR) family of proteins recognize different pathogen-associated molecular patterns (PAMPs) through their luminal leucine-rich repeats [1]. TLRs are type I trans-membrane proteins capable of forming homo- and heterodimers [2]. While their expression patterns often differ, some, like TLR1, are ubiquitously expressed [3]. They localize on cell surface (TLR1, 2, 4, 5, 6 and 11) or in endosomes (TLR3, 7, 8 and 9), in line with the subcellular accumulation of their specific ligands [4,5]. TLRs are essential in the early events of innate immunity as well as in the development of robust adaptive immune responses [6,7]. Microbial products, such as LPS and DNA, trigger signaling cascades through the cytoplasmic Toll/IL-1 receptor (TIR) domain and various adaptor proteins, which include MyD88, TIRAF, TRIM, TRAF and IRAK [7]. One exception is TLR3, which is MyD88-independent and thus signals through TRIF [8]. The recognition of PAMPs by TLRs ultimately leads to NF-κB and AP-1 activation and the production of many pro-inflammatory cytokines, such as TNF-α and IL-6 [9]. Additionally, type I interferons are induced through the phosphorylation of IRF3 and IRF7 [10]. Thus, TLRs are important in the early innate immune responses against pathogens. These initial mediators and the activation of antigen presenting cells (APCs) will also impact the ensuing adaptive immunity.

Many accessory molecules, which modulate the activity of TLRs, have been identified. Some are implicated, for instance, in the folding, trafficking and processing of the TLRs [11]. Other cofactors include CD14 and granulin, which have been shown to deliver specific ligands to TLR4 and 9 respectively. TLR signals are also regulated by molecules such as the suppressor of cytokine signaling 1 (SOCS1) and Toll-interacting protein (Tollip) [12–14]. While four isoforms of Tollip have been described in humans and mice, the canonical protein is composed of three domains [15] and is ubiquitously expressed [14]. A TBD (Tom1-binding domain) and a CUE (coupling of ubiquitin to endoplasmic reticulum degradation) domain, located on the N- and C-terminal regions respectively, confer a potential for multiple protein interactions [16]. Finally, a C2 (internal protein kinase C conserved

Abbreviations: TLR, toll-like receptor; PAMPs, pathogen-associated molecular patterns; TIR, Toll/IL-1 receptor; APCs, antigen presenting cells; SOCS1, suppressor of cytokine signaling 1; Tollip, Toll-interacting protein; TBD, Tom1-binding domain; CUE, coupling of ubiquitin to endoplasmic reticulum degradation domain; C2, internal protein kinase C conserved region 2; TGFBR1, TGF-beta type I receptor; IRAK, IL-1 receptor-associated kinase; MHC II, MHC class II; DCs, dendritic cells; iDCs, immature DCs; MARCH, membrane-associated RING-CH; MIR, modulator of immune recognition; CIITA, class II trans-activator; Tfr, transferrin receptor; Btk, Bruton tyrosine kinase; IL-1RI, IL-1 receptor; IL-1RAcP, IL-1R-associated protein; MFVs, mean fluorescence values.

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region 2) domain binds phosphoinositides and is responsible for the intracellular trafficking of the protein to the endocytic pathway [17].

While experiments on deficient mice suggested that Tollip was needed for maximal cytokine production in response to low doses of TLR agonists, most studies imply a negative regulatory role for Tollip in various signaling pathways [14,18–22]. For instance, Tollip has been shown to participate in IL-1β signaling as well as in the intracellular sorting and degradation of the ubiquitinated IL-1RI receptor [14,23]. Similarly, Tollip was recently shown to modulate TGF-β signaling through its interaction with Smad7 and by regulating the degradation of the activated TGF-β type I receptor (TβRI) [20]. Also, Tollip associates with TLRs and attenuates signaling by suppressing the activity of IL-1 receptor-associated kinase (IRAK) and NF-κB activation [18,22,24]. A high throughput shRNA screen identified Tollip as a potential regulator of MHC class II (MHC II) trafficking [25]. If ubiquitin links MHC II and Tollip pathways remains to be addressed. Interestingly, ubiquitination of MHC II molecules can occur in many different physiological conditions and cell types, allowing, for instance, maturation-dependent fine-tuning of antigen presentation in dendritic cells (DCs) [26,27].

Up to now, only two E3 ubiquitin ligases have been shown to modify MHC class II molecules. These are the membrane-associated RING-CH (MARCH) 1 and 8, two close homologues of viral modulator of immune recognition (MIR) proteins [28,29]. MARCH1 is mostly expressed in the spleen and more specifically in follicular B cells [30,31]. Comparably to the class II trans-activator (CIITA), which is the master regulator of MHC II gene transcription, MARCH1 appears to be the master regulator of MHC II expression at the post-translational level [32]. Indeed, the increased MHC II surface expression following activation of immature DCs (iDCs) is accompanied by the down-regulation of MARCH1 expression [33,34]. On the other hand, the immunosuppressive cytokine IL-10 up-regulates MARCH1 in monocytes and DCs to decrease MHC II expression and antigen presentation [35,36]. MARCH1 also ubiquitinates the transferrin receptor (Tfr), CD86, HLA-DM and Fas to modulate their expression and antigen presentation [30,31,37–39].

The impact of MARCH1 on the trafficking of MHC II has consequences beyond antigen presentation. It has been shown recently that DCs from MARCH1-deficient mice are impaired in the production of the pro-inflammatory cytokines IL-12 and TNF-α in response to LPS, suggesting that this ubiquitin ligase might be another accessory molecule involved in TLR signalling [40]. This activity of MARCH1 is dependent on the ubiquitination of MHC II molecules since the same phenotype was observed in MARCH1-proficient mice expressing non-ubiquitinable I-A^b β chain [40]. The fact that MHC II molecules potentiate LPS-induced signaling in human monocytes and mouse cells has been known for many years [41]. It was postulated that a LPS-binding receptor may interact with MHC II molecules to up-regulate TNFα secretion. Then, using MHC II-deficient primary cells from human patients or knock-out mice as well as reconstituted in vitro systems, Lauener and collaborators showed that MHC II molecules enhance TLR-induced responses [42,43]. More recently, it has been shown that MHC class II molecules promote TLR signaling in antigen presenting cells by maintaining activation of the Bruton tyrosine kinase (Btk) [44].

The cooperation between MHC II and TLRs promotes the innate as well as the adaptive immune response. Direct interactions between the MHC II and the TLRs have been observed and the generation of peptide-MHC class II complexes depends on endosomal trafficking of LPS-associated antigens in a phagosome-autonomous fashion [43,45,46]. Considering these clear functional links between innate and adaptive immunity and the interplay between the TLR4 signaling and the antigen presentation pathway, we hypothesized, as proposed recently, that Tollip might regulate the trafficking of MHC II molecules [47]. Our results demonstrate a direct interaction between MHC II and Tollip, which is reduced in the presence of MARCH1. Also, Tollip impairs the expression of MHC II and of MARCH1, in line with its previously described inhibitory functions.

2. Materials and methods

2.1. Antibodies

L243 (HLA-DR), XD5.117 (HLA-DRβ), CerCLIP.1 (CLIP/HLA-DR complexes), BU45 (human invariant chain), MaP.DM1 (HLA-DM) mAbs have been previously described [48–50]. The rabbit antisera against denatured HLA-DRα and HLA-DRβ were a kind gift from Dr. Rafick Sékaly (Vaccine and Gene Therapy Institute, Port St-Lucie, FL, USA). Rabbit polyclonal anti-GFP that recognizes both GFP and YFP, $Alexa^{488}$- and $Alexa^{633}$-fluor-coupled goat-anti mouse antibodies were purchased from Invitrogen (Laval, QC, Canada). The mouse anti-flag antibody was bought from Sigma (St-Louis, MS, USA). The rabbit anti-human Tollip was purchased from the cell signaling technology (Pickering, ON, Canada). The mouse anti-human Tfr OKT9 antibody was bought from ebioscience (San Diego, CA, USA). The mouse anti-MARCH1 (H1) was described previously [33]. The mouse anti-actin antibody was purchased from Millipore (Billerica, MA, USA).

2.2. Reagents

Poly(I:C) (Invivogen, San Diego, CA, USA) was used at a final concentration of 2 μg/mL. LPS was purchased from Sigma (St-Louis, MS, USA) and used at a concentration of 100 ng/mL. Benzyl Coelenterazine and luciferine were used at final concentrations of 5 uM and 20 μg/mL, respectively (Nanolight technology, Pinetop, AZ, USA).

2.3. Cell lines and mice

HeLa DR1, HeLa DR1 TM/TM, HeLa CIITA, HeLa CIITA/DO and HEK 293E CIITA stable transfectants were described previously [51,52]. Cells were cultured in DMEM supplemented with 5% FBS (Wisent, Saint-Jean-Baptiste, QC, Canada).

C57BL/6 (B6) mice were purchased from Charles River Laboratory (Wilmington, MA, USA). The M1K-O mice were described previously [31]. Xid mice were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Use of animals as described herein was approved by the University of Montreal's Institutional Animal Care and Use Committee (CDEA; protocol #12–042).

2.4. Plasmids and constructs

The flag, Rluc, $EGFP_2$ or EYFP tags were fused by PCR overlap to the N-terminus of HLA-DRβ or TLR3 molecules using pcDNA3.1_flag_MCS, pcDNA3.1_Rluc_MCS, pcDNA3.1_EYFP_MCS or pcDNA3.1_$EGFP_2$_MCS constructs. The cDNAs for the DRαTM and DRβTM chains include a stop codon immediately after the transmembrane coding regions, as described previously [53,54]. The GFP-SOCS1 and the GFP-Tollip constructs were obtained from Dr. Gerardo Ferbeyre (Université de Montréal, Montreal, QC, Canada) and Dr. Liwu Li (Virginia Polytechnic Institute and State University, Virginia, USA) respectively. The pcDNA3.1_MARCH1, pcDNA3.1_EYFP-MARCH1 and pcDNA-3.1_EYFP-MARCH1K-0 were described previously [48]. For the luciferase assay, we used the P2(2x)TK-pGL3_NF-κB reporter plasmid that was describe previously [55].

2.5. Transfections

For HeLa, 1×10^6 cells were plated 24 h prior to transfection in 10 cm petri dishes and transfected using lipofectamine LTX and Plus reagents according to the manufacturer's protocol (Invitrogen, Laval, QC, Canada). For HEK 293T and HEK 293E CIITA, 1.5×10^6 cells were