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CD300F Blocks Both MyD88 and TRIF-Mediated TLR Signaling through Activation of Src Homology Region 2 Domain-Containing Phosphatase 1

Sang-Min Lee,* Eun-Ju Kim,* Kyoungho Suk, † and Won-Ha Lee*

CD300F is known to exhibit inhibitory activity in myeloid cells through its intracellular ITIM. To investigate the effect of CD300F stimulation on TLR signaling, the human acute monocytic leukemia cell line THP-1 was treated with CD300F-specific mAbs or two synthetic peptides that represented the ITIM-like domains of CD300F. Treatment with these agents blocked TLR2-, 3-, 4-, and 9-mediated expression of proinflammatory mediators such as IL-8 and matrix metalloproteinase-9. The luciferase reporter assay in 293T cells and Western blot analysis of THP-1 cells revealed that these inhibitory actions were effective in pathways involving MyD88 and/or TRIF of TLR signaling and associated with marked suppression of IκB kinase activation, phosphorylation/degradation of IκB, and subsequent activation of NF-κB. Use of specific inhibitors and immunoprecipitation analysis further indicated that the inhibitory effects were mediated by Src homology 2 domain-containing phosphatase-1, a protein tyrosine phosphatase with inhibitory activity in hematopoietic cells. These data indicate that CD300F is an active regulator of TLR-mediated macrophage activation through its association with Src homology 2 domain-containing phosphatase-1 and that the synthetic peptides can be applied for the regulation of immune responses that are induced by TLRs. The Journal of Immunology, 2011, 186: 6296–6303.

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leads to two signaling pathways: one passes through TANK-binding kinase 1 and leads to the activation of IFN regulatory factor 3, which is responsible for the expression of type I IFN (16), whereas the other is mediated by receptor-interacting protein 1 and Pellino homolog 1, which induces IKK activation, degradation of IκB, and subsequent activation of NF-κB (17, 18).

To analyze the CD300F-mediated regulation of TLR signaling, THP-1 cells were treated with CD300F-specific mAbs or synthetic peptides representing the ITIM-like domains of CD300F that has been fused with HIV-TAT48–57 to facilitate the internalization of the peptide into the cells. Treatment with these agents blocked the production of inflammatory mediators that were induced by ligands for TLR2, 3, 4, and 9. The molecular mechanisms responsible for the CD300F-mediated inhibition of macrophage activation were then investigated.

Materials and Methods

Abs and reagents

CD300F-specific mAbs used for the inhibition assay were either generated in our laboratory (clones 2-3, 6D and 2-17B) as previously described (6) or purchased from BioLegend (clone UP-D2) (San Diego, CA). For the Western blot analysis or immunoprecipitation of CD300F, clone UP-D2 was used. Polyclonal Abs against ERK (p42/44 MAPK), phospho-ERK (Thr202/Tyr204), IκB, IKK, phospho-IKK, p38, and phospho-p38 as well as JNK and mAbs for phospho-IκB (Ser32/36) (clone 5A5), phospho-JNK (Thr183/Tyr185), p53, and phospho-p53 as well as p38 and phospho-p38 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). PP2 was obtained from Sigma, Aldrich (St. Louis, MO); palmitytoyl-Cys((R,S)-2,3-di(palmityloxy)-propyl)-Ala-Gly-Oh (PAM-CSDK) was obtained from Bachem AG (Budendorf, Switzerland); polyinosinic-polycytidylic acid (poly(I)-poly(C)) dsRNA was obtained from GE Healthcare (Little Chalfont, Buckinghamshire, U.K.), and CpG 1826 (TLR9 ligand) was purchased from InvivoGen (San Diego, CA). For the construction of a CD300F-expressing construct, the CD300F full length or its cytoplasmic tail deletion mutant was PCR amplified and cloned into a pGEM-T Easy vector, which was further digested with EcoRI to clone into a corresponding site in pC1-neo mammalian expression vector. Expression constructs for CD4-TLR4, the death domain of MyD88, wild-type TRIF, and TRAF6, the luciferase reporter gene under the control of NF-κB binding sites, and the Renilla-luciferase construct for transfection control were described previously (19–22).

Immunoprecipitation and Western blot analysis

For the immunoprecipitation, cells (5 × 10^6 cells/well in a 6-well plate) were incubated with 1 nM sodium sordavale for 15 min at 37°C and treated with either 1 μg/ml anti-CD300F mAb or 10 μM synthetic peptides for 3–30 min and lysed with Nonident P-40 buffer (150 mM NaCl, 50 mM Tris-Cl [pH 7.5], 5 mM EDTA, 1% Nonidet P-40 [IGEPAL, CA-630; Sigma-Aldrich], 0.5% sodium deoxycholate, and 1% of a protease inhibitor mixture [Calbiochem]). Cellular debris was removed by centrifugation at 10,000 × g for 15 min at 4°C, and the supernatants were precluded with 30 μl protein G-Sepharose beads for 1 h at 4°C. Immunoprecipitation with 1 μg/ml anti-SHP-1 or anti-CD300F mAb was performed overnight at 4°C, and after which, 50 μl protein G-Sepharose beads was added and incubated for 1 h at 4°C. After they were washed twice with lysis buffer, the beads were mixed with 50 μl SDS-PAGE loading buffer. Western blot analysis was performed as described previously (22, 23).

Luciferase reporter assay

The 293T cells were seeded (1 × 10^6 cells/well, triplicate/sample) in 96-well plates and incubated overnight before transfection with 800 ng/well total DNA, which had been mixed with 2.5 μl Superfect transfect reagent (Qiagen, Valencia, CA), and the rest of the reactions were performed as suggested by the manufacturer. Synthetic peptides were added 2 h after transfection if required. Cell lysates were obtained 24 h after transfection in lysis buffer, and the luciferase activities were determined using the

<table>
<thead>
<tr>
<th>Name</th>
<th>Sequence</th>
<th>Amino Acid Number in CD300F</th>
</tr>
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<tbody>
<tr>
<td>TAT-YADL</td>
<td>GRKKRRQRRR–GDLCYADLTL</td>
<td>201–210</td>
</tr>
<tr>
<td>TAT-FADL</td>
<td>GRKKRRQRRR–GDLCFADLTL</td>
<td>201–210 (Y205F)</td>
</tr>
<tr>
<td>TAT-YADA</td>
<td>GRKKRRQRRR–GDLCYADLTL</td>
<td>201–210 (L210A)</td>
</tr>
<tr>
<td>TAT-YASL</td>
<td>GRKKRRQRRR–EDISYASLTL</td>
<td>245–254</td>
</tr>
<tr>
<td>TAT-YASA</td>
<td>GRKKRRQRRR–EDISYASLTL</td>
<td>245–254 (L254A)</td>
</tr>
<tr>
<td>TAT</td>
<td>GRKKRRQRRR</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table I. TAT-ITIM hybrid peptides used in the experiments
Dual-Luciferase reporter Assay System (Promega, Madison, WI), according to the manufacturer’s recommended protocol. Relative firefly luciferase activity was determined by normalization with Renilla luciferase activity.

Gelatin zymogram and ELISA

The cells were activated by adding TLR ligands to the medium containing 1 × 10⁶/ml THP-1 cells in RPMI 1640 medium supplemented with 0.1% FBS. A sandwich ELISA (R&D Systems, Minneapolis, MN) measured the levels of IL-8 in the supernatants. The detection limit was <10 pg/ml. The matrix metalloproteinase (MMP) activity in the culture supernatant was determined via substrate gel electrophoresis, as described previously (23).

Statistical analysis

Statistical analysis of the data was performed using the two-tailed Student t test. All data are presented as mean values ± SEM, with the number of independent experiments indicated in the figure legends. Differences between experimental groups were considered significant for \( p < 0.05 \).

Results

Cross-linking of CD300F blocks TLR-induced expression of MMP-9 and IL-8 in THP-1 cells through blocking both MyD88 and TRIF-mediated cellular activation

The expression of CD300F has been detected in various cell types including macrophages, dendritic cells, and granulocytes (1–3). THP-1, as a human macrophage-like cell line, has been reported to express CD300F (6, 9). To analyze the inhibitory activities of CD300F in processes associated with TLR signaling, THP-1 cells were treated with anti-CD300F mAb for 30 min to cross-link CD300F and then were treated with LPS. Pretreatment with CD300F-specific mAb, but not with isotype-matching mouse IgG, completely blocked LPS-induced expression of IL-8 (Fig. 1A) and MMP-9 (Fig. 1B). LPS stimulates TLR4, which induces cellular signaling through both MyD88/TIRAP and TRIF/TRAM. The stimulation of TLR2 also induces signaling through these signaling adaptors. When THP-1 cells were stimulated with Pam3-CSK₄ as a ligand for TLR2, IL-8 expression was detected, and it was completely suppressed by pretreatment with anti-CD300F mAb (Fig. 1A). To find out the minimal amount of mAb required for this inhibitory effect, different doses of anti-CD300F mAb were then used. As shown in Fig. 1B, inhibitory effect was shown in cells pretreated with as little as 0.01 μg/ml anti-CD300F mAb, and 1 μg/ml was enough for complete blockage. The inhibitory activities of anti-CD300F mAb were observed when the cells were treated with the CD300F-specific mAbs generated in our laboratory (clones 2-3.6D and 2-1.7B), as well as a commercially available mAb (clone UP-D2) (data not shown). This indicates that the inhibitory effect of the mAb is not restricted to certain types of mAb, and cross-linkage of CD300F is enough for the induction of its inhibitory action.

The inhibitory effect of CD300F cross-linkage was then tested after stimulation of cells with other TLR ligands. Poly(I)-poly(C) mimos dsRNA and serves as the ligand for TLR3, which is known to induce signaling through TRIF. As shown in Fig. 1C, stimulation of THP-1 cells with poly(I)-poly(C) induced the expression of IL-8, which was blocked by pretreatment of the cells with anti-CD300F mAb. Likewise, the expression of IL-8 in cells stimulated with CpG oligodeoxynucleotides (ODNs), as a ligand for TLR9 that induces signaling through MyD88, was also inhibited by the cross-linkage of CD300F (Fig. 1C).

Synthetic peptides containing the ITIM domains of CD300F mimicked the inhibitory effect of anti-CD300F mAb

The inhibitory effect of CD300F is known to be mediated by ITIMs at its intracellular region. Two potential ITIM domains (encompassing Y205 and Y249) have been reported to be responsible for the inhibitory effect of CD300F through the interaction with SHP-1 (8). To compare the inhibitory potentials of individual ITIMs against TLR-mediated induction of IL-8 and MMP-9 expression, hybrid peptides (Table I) were synthesized. Each of these peptides contains decapetide spanning the ITIM region and HIV-TAT₄₈–₅₇, which serves as a carrier for the internalization of the peptides (24). Treatment of THP-1 cells with these peptides induced internalization of the peptides within 20 min (Supplemental Data 1). As expected, pretreatment of THP-1 cells with a synthetic peptide containing Y205 (TAT-YADL) and Y249 (TAT-YASL) blocked the LPS-induced expression of IL-8 (Fig. 2A) and MMP-9 (data not shown). The addition of different doses of TAT-YADL revealed that inhibition was evident at concentrations as little as 0.1 and 10 μM TAT-YADL or TAT-YASL, which was enough for >90% inhibition of LPS-induced expression of IL-8 (Fig. 2A). According to previous publications, substitution of the tyrosine with phenylalanine residue in each ITIM abolished the inhibitory activity (8). When the tyrosine residue in TAT-YADL was substi-
tuted with phenylalanine (TAT-FADL), the inhibitory effect was completely lost (Fig. 2B). This confirms the essential role of Y205 in the inhibitory activity of TAT-YADL. The inhibitory activity of TAT-YADL was then tested in another monocytic cell line, U937. The U937 cells responded to LPS and expressed high levels of both MMP-9 and IL-8 that were completely blocked by the addition of TAT-YADL and partially blocked by the treatment with anti-CD300F mAb (Supplemental Data 2). This indicates that the inhibitory action of the synthetic peptide is not restricted to THP-1 but more likely to be common in cells of the monocytic lineage.

The inhibitory effects of TAT-YADL were then tested in THP-1 cells stimulated with other TLR ligands. As shown in Fig. 3, TAT-YADL blocked IL-8 production that was induced by Pam3CSK4, CpG ODN, and poly(I)-poly(C). This indicates that TAT-YADL, similar to the cross-linkage of CD300F, can exert its inhibitory activity toward both MyD88- and TRIF-mediated signaling pathways.

To find out whether the inhibitory action of CD300F or TAT-ITIM peptides affect IL-8 expression at transcription or post-transcriptional level, RT-PCR analysis of IL-8 mRNA was performed. The levels IL-8 mRNA was induced by LPS treatment, and pretreatment of the cells with anti-CD300F or TAT-YADL inhibited the LPS-induced increase of IL-8 mRNA levels (Supplemental Data 3). In addition, LPS-induced transcriptional activation of another proinflammatory cytokine, TNF-α, was also inhibited by the pretreatment with anti-CD300F or TAT-YADL (Supplemental Data 3). On the basis of these observations, it is most likely that CD300F and TAT-YADL inhibit signaling pathways that are responsible for the transcriptional activation of inflammatory mediators including TNF-α, IL-8, and MMP-9.

Luciferase reporter assay confirmed that CD300F and TAT-YADL blocked TLR-mediated NF-κB activation through blocking both MyD88 and TRIF-mediated signaling pathways.

Pretreatment of THP-1 cells with anti-CD300F mAb blocked not only the TLR4 (or TLR2)-mediated induction of IL-8 expression but also TLR3- and TLR9-mediated events (Fig. 1). These data indicate that CD300F blocks both MyD88- and TRIF-mediated cellular responses. To confirm that CD300F inhibits signaling pathways mediated by these signaling adaptors, a reporter assay was performed with HEK293T cells, which were transiently transfected with the luciferase reporter gene under the control of NF-κB binding sites. Transfection of the cells with an expression construct for the CD4-TLR4 fusion protein (extracellular domain of CD4 and transmembrane and intracellular domains of TLR4), which is constitutively active (CA) (25), induced the luciferase gene expression and additional transfection of CD300F full length, but not the cytoplasmic tail deletion mutant of CD300F, and blocked the induction (Fig. 4A). Similarly, cotransfection of the CD300F full-length expression construct blocked the reporter gene expression that was induced by the CA form of MyD88 (death domain of MyD88) (26), overexpression of wild-type TRIF (27–29), or overexpression of wild-type TRAF6 (Fig. 4A) (26, 30). Transfection of increasing doses of CD300F exerted a dose-dependent inhibition of reporter activity induced by the CA form of MyD88 (Fig. 4B). These data confirm that CD300F blocks the activation of NF-κB through blocking both MyD88- and TRIF-mediated signaling pathways.

The inhibitory activities of synthetic peptides were then tested. As expected, treatment with TAT-YADL resulted in a significant and dose-dependent reduction of reporter gene expression that had been induced by the CA form of TLR4, MyD88, and TRIF, whereas treatment with TAT-FADL did not (Fig. 5).

CD300F cross-linking or TAT-YADL treatment blocks the activation of IKK and subsequent phosphorylation/degradation of IκB

LPS-induced expression of proinflammatory mediators requires the activation and nuclear translocation of NF-κB. Activation of NF-κB, which is in complex with IκB before activation, requires phosphorylation and degradation of IκB. Phosphorylation of IκB...
is mediated by an IKK complex that contains a regulatory IKK-γ subunit and two catalytic subunits (IKK-α and IKK-β). Activation status of IKK complex can be detected through the phosphorylation of IKK-α and IKK-β. As shown in Fig. 6A, pretreatment of THP-1 cells with anti-CD300F mAb or TAT-YADL blocked LPS-induced phosphorylation of IKK-α and IKK-β. In contrast, pretreatment with mouse IgG or TAT peptide failed to affect the LPS-induced phosphorylation of the IKK complex (Fig. 6A). Accordingly, pretreatment with anti-CD300F mAb or the TAT-YADL peptide inhibited LPS-induced phosphorylation and degradation of the IκB (Fig. 6B). Pretreatment with TAT-FADL failed to inhibit LPS-induced phosphorylation of IKK and phosphorylation/degradation of IκB (data not shown).

In addition to the activation of IKK, LPS also induces activation of MAPKs (31). Pretreatment with anti-CD300F mAb or the TAT-YADL peptide resulted in a partial inhibition of JNK MAPK, whereas the activation of p38 MAPK (Fig. 6C) and ERK MAPK (data not shown) was not affected. This raises the possibility that CD300F may affect, at least partially, other cellular processes that are associated with the activation of JNK.

The inhibitory action of the CD300F and the synthetic peptides requires the activity of SHP-1

To confirm that the inhibitory actions of CD300F and TAT-ITIM peptides are mediated by their interaction with SHP-1, an agent specialized in SHP-1 inhibition was used. PTP inhibitor III has been shown to inactivate SHP-1 through binding to its catalytic domain (32). Presence of PTP inhibitor III reversed the inhibitory effect of CD300F-specific mAb, TAT-YADL (Fig. 7A), and TAT-YASL (data not shown). This indicates that the inhibitory action of TAT-ITIM peptides requires the activity of SHP-1. To confirm the role of SHP-1 in the inhibitory action of CD300F and/or TAT-ITIM peptides, SHP-1 activation levels were directly measured in THP-1 cells after treatment with anti-CD300F mAb or TAT-YADL. As shown in Fig. 7B and 7C, SHP-1 phosphorylation was observed in cells treated with CD300F-specific mAb or the TAT-YADL peptide but not in cells treated with mouse IgG, TAT, or TAT-FADL.

The 293T cells were then transfected to confirm the involvement of SHP-1 in the inhibitory action of CD300F. 293T cells expressed SHP-1, but the levels were much lower than that of THP-1 (Fig. 8A). The cells were then transfected with full length or cytoplasmic tail deletion mutant of CD300F. Although the predicted molecular mass of CD300F is 32 kDa, anti-CD300F mAb detected a band of ∼45 kDa in cells transfected with the full length of CD300F, probably because of N- and/or O-linked glycosylation (1). Immunoprecipitation with anti-CD300F mAb and Western blot analysis of the precipitates with phosphotyrosine-specific mAb indicated full-length CD300F, but not the cytoplasmic tail deletion mutant, was phosphorylated at its tyrosine residues (Fig. 8B). This indicates that overexpression of CD300F, without the treatment with triggering anti-CD300F mAb, was sufficient to induce phosphorylation of its cytoplasmic tail. When the immunoprecipitated samples were tested for the presence of SHP-1, it was detected in the cells transfected with the full length, but not the deletion mutant, of CD300F. Furthermore, Western blot analysis with phosphotyrosine-specific Abs revealed that bound SHP-1 was phosphorylated (Fig. 8B). In contrast, immunoprecipitation with anti–SHP-1 mAb revealed that SHP-1 is not phosphorylated at the basal level, and transfection of full-length CD300F, but not the deletion mutant, results in the phosphorylation of its tyrosine residue (Fig. 8B). This indicates that overexpression of CD300F can induce its phosphorylation in the cytoplasmic tail, which subsequently recruits and activates SHP-1.

**FIGURE 7.** CD300F and synthetic peptide-mediated inhibition of LPS signaling requires SHP-1 activity. A, THP-1 cells were pretreated with 1 mM PTP inhibitor III (PI) for 30 min and then 1 μg/ml anti-CD300F mAb/mouse IgG (M) or 5 μM TAT/TAT-YADL for another 30 min, after which, cells were stimulated with 1 μg/ml LPS. DMSO (0.2%) was used as a vehicle control (VC). Culture supernatants were collected in 24 h for the analysis of MMP-9 activities and the measurement of IL-8 (n = 3; ***p < 0.001). B, THP-1 cells were treated with 1 μg/ml anti-CD300F mAb or mouse IgG for indicated times. Cell lysates were then collected, and SHP-1 was immunoprecipitated with specific mAbs. The amounts of SHP-1 and its phosphorylation levels were detected using Western blot analysis with SHP-1 or phosphotyrosine-specific mAb. C, THP-1 cells were treated with 5 μM TAT, TAT-YADL, or TAT-FADL (T-F) for indicated times, and the phosphorylation levels of SHP-1 was detected as in B. These experiments were repeated twice with essentially the same results.
Tyrosine phosphorylation of ITIMs is mediated by src-related kinases. When THP-1 cells were treated with a well-known src kinase inhibitor, PP2, LPS-induced expression of IL-8 was not affected, whereas the inhibitory action of CD300F was blocked (data not shown). When the luciferase assay was performed in the presence of PP2, CD300F-mediated inhibition was blocked without affecting the activation of NF-κB induced by the CA form of MyD88 (Fig. 8C). This indicates that phosphorylation of CD300F by src kinase is an essential step for its inhibitory action in THP-1 cells as well as 293T cells.

Specific sequences in addition to tyrosine are required for the inhibitory action of the synthetic peptides

The tyrosine residue plays an essential role in the inhibitory action of the ITIM sequences. In addition to it, flanking sequences are also important for the ITIM activity. To make sure that the inhibitory action of synthetic peptides requires the presence of specific amino acid sequences other than tyrosine, another tyrosine-containing peptide (TAT-YMMN) was synthesized. YMMN represents a sequence motif associated with the costimulatory action in the cytoplasmic tail of CD28 (33). When TAT-YADL was compared with TAT-YMMN, only TAT-YADL exhibited inhibitory effect, whereas TAT-YMMN did not. Furthermore, addition of TAT-YMMN alone resulted in the induction of MMP-9 and IL-8 expression (Fig. 9A). These data indicate that the inhibitory action of the ITIM-representing peptides requires not only the presence of tyrosine but also the presence of certain sequences that accompany the tyrosine residue. The common sequence motif for ITIMs is known to be S/I/V/LxYxxI/V/L (34). To make sure that YADL and YASL sequence comply with this general role, the final leucine residue in TAT-YADL and TAT-YASL was substituted with alanine and compared with respective wild-type sequences. As shown in Fig. 9B and 9C, substitution of leucine with alanine abolished the inhibitory action of the synthetic peptides as well as their ability to associate with SHP-1 (Fig. 9D). These data further confirm that the synthetic peptides exert their inhibitory activity through ITIM sequences in a manner similar to its original molecule, CD300F.

Discussion

Current data indicate that stimulation of CD300F can block the TLR-mediated induction of proinflammatory mediator expression though blocking the MyD88- and/or TRIF-mediated activation of the IKK complex and subsequent phosphorylation/degradation of IkB. Five tyrosine residues (Y205, Y236, Y249, Y284, and Y263) have been identified in the intracellular portion of CD300F, and substitution of tyrosine into phenylalanine revealed that two of them (Y205 and Y249) were required for the CD300F-mediated inhibitory effects, whereas the other two (Y236 and Y263) were responsible for the interaction with PI3K and subsequent induction of degranulation in rat basophilic leukemia cells (8). Studies using inhibitors that are specific for SHP-1 or PI3K indicated that only SHP-1, which interacts with phosphorytrosine residues of CD300F ITIM through its SH2 domain (1), mediates the inhibitory action CD300F. In addition, synthetic peptides that contained ITIM-like domains encompassing Y205 (TAT-YADL) and Y249 (TAT-YASL) mimicked the inhibitory action of CD300F, and substitution of tyrosine into phenylalanine in the TAT-YADL peptide abolished its interaction with SHP-1 as well as the inhibitory function. These data confirm the essential role of ITIM-like domains and their interaction with SHP-1 in the inhibition of TLR signaling by CD300F.

SHP-1, a PTP that is known to have roles in various cellular functions, is composed of two SH2 N-terminal domains and a C-terminal PTP domain. In lymphocytes and myeloid cells, SHP-1 has been demonstrated to modulate cellular signals that involve PI3K, Janus kinase 2, STATs, MAPKs, ERK, and NF-κB (35, 36). As a result, SHP-1 deficiency resulted in enhanced macrophage activities in clinical cases and experimental models (37–39). Phosphorylation of CD300F and its subsequent interaction with SHP-1 lead to the phosphorylation and activation of SHP-1. Currently, it is not known how SHP-1 suppresses either MyD88- or TRIF-mediated cellular signaling. However, several previous observations demonstrated the inhibitory function of SHP-1 in LPS signaling. LPS-induced expression of proinflammatory mediators was inhibited by the overexpression of SHP-1 in murine macrophages (40). In addition, SHP-1 was shown to be involved in the inhibition of LPS signaling by leukocyte Ig-like receptors (LILRs) (reviewed in Ref. 41). LILRs, as the family of 11 innate immune receptors, are predominantly expressed on APCs and B cells. The ITAM-containing cytoplasmic tails of activating members of LILRs (LILRα1–2, 4–6) associate with FceRγ, whereas the ITIM domains of inhibitory members of LILRs (LILRβ1–5) interact with inhibitory adaptor molecules including SHP-1, SHP-2, and SHIP. These ITIM-interacting molecules are believed to be responsible for the suppression of signaling adaptors.
through their phosphatase activity. Similarly, TLR-mediated inflammatory cytokine production in macrophage was inhibited by SH2 domain-containing PTP substrate-1, which exerts its effect through its association with SHP-1 and SHP-2 (42).

CD300F is expected to have regulatory function in the inflammatory activation of macrophages during normal immune response against pathogens or pathogenesis of chronic inflammatory diseases. In an effort to confirm the macrophage expression of CD300F in vivo, atherosclerotic plaques obtained from human carotid endarterectomy were analyzed. Preliminary data detected the expression of CD300F, as shown in Supplementary Data 4. First, in situ hybridization using a CD300F-specific antisense riboprobe detected high-level hybridization to the macrophage-rich areas that were also stained with mAb specific to CD68, the macrophage-specific marker. Furthermore, immunohistochemical analysis of another atherosclerotic plaque also demonstrated the colocalization of macrophage-specific staining with CD300F-specific staining. The in vivo expression of CD300F in macrophages further supports the involvement of CD300F in the regulation of macrophage activity during inflammation. Macrophages express most of the TLRs (43). TLR ligands of microbial origin as well as endogenous ligands such as fibronectin and heat shock proteins are also present in atherosclerotic plaques (44–47). TLR-mediated inflammatory activation of macrophages is believed to provide one of the major driving forces for the progression of atherosclerosis. The expression of CD300F by macrophages in atherosclerotic plaques may serve a regulatory role for the suppression of excess inflammatory activation through its interaction with a yet unidentified counterpart molecule, which can be either in membrane-bound or soluble forms.

In conclusion, CD300F and synthetic peptides representing the ITIM-like domains of CD300F blocks both MyD88- and TRIF-mediated TLR signaling pathways through activation of SHP-1, which blocks IKK activation, phosphorylation/degradation of IkB, and subsequent activation of NF-κB. The synthetic peptides that mimic the inhibitory action of CD300F could be useful for the regulation of inflammatory activation of macrophages that are closely associated with the pathogenesis of diseases such as atherosclerosis, rheumatoid arthritis, and cancer.

Disclosures

The authors have no financial conflicts of interest.

References


