Shedding of the Type II IL-1 Decoy Receptor Requires a Multifunctional Aminopeptidase, Aminopeptidase Regulator of TNF Receptor Type 1 Shedding

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Proteolytic cleavage of the extracellular domain of the type II IL-1 decoy receptor (IL-1RII) generates soluble IL-1-binding proteins that prevent excessive bioactivity by binding free IL-1. In this study we report that an aminopeptidase, aminopeptidase regulator of TNFR1 shedding (ARTS-1), is required for IL-1RII shedding. Coimmunoprecipitation experiments demonstrate an association between endogenous membrane-associated ARTS-1 and a 47-kDa IL-1RII, consistent with ectodomain cleavage of the membrane-bound receptor. A direct correlation exists between ARTS-1 protein expression and IL-1RII shedding, as cell lines overexpressing ARTS-1 have increased IL-1RII shedding and decreased membrane-associated IL-1RII. Basal IL-1RII shedding is absent from ARTS-1 knockout cell lines, demonstrating that ARTS-1 is required for constitutive IL-1RII shedding. Similarly, PMA-mediated IL-1RII shedding is almost entirely ARTS-1-dependent. ARTS-1 expression also enhances ionomycin-induced IL-1RII shedding. ARTS-1 did not alter levels of membrane-associated IL-1RI or IL-1R antagonist release from ARTS-1 cell lines, which suggests that the ability of ARTS-1 to promote shedding of IL-1R family members may be specific for IL-1RII. Further, increased IL-1RII shedding by ARTS-1-overexpressing cells attenuates the biological activity of IL-1β. We conclude that the ability of ARTS-1 to enhance IL-1RII shedding represents a new mechanism by which IL-1-induced cellular events can be modulated. As ARTS-1 also promotes the shedding of the structurally unrelated 55-kDa, type I TNF receptor and the IL-6R, we propose that ARTS-1 may play an important role in regulating innate immune and inflammatory responses by increasing cytokine receptor shedding. ***The Journal of Immunology,* 2003, 171: 6814–6819.**

Interleukin-1 is a proinflammatory cytokine that plays an important role in inflammation, host defense, and immunity as well as in the pathogenesis of inflammatory diseases, such as rheumatoid arthritis (1–3). Two distinct IL-1Rs exist. IL-1 signaling is mediated by the 80-kDa type I IL-1R (IL-1RI),[2] a member of the IL-1R/Toll-like receptor superfamily, that possesses a characteristic cytosolic Toll-IL-1R domain and extracellular Ig-like domains (1, 4, 5). Following IL-1 binding, the IL-1R accessory protein (IL-1RAcP) is recruited to IL-1RI to form a high affinity complex that mediates signaling via the adaptor protein MyD88 and the IL-1R-associated kinase (1, 6). In contrast, the 60-kDa type II IL-1R functions as a nonsignaling decoy receptor, because its short 29-aa cytoplasmic domain lacks a Toll-IL-1R domain (1, 4, 7). IL-1RII can also form a nonsignaling trimeric complex with IL-1 and IL-1RAcP, thereby exerting a dominant negative effect on IL-1RI signaling via sequestration of essential components of the IL-1RI signaling complex (8–10). Further, the low binding affinity of IL-1RII for the IL-1R antagonist (IL-1Ra) allows these molecules to function in a synergistic fashion (1, 10).

Generation of soluble type II IL-1Rs (sIL-1RII) that function as sIL-1-binding proteins represents another mechanism by which excessive IL-1 bioactivity can be attenuated (11, 12). Further, the ability of sIL-1RII to bind IL-1 and inhibit its bioactivity is enhanced by sIL-1RAcP, which is generated by alternative splicing, rather than ectodomain cleavage (13). In addition, sIL-1RII can bind to and inhibit the processing of pro-IL-1β precursor to its mature form by the IL-1-converting enzyme (caspase-1) (10, 14). Two distinct patterns of regulated IL-1RII shedding have been identified (10). The first type of regulated IL-1RII shedding occurs in response to IL-4, IL-13, or glucocorticoid stimulation and is slow, occurring over hours (13, 15, 16). The second type of regulated IL-1RII shedding occurs in response to fMLP, LPS, TNF, reactive oxygen species, and phorbol ester and is rapid, occurring over minutes (17–21).

Soluble IL-1RII has typically been described as a 45- to 47-kDa protein that is generated via proteolytic cleavage and release of the IL-1RII extracellular domain (20, 22, 23). This is supported by the inhibition of both slow and rapid IL-1RII shedding by hydroxamic acid-based zinc metalloprotease inhibitors (20). Further, TNF-α-converting enzyme (TACE; ADAM 17), a member of the disintegrin-metalloprotease family, was identified as having IL-1RII shedding activity based upon experiments demonstrating restoration of IL-1RII shedding following reconstitution of TACE-deficient murine cell lines (24). Although an alternatively spliced IL-1RII mRNA that does not contain a transmembrane or intracytoplasmic domain has been identified, its contribution to physiological IL-1RII shedding is unclear (20, 25).

The goal of this study was to define further the mechanisms by which IL-1RII shedding is regulated. We hypothesized that additional proteins exist that regulate IL-1RII shedding. In this study we report that an aminopeptidase, aminopeptidase regulator of TNFR1 shedding (ARTS-1), is required for constitutive IL-1RII

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Received for publication May 29, 2003. Accepted for publication October 8, 2003.

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[2] Abbreviations used in this paper: IL-1RI, type I IL-1R; IL-1Ra, IL-1R antagonist; IL-1RAcP, IL-1R accessory protein; sIL-1R, soluble type II IL-1R; TACE, TNF-α-converting enzyme; TAPI, TNF-α protease inhibitor.

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shedding and significantly enhances inducible IL-1RII shedding in response to stimulation with phorbol ester or calcium mobilization. Further, ARTS-1-mediated IL-1RII shedding attenuates the biological activity of IL-1β. As ARTS-1 also promotes the shedding of receptors of the TNF (TNFR1) and the type I cytokine receptor superfamilies (IL-6Rα), we propose that ARTS-1 plays an important role in modulating innate immune and inflammatory responses by regulating the shedding of three distinct and structurally dissimilar types of cytokine receptor superfamilies (26, 27).

Materials and Methods

ARTS-1 cell lines and reagents

NCI-H292 cells, a human pulmonary mucoepidermoid carcinoma cell line, were purchased from American Type Culture Collection (Manassas, VA) and grown in RPMI 1640 supplemented with 10% FBS under 5% CO_2 at 37°C. Stably transfected NCI-H292 cell lines that express either the full-length human ARTS-1 coding sequence or antisense ARTS-1 (bases 61–213) were used (26). NCI-H292 ARTS-1 knockout cell lines (*arts-1*$^{-/-}$) that contain a substitution of the neomycin phophotransferase gene, in the antisense direction, for *arts-1* exons 5 and 6 that encode the consensus zinc metalloprotease catalytic motif, were also used (27). These *arts-1*$^{-/-}$ cell lines do not express ARTS-1 mRNA or protein. The *arts-1*$^{-/-}$ cell lines were reconstituted by transient transfection with plasmids encoding either full-length ARTS-1 or ARTS-1 catalytic site mutants (H353P, H357V, H353P/E354V), using Gene Porter II and Booster (Gene Therapy Systems, San Diego, CA) (26).

Coimmunoprecipitation of endogenous ARTS-1 and IL-1RII

Immunoprecipitation experiments were performed on membrane fractions of NCI-H292 cells. Cells were harvested for membrane isolation by scraping and disrupted by sonicating twice (10 s) in lysis buffer (50 mM Tris-HCl (pH 7.2), 120 mM NaCl, 0.1% Triton X-100, and Complete protease inhibitor (Roche, Indianapolis, IN), followed by centrifugation (1,000 $\times$ *g*, 5 min) to remove nuclei and cellular debris. Postnuclear supernatants were centrifuged (100,000 $\times$ *g*, 1 h) to recover membrane pellets that were suspended by sonicating three times (2 s each time) in lysis buffer. Protein concentrations were determined using a bicinchoninic acid protein determination kit (Pierce, Rockford, IL) with BSA as a standard. For immunoprecipitation, samples of membrane proteins (200 μg) were incubated (4°C) overnight with 20 μg of murine mAb against the extracellular domain of IL-1RII (R&D Systems, Minneapolis, MN) or 1 μl of rabbit anti-human ARTS-1 immune or preimmune serum, followed by addition of 200 μl of immobilized protein A/G beads (Pierce) for 2 h at room temperature. After the beads were washed eight times with lysis buffer, bound proteins were separated by SDS-PAGE, electroblotted onto nitrocellulose membranes, and incubated overnight (4°C) with ARTS-1 immune or pr-immune serum diluted 1/20,000 or the murine anti-IL-1RII mAb, 2 μg/ml. Detection was performed with HRP-conjugated secondary Abs and SuperSignal West Pico chemiluminescent substrate (Pierce).

Immunoblotting of IL-RI and IL-1RII

To quantify membrane-associated IL-1Rs, 20 μg of membrane proteins were separated via SDS-PAGE and electroblotted onto nitrocellulose membranes, which were incubated (4°C) overnight with rabbit anti-human IL-1RII polyclonal Ab (Rockland Immunochemicals, Gilbertsville, PA) at a concentration of 0.1 mg/ml, followed by incubation with a goat anti-rabbit secondary Ab (Pierce), for 2 h at room temperature. For IL-1RI immunoblotting, nitrocellulose membranes were incubated with rabbit anti-human IL-1RI Ab (Santa Cruz Biotechnology, Santa Cruz, CA; 1 μg/ml), followed by incubation with a goat anti-rabbit secondary Ab (Pierce), for 2 h at room temperature. Detection was performed with HRP-conjugated secondary Abs and SuperSignal West Pico chemiluminescent substrate (Pierce).

Analysis of cytokine secretion and receptor shedding

Soluble IL-1RII, IL-1Ra, and IL-8 in NCI-H292 cell culture supernatants were quantified by ELISA with a sensitivity of 7.8 pg/ml (R&D Systems). PMA and ionomycin were purchased from Sigma-Aldrich (St. Louis, MO). TNF-α protease inhibitor (TAPI)-0, TAPI-1, and TAPI-2 were purchased from Peptides International (Louisville, KY). Statistical analysis was performed using Student's *t* test with Bonferroni's correction for multiple comparisons. A value of $p < 0.05$ was considered significant.

Results

Endogenous membrane-associated ARTS-1 coimmunoprecipitates IL-1RII

Experiments were conducted to determine whether membrane-associated ARTS-1 coimmunoprecipitates IL-1RII. As shown in Fig. 1*A*, immunoprecipitation of membrane fractions of wild-type NCI-H292 cells with an anti-IL-1RII mAb pulled down the 100-kDa ARTS-1 isoform. In the reciprocal experiment, immunoprecipitation with ARTS-1 antiserum pulled down a 47-kDa IL-1RII, which is consistent with sIL-1RII generated by proteolytic cleavage of the IL-1RII extracellular domain (Fig. 1*B*). Based upon these experiments, we conclude that endogenous ARTS-1 is associated with the 47-kDa sIL-1RII in membrane fractions of NCI-H292 cells.

ARTS-1 promotes IL-1RII shedding

Because of the association between endogenous ARTS-1 and sIL-1RII, we investigated whether ARTS-1 expression promotes IL-1RII shedding. Soluble IL-1RII in culture supernatants from wild-type NCI-H292 cells and ARTS-1 cell lines were quantified by ELISA. The ARTS-1 cell lines had been generated by stable transfection of NCI-H292 cells with mammalian expression plasmids encoding the full-length ARTS-1-coding sequence in the sense orientation or ARTS-1 bases 61–213, which includes the putative translation start site and the intracellular and transmembrane domains, in the antisense orientation (26). The overexpressing cell lines express increased amounts of ARTS-1 compared with wild-type and mock-transfected cells, whereas the antisense cell lines demonstrate reduced ARTS-1 protein levels (26). As shown in Fig. 2*A*, the amount of sIL-1RII in culture supernatants from cell lines overexpressing ARTS-1 was significantly greater than that from mock-transfected cells, whereas that in supernatants from antisense ARTS-1 cell lines had significantly less sIL-1RII than did mock-transfected cells. Therefore, changes in ARTS-1 protein levels correlated directly with changes in sIL-1RII protein.

To characterize further the effect of ARTS-1 on IL-1RII shedding, membrane-associated IL-1RII was analyzed by immunoblotting. As shown in Fig. 2*B*, membrane fractions from ARTS-1 an-

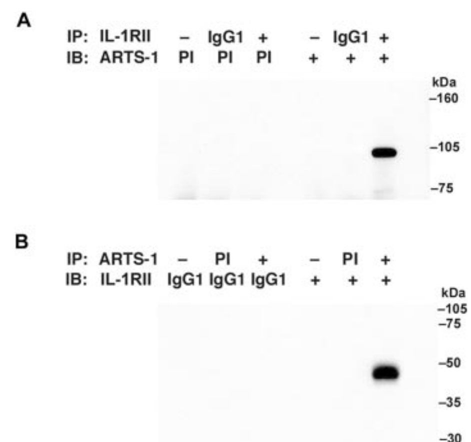

FIGURE 1. Endogenous membrane-associated ARTS-1 coimmunoprecipitates IL-1RII from NCI-H292 cells. *A*, Coimmunoprecipitations were performed with either an anti-IL-1RII mAb directed against the IL-1RII extracellular domain (+) or a murine IgG1 isotype control (IgG1) and immunoblotted with anti-ARTS-1 preimmune (PI) or immune (+) serum. *B*, Reciprocal coimmunoprecipitations were performed with either anti-ARTS-1 preimmune (PI) or immune (+) serum and immunoblotted with either an anti-IL-1RII mAb (+) or a murine IgG1 isotype control (IgG1).

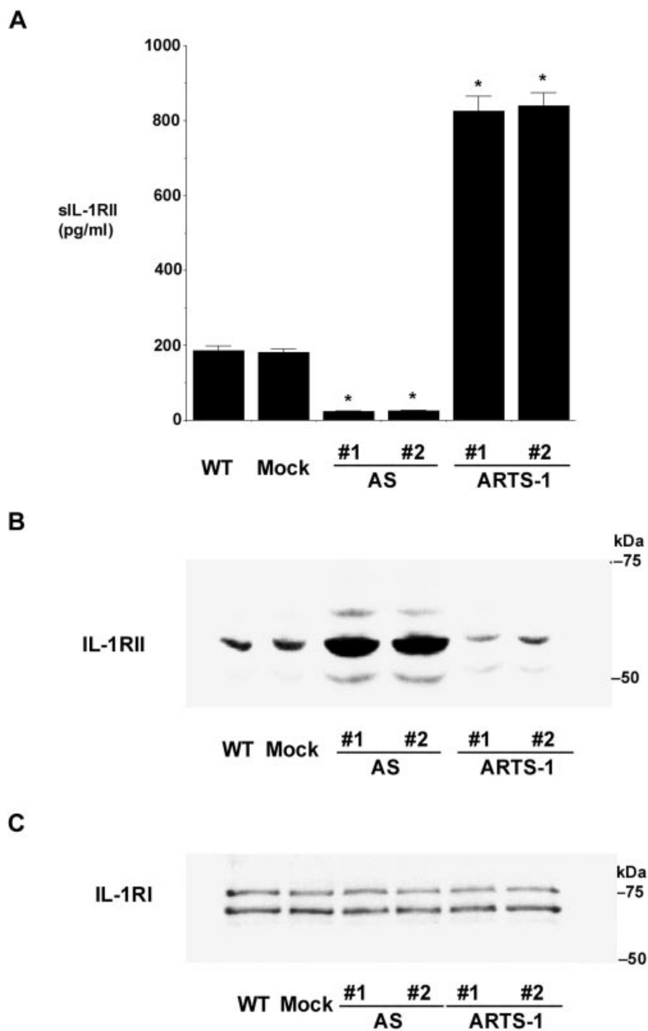

FIGURE 2. ARTS-1 promotes IL-1RII shedding. *A*, Concentrations of sIL-1RII present in 24-h culture supernatants from two antisense (AS) and two sense (ARTS-1) cell lines were determined by ELISA ($n = 5$). *, $p < 0.05$ vs mock-transfected cells. WT, wild-type NCI-H292 cells. *B*, Effect of ARTS-1 on membrane-associated IL-1RII in ARTS-1 cell lines. Samples (20 μg) of membrane proteins from ARTS-1 cell lines were separated by SDS-PAGE, transferred to nitrocellulose, and reacted with Abs against IL-1RII. *C*, Effect of ARTS-1 on membrane-associated IL-1RI in ARTS-1 cell lines. Samples (20 μg) of membrane proteins from ARTS-1 cell lines were separated by SDS-PAGE, transferred to nitrocellulose, and reacted with Abs against IL-1RI.

tisense cell lines had significantly greater quantities of full-length, 60-kDa IL-1RII than was present in membrane fractions from either wild-type or mock-transfected NCI-H292 cells. In contrast, membrane fractions from ARTS-1-overexpressing cell lines had significantly lower quantities of full-length, 60-kDa IL-1RII protein. Taken together, these experiments demonstrate a reciprocal relationship between ARTS-1 protein expression and levels of membrane-associated IL-1RII.

To investigate the specificity of the ARTS-1 effect, experiments were performed to assess whether ARTS-1 protein expression correlated with levels of membrane-associated IL-1RI. As shown in Fig. 2*C*, there was no significant change in levels of membrane-associated IL-1RI among wild-type, mock-transfected, ARTS-1, or antisense ARTS-1 cell lines. These data suggest that ARTS-1 protein expression does not promote IL-1RI shedding.

IL-1RII shedding is absent from ARTS-1 knockout cell lines

NCI-H292 ARTS-1 knockout cell lines (*arts-1*$^{-/-}$), which contain a targeted deletion of the zinc metalloprotease catalytic domain, were used to characterize further the effect of ARTS-1 on IL-1RII shedding (27). The *arts-1*$^{-/-}$ cell lines do not express ARTS-1 mRNA or protein, because *arts-1* exons 5 and 6, which encode the ARTS-1 catalytic domain, are replaced by a neomycin phosphotransferase cassette in the antisense orientation. As shown in Fig. 3*A*, soluble IL-1RII was not detected in the culture supernatants from *arts-1*$^{-/-}$ cell lines, whereas wild-type NCI-H292 cells demonstrated constitutive IL-1RII shedding. Additional experiments were performed to assess the effect of ARTS-1 on PMA-induced IL-1RII shedding. Treatment of wild-type NCI-H292 cells with 0.1 μM PMA for 24 h resulted in significant increases in the quantity of sIL-1RII detected in culture supernatants over that from nonstimulated cells. Although PMA stimulation also induced IL-1RII shedding from *arts-1*$^{-/-}$ cell lines, the quantity of shed receptor

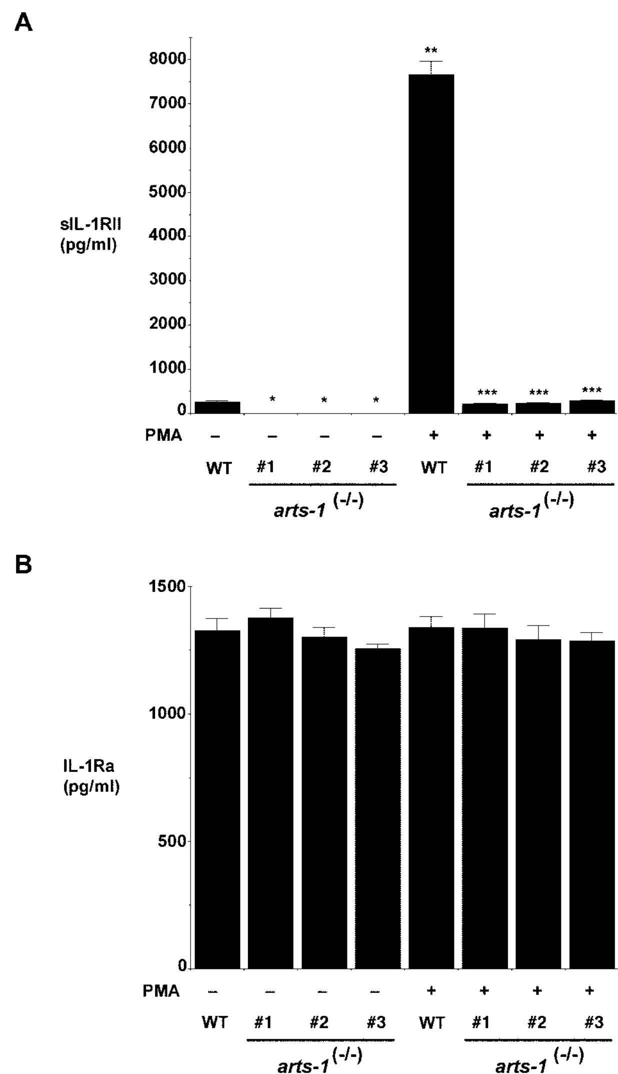

FIGURE 3. ARTS-1 is required for IL-1RII shedding. Concentrations of sIL-1RII (*A*) and IL-1Ra (*B*) in culture supernatants from wild-type NCI-H292 cells and *arts-1*$^{-/-}$ cell lines, incubated for 24 h with or without 0.1 μM PMA, were determined by ELISA ($n = 5$). *, $p < 0.05$, *arts-1*$^{-/-}$ cell lines vs wild-type NCI-H292 cells. **, $p < 0.05$, PMA-treated wild-type NCI-H292 cells vs untreated wild-type NCI-H292 cells. ***, $p < 0.05$, PMA-treated *arts-1*$^{-/-}$ cell lines vs PMA-treated wild-type NCI-H292 cells.

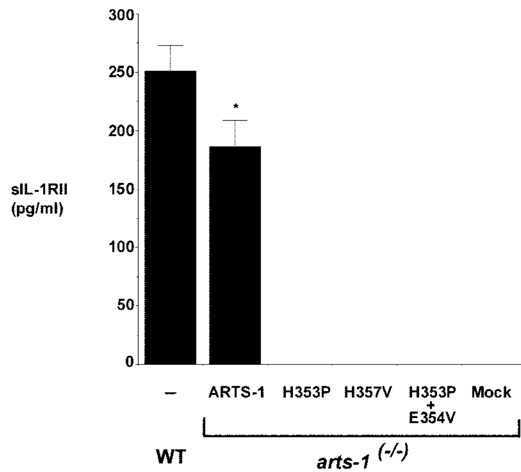

FIGURE 4. IL-1RII shedding is restored by ARTS-1 expression in *arts-1*$^{-/-}$ cell lines. *arts-1*$^{-/-}$ cell lines were transiently transfected with plasmids encoding either full-length ARTS-1 or ARTS-1 catalytic site mutants (H353P, H357V, H353P, and E354V). Concentrations of sIL-1RII in 24-h culture supernatants from wild-type NCI-H292 cells and transiently transfected *arts-1*$^{-/-}$ cell lines were determined by ELISA (n = 5). *, $p < 0.05$, *arts-1*$^{-/-}$ cell lines transfected with full-length ARTS-1 vs mock-transfected cells.

was significantly less than that from PMA-stimulated wild-type NCI-H292 cells. These experiments demonstrate that ARTS-1 is required for constitutive IL-1RII shedding and that ARTS-1 expression markedly enhances PMA-induced IL-1RII shedding.

In contrast, there was no significant change in the quantity of IL-1Ra present in supernatants of *arts-1*$^{-/-}$ cell lines compared with wild-type NCI-H292 cell lines over 24 h, with or without 0.1 μM PMA (Fig. 3*B*). This demonstrates that ARTS-1 protein expression does not regulate IL-1Ra release from NCI-H292 cell lines. Taken together with the data regarding IL-1RI, these experiments suggest that the ability of ARTS-1 to regulate members of the IL-1R family may be restricted to IL-1RII.

The ARTS-1 catalytic domain is required for IL-1RII shedding

Experiments were performed next to determine whether the ARTS-1 catalytic domain is required for IL-1RII shedding. These experiments used *arts-1*$^{-/-}$ knockout cell lines that had been reconstituted by transient transfection with plasmids encoding either the full-length ARTS-1-coding sequence or catalytically inactive, full-length ARTS-1 mutants, both of which restore ARTS-1 protein expression, as previously demonstrated by immunoblotting (27). The ARTS-1 catalytic site mutants have either single or double amino acid substitutions of key residues in the consensus zinc metalloprotease catalytic site, HELAH(Y)$_{18}$E. Mutation of the first glutamic acid (E354) abolishes zinc metalloprotease catalytic activity, whereas mutation of the two histidines (H353 and H357) abolishes catalytic activity and zinc binding (28, 29). As shown in Fig. 4, IL-1RII shedding was restored when *arts-1*$^{-/-}$ cell lines were reconstituted by transfection with plasmids encoding full-length ARTS-1 cDNA. In contrast, IL-1RII shedding was not detected when *arts-1*$^{-/-}$ cell lines were reconstituted by transfection with plasmids encoding the ARTS-1 catalytic site mutants. These experiments demonstrate that ARTS-1-mediated IL-1RII shedding is dependent upon its zinc metalloprotease catalytic domain.

Additional experiments were performed to confirm that zinc metalloprotease activity is required for ARTS-1-mediated IL-1R shedding. Treatment of wild-type, mock-transfected, and ARTS-1 cell lines with 25 μM of the hydroxamic acid-based metalloprotease inhibitors, TAPI-0, TAPI-1, or TAPI-2, significantly decreased IL-1RII shedding (Fig. 5). Thus, a hydroxamic acid-sensitive zinc metalloprotease activity is required for IL-1R shedding.

Effect of calcium mobilization on IL-1RII shedding

Experiments were performed to assess whether calcium mobilization induces IL-1RII shedding from NCI-H292 cells. As shown in Fig. 6, treatment of wild-type, mock-transfected, or antisense NCI-H292 cells with 1 μM ionomycin for 24 h resulted in significant increases in the quantity of sIL-1RII detected in culture supernatants over that from nonstimulated cells. The quantity of shed receptor from ionomycin-stimulated antisense cell lines, however, was significantly reduced compared with that from ionomycin-stimulated mock-transfected cells. These experiments demonstrate that calcium mobilization induces IL-1RII shedding and that ARTS-1 expression significantly enhances this effect.

Effect of ARTS-1-mediated IL-1RII shedding on IL-1β-induced IL-8 secretion

As sIL-1RII functions as a sIL-1-binding protein, we assessed whether ARTS-1-mediated IL-1RII shedding can attenuate IL-1β-induced IL-8 secretion. As shown in Fig. 7, treatment of wild-type, mock-transfected, or ARTS-1 NCI-H292 cell lines with 10 ng/ml IL-1β for 24 h resulted in significant increases in the quantity of

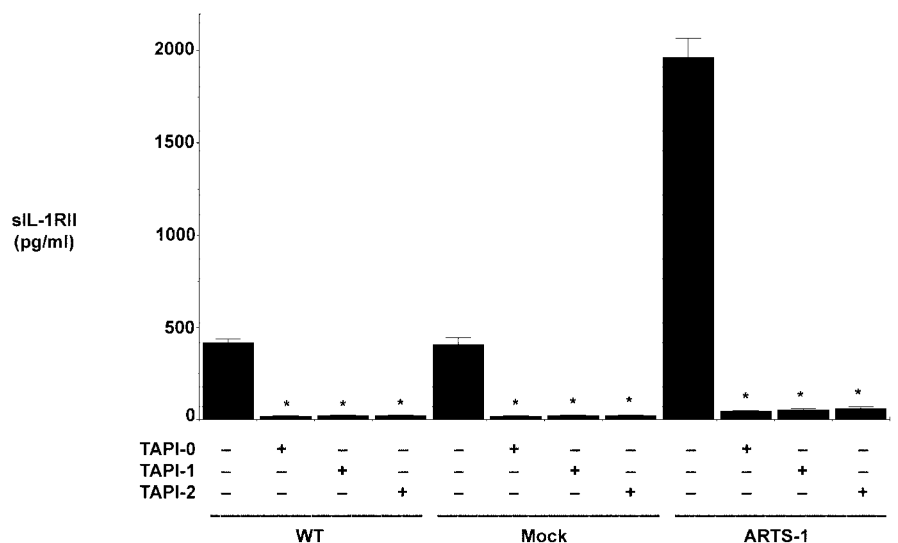

FIGURE 5. TAPI inhibits ARTS-1-mediated increases in IL-1RII shedding. ARTS-1 cell lines were treated for 24 h with 25 μM TAPI-0, TAPI-1, or TAPI-2. Soluble IL-1RII in cell culture supernatants was quantified by ELISA and compared with that in supernatants from untreated cells (n = 5). *, $p < 0.05$.

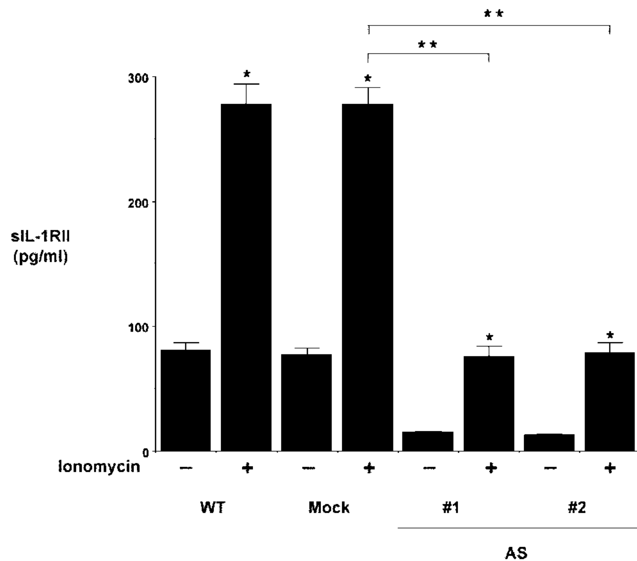

FIGURE 6. ARTS-1 enhances calcium-mediated increases in IL-1RII shedding. Concentrations of sIL-1RII present in culture supernatants from two antisense ARTS-1 (AS) NCI-H292 cell lines, incubated for 24 h with or without 1 μM ionomycin, were determined by ELISA ($n = 5$). WT, wild-type NCI-H292 cells. *, $p < 0.05$, ionomycin-treated vs untreated cells. **, $p < 0.05$, ionomycin-treated mock-transfected NCI-H292 cells vs ionomycin-treated antisense cell lines.

IL-8 detected in culture supernatants over that from nonstimulated cells. The quantity of IL-8 secreted from IL-1β-stimulated ARTS-1 cell lines, however, was significantly reduced compared with that from IL-1β-stimulated mock-transfected cells. This experiment is consistent with the conclusion that increased IL-1RII shedding from ARTS-1 cell lines can attenuate IL-1β-mediated biologic effects, such as IL-8 secretion.

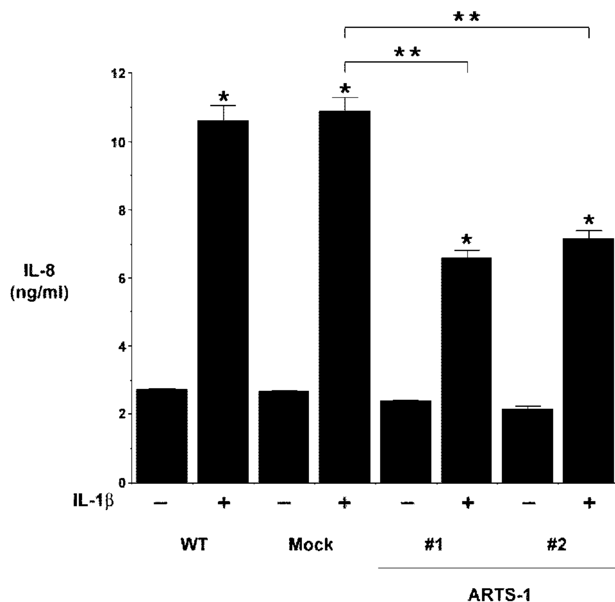

FIGURE 7. ARTS-1-induced IL-1RII shedding attenuates IL-1β-mediated IL-8 secretion. Concentrations of IL-8 present in culture supernatants from two ARTS-1-overexpressing NCI-H292 cell lines, incubated for 24 h with or without IL-1β (10 ng/ml), were determined by ELISA ($n = 5$). WT, wild-type NCI-H292 cells. *, $p < 0.05$, IL-1β-treated vs untreated cells. **, $p < 0.05$, IL-1β-treated mock-transfected NCI-H292 cells vs IL-1β-treated ARTS-1-overexpressing cell lines.

Discussion

The biological activity of IL-1 can be regulated at several levels, including proteolytic cleavage of the IL-1RII ectodomain to generate sIL-1RII that bind to and sequester IL-1. We now report that a multifunctional aminopeptidase, ARTS-1, promotes IL-1RII shedding. First, ARTS-1 coimmunoprecipitates with a 47-kDa IL-1RII protein, a size consistent with sIL-1RII generated by ectodomain cleavage of the membrane-bound receptor. Second, there was a direct correlation between ARTS-1 protein expression and IL-1RII shedding. Third, constitutive IL-1RII shedding was absent in ARTS-1 knockout cells. Fourth, reconstitution of the ARTS-1 knockout cells with wild-type ARTS-1 restored IL-1RII shedding. Further, ARTS-1-mediated IL-1RII shedding attenuates the biological activity of IL-1β. Taken together, these data demonstrate that ARTS-1 promotes IL-1RII shedding and thereby regulates IL-1-induced cellular events.

Although ARTS-1 is required for constitutive IL-1RII shedding, our data demonstrate that ARTS-1 expression also promotes inducible IL-1RII shedding. For example, PMA-mediated IL-1RII shedding is almost entirely dependent upon ARTS-1 expression. An ARTS-1-independent pathway also exists, as evidenced by the low level of IL-1RII shedding from ARTS-1 knockout cell lines in response to PMA. Similarly, we demonstrate that calcium mobilization, which is known to induce IL-6R shedding (30), also stimulates IL-1RII shedding, and that ARTS-1 expression significantly enhances this effect.

ARTS-1-mediated IL-1RII shedding is also dependent upon an intact ARTS-1 zinc metalloprotease catalytic motif. Although TACE has been identified as possessing IL-1RII sheddase activity, we previously found that ARTS-1 neither affects levels of TACE protein nor alters removal of the TACE prodomain or processing to a mature form (24, 26). Consequently, ARTS-1 does not appear to promote receptor shedding by altering TACE expression or maturation. Further, as ARTS-1 is an aminopeptidase with no known endopeptidase activity, we propose that it indirectly promotes IL-1RII shedding via the activation of IL-1R sheddases (26, 27).

We also report that the ability of ARTS-1 to promote shedding of IL-1R family members appears specific for IL-1RII. Overexpression of ARTS-1 was associated with increased IL-1RII shedding and reciprocal decreases in membrane-associated IL-1RII. In contrast, changes in ARTS-1 expression were not associated with altered levels of IL-1RI protein in membrane preparations. Further, the targeted deletion of ARTS-1 did not alter the extracellular release of the IL-1R antagonist by NCI-H292 cells (31, 32). Similarly, ARTS-1 specifically promoted shedding of the 55-kDa, type I (TNFR1), but not the 75-kDa, type II (TNFR2), TNFR (26). Therefore, our data suggest that ARTS-1 contributes specificity to the regulation of cytokine receptor shedding.

Although IL-1RII and IL-6Rα are structurally dissimilar and belong to different cytokine receptor superfamilies, there exist many similarities in the mechanisms by which their shedding is regulated by ARTS-1 (27). First, ARTS-1 binds to truncated forms of both IL-1RII and IL-6Rα, consistent with proteolytic cleavage of their extracellular domains (27). In contrast, ARTS-1 binds to a full-length 55-kDa TNFR1 isoform (26). Second, constitutive shedding of both IL-1RII and IL-6Rα is ARTS-1 dependent. Lastly, constitutive shedding of both IL-1RII and IL-6Rα require ARTS-1 catalytic activity. Taken together, these data demonstrate that ARTS-1 promotes shedding of IL-1RII and IL-6Rα by similar, or perhaps common, mechanisms.

These findings expand the known repertoire of ARTS-1-mediated functions to include the regulation of IL-1RII shedding. Our

laboratory initially identified ARTS-1 as a type II integral membrane protein that was capable of binding to and promoting shedding of TNFR1 and IL-6Rα (26, 27). In addition, recombinant ARTS-1 has aminopeptidase activity, which was selective for nonpolar amino-terminal residues. ARTS-1 was also independently identified as a soluble aminopeptidase (adipocyte-derived leucine aminopeptidase and puromycin-insensitive leucyl-specific aminopeptidase) with substrate specificity for leucine and methionine as well as peptide hormones, including angiotensin II and kallidin (33–36). Therefore, ARTS-1 is a member of the MA clan of the M1 family of zinc metalloproteases that contain a highly conserved consensus catalytic motif (HEXXH(Y)$_{18}$E) (37, 38). Importantly, ARTS-1 homologues have been identified that reside in the endoplasmic reticulum and play a key role in processing peptides to an optimal length of eight or nine amino acids before their presentation in the context of MHC class I (38–40). These homologues, endoplasmic reticulum aminopeptidase associated with Ag processing and endoplasmic reticulum aminopeptidase I, catalyze a key step in peptide processing after proteosomal degradation and play an important role in Ag presentation and immune surveillance (41).

In summary, we have identified ARTS-1 as an important regulator of constitutive and inducible shedding of the decoy receptor IL-1RII. We propose that the ability of ARTS-1-mediated IL-1RII shedding to attenuate the biological activity of IL-1β represents a new mechanism by which IL-1-induced cellular events may be modulated.

Acknowledgments

We thank Drs. Martha Vaughan and Joel Moss for their helpful advice and critical review of the manuscript.

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