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MALT1 Protease Plays a Dual Role in the Allergic Response by Acting in Both Mast Cells and Endothelial Cells

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The signaling protein MALT1 plays a key role in promoting NF- κ B activation in Ag-stimulated lymphocytes. In this capacity, MALT1 has two functions, acting as a scaffolding protein and as a substrate-specific protease. MALT1 is also required for NF- κ B-dependent induction of proinflammatory cytokines after Fc ϵ R1 stimulation in mast cells, implicating a role in allergy. Because MALT1 remains understudied in this context, we sought to investigate how MALT1 proteolytic activity contributes to the overall allergic response. We compared bone marrow–derived mast cells from MALT1 knockout (MALT1^{−/−}) and MALT1 protease-deficient (MALT1^{PD/PD}) mice to wild-type cells. We found that MALT1^{−/−} and MALT1^{PD/PD} mast cells are equally impaired in cytokine production following Fc ϵ R1 stimulation, indicating that MALT1 scaffolding activity is insufficient to drive the cytokine response and that MALT1 protease activity is essential. In addition to cytokine production, acute mast cell degranulation is a critical component of allergic response. Intriguingly, whereas degranulation is MALT1-independent, MALT1^{PD/PD} mice are protected from vascular edema induced by either passive cutaneous anaphylaxis or direct challenge with histamine, a major granule component. This suggests a role for MALT1 protease activity in endothelial cells targeted by mast cell–derived vasoactive substances. Indeed, we find that in human endothelial cells, MALT1 protease is activated following histamine treatment and is required for histamine-induced permeability. We thus propose a dual role for MALT1 protease in allergic response, mediating 1) IgE-dependent mast cell cytokine production, and 2) histamine-induced endothelial permeability. This dual role indicates that therapeutic inhibitors of MALT1 protease could work synergistically to control IgE-mediated allergic disease. *The Journal of Immunology*, 2020, 204: 2337–2348.

Mast cells are innate immune cells that are widely distributed throughout vascularized tissues in the human body. Their activation via the Fc ϵ R1 receptor is widely

recognized for playing a role in IgE-mediated allergic disease (1–3). Aggregation of receptors leading to the release of numerous proinflammatory mediators, including histamine, arachidonic acid metabolites, and cytokines, precipitates the various signs and symptoms experienced during an allergic reaction. Although much is known about how these cells contribute to the allergic response, less is known about the key intracellular signaling pathways that control the mast cell response.

The cytoplasmic proteins B cell lymphoma 10 (Bcl10) and MALT1 are part of a signaling complex that mediates NF- κ B activation in both immune and nonimmune cells and in both normal physiologic as well as pathologic settings (4–7). In mast cells, Bcl10 and MALT1 are required for IgE-mediated, NF- κ B–induced proinflammatory cytokine production (8, 9). MALT1 is considered the downstream effector protein of the complex and is known to promote activation of NF- κ B by serving as a scaffold to bind and recruit downstream signaling proteins that interface with I κ B kinase (IKK), a master regulator of NF- κ B (4). More recently, several groups demonstrated that in addition to scaffolding activity MALT1 also possesses proteolytic activity (10, 11). Importantly, although MALT1 scaffolding activity is required for Bcl10/MALT1-mediated IKK activation, MALT1 protease activity is dispensable. Instead, it is thought that MALT1 proteolytically cleaves specific substrates, such as the NF- κ B subunit, RelB, and the deubiquitinases A20 and CYLD, that are negative regulators of NF- κ B signaling (10, 12, 13). When the MALT1 protease is active, cleavage of these key regulatory substrates disrupts their inhibitory effects, optimizing and sustaining the overall NF- κ B signal that is directly induced by the MALT1 scaffolding activity. Although MALT1 deficiency is known to impair mast cell responsiveness, MALT1 proteolytic activity in mast cells has not yet

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Abbreviations used in this article: Bcl10, B cell lymphoma 10; BMDC, bone marrow–derived mast cell; CBM, CARMA3–Bcl1–MALT1; CST, Cell Signaling Technology; DNP-HSA, DNP-Albumin; ECIS, electric cell–substrate impedance sensing; GPCR, G protein–coupled receptor; HDMVEC, human dermal microvascular endothelial cell; H1R, histamine receptor H1; IKK, I κ B kinase; IKK-VI, IKK-2 inhibitor VI; PAR1, protease activated receptor 1; PCA, passive cutaneous anaphylaxis; siRNA, small interfering RNA; SNV, single nucleotide variant.

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been evaluated. In this study, we show that IgE-mediated mast cell activation induces MALT1 protease-dependent cleavage of RelB and that loss of MALT1 protease activity abrogates IgE-driven cytokine production.

Klemm et al. (9) demonstrated that MALT1 is not required for mast cell degranulation and subsequent histamine release, which, in contrast to cytokine production, represents a more acute component of the allergic response. The effects of histamine and other preformed vasoactive mediators released from mast cells during an allergic reaction on target tissues that include the microvasculature can be dramatic and even life threatening (14–16). We recently demonstrated that MALT1 proteolytic activity plays a critical role in triggering acute endothelial barrier disruption in response to thrombin treatment (17). Specifically, in vascular endothelial cells, thrombin serves as an agonist for protease activated receptor 1 (PAR1), a G protein-coupled receptor (GPCR). Stimulation of PAR1 by thrombin triggers the formation and activation of a signaling complex composed of the CARMA3 scaffolding protein along with Bcl10 and MALT1 (18). Active MALT1 protease then cleaves CYLD, leading to microtubule disruption and a cascade of events culminating in an acute permeability response. Preliminary data from our laboratory suggested that MALT1 protease activity in endothelial cells might also play a role in mediating the effects of other GPCRs similar to PAR1, including histamine receptor H1 (HR1; also known as HRH1). In this study, we now demonstrate that MALT1 protease activity is required for histamine-induced endothelial barrier disruption, both in vitro and in vivo.

Based on these findings, we propose a dual role for the MALT1 protease in allergic response. First, in the initiator mast cell, MALT1 protease activity promotes NF- κ B-dependent cytokine expression and release, thus contributing to late-phase hypersensitivity reactions. Second, in an end-target organ, the vascular endothelium, MALT1 protease activity promotes the loss of endothelial barrier integrity in response to histamine, leading to acute phase endothelial dysfunction and increased vascular permeability. As the MALT1 protease is a druggable target, our findings suggest that pharmaceutical targeting of MALT proteolytic activity could be broadly beneficial in the treatment of allergic disease by inhibiting both chronic mast cell cytokine secretion and acute endothelial cell permeability.

Materials and Methods

Mice

MALT1^{PD/PD} mice were generated with the assistance of genOway and have been recently described (19). Briefly, the mouse MALT1 locus was modified by homologous recombination in C57BL/6-derived embryonic stem cells to introduce a C472A alteration (TGT > GCC) in exon 12, resulting in knock-in of a protease-dead allele. Additionally, exon 12 is flanked by loxP sites. MALT1^{-/-} mice were generated by crossing mice with a C57BL/6-Cre deleter line to excise exon 12. This results in a premature STOP codon in exon 13 and induces nonsense-mediated mRNA decay, leading to loss of MALT1 protein expression. Heterozygous mice were interbred, and progeny were born at the expected Mendelian ratio. Mice were maintained under specific pathogen-free conditions. All animal work conducted at genOway was ethically reviewed and carried out in accordance with European Directive 2010/63/EU, and all studies carried out at the University of Pittsburgh were reviewed and approved by the Institutional Animal Care and Use Committee (protocol number 15096981).

Cell lines, Abs, and reagents

The cells used are described in this section. Pooled, primary human dermal microvascular endothelial cells (HDMVEC) were obtained from Lonza, cultured in Vasculife EnGS-Mv media (Lifeline Cell Technology) on 0.2% gelatin-coated plates and used for no more than eight passages. SVEC (SVEC4-10) cells were obtained from American Type Culture Collection and cultured in DMEM supplemented with 10% FBS.

Abs. The following Abs were used: phospho-I κ B α (Ser32/36, 9246; Cell Signaling Technology [CST]), RelB (C1E4, 4922; CST), GAPDH (D16H11, 5174; CST), CYLD (E-10, SC-74435; Santa Cruz Biotechnology), MALT1 (2494; CST), phospho-SAPK/JNK (Thr183/Tyr185, 9251; CST), SAPK/JNK (9252; CST), phospho-p44/42 MAPK (ERK1/2, Thr202/Tyr204, 9101; CST), p44/42 MAPK (ERK1/2, 9102; CST), phospho-AKT (Ser473, 9271; CST), and AKT (pan, C67E7, 4691; CST).

Reagents. The following reagents were used: histamine (H7125; Sigma-Aldrich), z-VRPR-fmk (ALX-260-166; Enzo Life Sciences), Evans blue dye (E2129; Sigma-Aldrich), Monoclonal Anti-DNP Ab produced in mouse IgE isotype (clone SPE-7, D8406; Sigma-Aldrich), DNP-Albumin (DNP-HSA) (A6661; Sigma-Aldrich), Recombinant Murine IL-3 (213-13; PeproTech), Fc ϵ RI α mAb, FITC conjugate (clone MAR-1, A18400; Thermo Fisher Scientific), allophycocyanin-Rat Anti-Mouse CD117 (clone 2B8, 553356; BD Pharmingen), 4-Nitrophenyl *N*-acetyl- β -D-glucosaminide (N9376; Sigma-Aldrich), mepazine (5.00500.0001; EMD), and IKK-2 inhibitor VI (IKK-VI) (17276; Cayman Chemical).

Bone marrow-derived mast cell culture and FACS analysis

Mast cells were derived from bone marrow cells as previously described (20, 21). Briefly, bone marrow cells from wild-type (MALT1^{WT/WT}), MALT1^{PD/PD}, or MALT1^{-/-} mice were isolated and cultured in RPMI 1640 media containing 1% penicillin/streptomycin, 10% FBS, 25 mM HEPES, 1 mM sodium pyruvate, 1 $\times$ nonessential amino acids, 50 μ M 2-ME, and 30 ng/ml recombinant murine IL-3 (PeproTech) for 4–6 wk with medium replaced every 3–4 d. To characterize the resulting population for purity, cells were incubated with FITC-conjugated anti-Fc ϵ RI and allophycocyanin-conjugated anti-CD117 (c-Kit), and expression levels were measured by flow cytometry. CD117 and Fc ϵ RI double-labeling was used to gate on mast cell populations. Cells were analyzed on an LSR II (BD Biosciences) flow cytometer and analyzed using BD FACSDiva.

Mast cell degranulation

To induce degranulation, 5 $\times$ 10⁵ bone marrow-derived mast cells (BMMCs)/ml were loaded with 100 ng/ml anti-DNP IgE mAb overnight in cytokine-free culture medium. Following sensitization, cells were washed and resuspended in HEPES buffer (10 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 0.4 mM sodium phosphate dibasic, 5.6 mM glucose, 1.8 mM CaCl₂·2H₂O, 1.3 mM MgSO₄·7H₂O, 0.04% BSA). Cells were then stimulated with the concentrations of DNP-HSA indicated in the figures. The enzymatic activity of β -hexosaminidase in the supernatant and cell lysates solubilized with 0.1% Triton X-100 were measured with 4-Nitrophenyl *N*-acetyl- β -D-glucosaminide, and the percentage of degranulation was calculated as previously described (21, 22).

Measurement of cytokines

A total of 1 $\times$ 10⁶ BMMCs/ml were loaded with 100 ng/ml anti-DNP IgE mAb overnight (~18 h) in cytokine-free culture medium. Following sensitization, cells were washed, then stimulated with 20 ng/ml DNP-HSA as indicated in the figures. Cell supernatants were collected, and IL-6 and TNF- α were determined by ELISA MAX Deluxe Sets (BioLegend) per manufacturer's instructions.

RT-PCR

A total of 2 $\times$ 10⁶ BMMCs/ml were loaded with 100 ng/ml anti-DNP IgE mAb overnight in cytokine-free culture medium. Following sensitization, cells were washed and then stimulated with 20 ng/ml DNP-HSA for 60 min. Total RNA was isolated by RNeasy Mini Kit (QIAGEN) following manufacturer's directions. RNA was reverse-transcribed using an ABI High-Capacity cDNA Reverse Transcription Kit, and PCR was performed using TaqMan probes for IL-6 (Mm00446190), TNF- α (Mm00443258), and GAPDH (Mm99999915) (Thermo Fisher Scientific).

Passive cutaneous anaphylaxis

For immediate-phase passive cutaneous anaphylaxis (PCA), MALT1^{PD/PD}, MALT1^{-/-}, and litter-matched wild-type mice, age 5–7 wk old, were passively sensitized by intradermal injection of 250 ng anti-DNP IgE mAb in 20 μ l of PBS into the left ear and shaved left flank. The contralateral ear and flank were injected with 20 μ l of vehicle (PBS) and served as control. Twenty-four hours later, mice were challenged with i.v. injection of 150 μ g of DNP-HSA in 100 μ l of 1% Evans blue dye. Mice were euthanized 60 min after challenge. Ear biopsy specimens and ~1 cm² of flank skin containing each injection site were collected. Extravasated Evans blue dye was extracted from excised tissue with 700 μ l of formamide incubated at 55°C overnight and then the OD of the supernatant was quantified by

spectrophotometry at 620 nm. Data were expressed as the difference in the amount of Evans blue extravasation (micrograms) per microgram of tissue in IgE-injected versus control-injected tissue from the same mouse.

Eight-to-twelve-week-old female $MALT1^{WT/WT}$, $MALT1^{PD/PD}$, or $MALT1^{-/-}$ mice were used for late-phase PCA analysis. On day 1, ear thickness measurements were performed using a Mitutoyo PK-0505 CPX (700-118-20; Transcat) Digital Mini Thickness Gage prior to intradermally injecting 50 μ l of 20 ng anti-DNP IgE in each mouse ear. Twenty-four hours later, mice were injected via tail vein with 50 μ l of 2 mg/ml DNP-HSA. Ear thickness measurements were performed prior to ear anti-DNP IgE injections and tail vein DNP injection and 2, 6, and 24 h after HSA injection. Data are expressed as the percentage change relative to baseline: [(postchallenge measurement – prechallenge baseline measurement)/baseline] $\times$ 100.

Miles assay: *in vivo* permeability assay

$MALT1^{PD/PD}$ or litter-matched wild-type mice (6–8 wk old) were anesthetized, and 100 μ l of 1% Evans blue dye in PBS was injected into the external jugular vein. After 1 min, various doses of histamine in 20 μ l PBS were injected s.c. into the shaved left flank. PBS was injected as control into the contralateral flank. After 10 min, mice were euthanized, and $\sim$ 1 cm² of skin containing each injection site was removed (23). Extravascular Evans blue dye was extracted from excised tissue using 500 μ l of formamide. Tissue was incubated at 55°C for 48 h and OD of the supernatant was determined by spectrophotometry at 620 nm (24). Data were expressed as the ratio of the amount of Evans blue extravasation promoted by histamine versus control injection (PBS) in the same mouse.

Cell activation and Western blotting

For mast cell experiments, $\sim$ 3 $\times$ 10⁶ BMBCs/ml were loaded with 100 ng/ml anti-DNP IgE mAb overnight in cytokine-free culture medium. Following sensitization, cells were washed and then stimulated with 20 ng/ml DNP-HSA, as indicated in figures. Cells were collected, placed on ice, and resuspended in ice-cold stop buffer (10 mM Tris–HCl [pH 7.4], 10 mM EDTA, 5 mM EGTA, 0.1 M NaF, 0.2 M sucrose, 100 μ M Na-orthovanadate, and 5 mM pyrophosphate) with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fisher Scientific) added fresh. For HDMVEC experiments, cells were grown in monolayer to confluence in gelatin-coated six-well plates. For activation, HDMVECs were treated with histamine as indicated in figures. When indicated, 50 μ M z-VRPR-fmk was added 4 h before agonist, or 1 μ M mepazine or 5 μ M IKK-VI was added to cells 1 h before agonist. Both BMBCs and HDMVECs were lysed in RIPA buffer containing Halt Protease and Phosphate Inhibitor Cocktail (Thermo Fisher Scientific). Proteins were resolved by SDS-PAGE using Bio-Rad Criterion TGX 4–12% gels or 8% gels for RelB and transferred to PVDF Immobilon Membrane (EMD Millipore) followed by blocking in 5% nonfat dry milk or BSA in TBS–Tween for 60 min at room temperature. Primary Abs were diluted to the recommended concentrations and incubated with membranes for 1 h at room temperature or overnight at 4°C. Secondary Abs were added for 1 h at room temperature before immuno-reactive proteins were visualized using ECL Reagent (Thermo Fisher Scientific).

Cell transfection

A total of 2.5 $\times$ 10⁵ SVEC cells per well of a six-well plate were reverse transfected using 30 pmol (ON-TARGETplus Mouse Malt1 [240354] small interfering RNA [siRNA]–SMARTpool or ON-TARGETplus Nontargeting Control Pool [L-051221-00-0005 and D-001810-10-05]; Dharmacon, respectively) with 6 μ l of RNAiMAX (13778150; Life Technologies) in Optimum and antibiotic free complete media. After 72 h, transfected cells were trypsinized, counted, and 40,000 cells per electric cell-substrate impedance sensing (ECIS) slide well were plated, and the remaining cells were lysed in RIPA buffer for MALT1 (2494s; CST) protein analysis by Western blot.

ECIS

Endothelial cell permeability was measured using an ECIS Z Theta instrument (Applied BioPhysics), with a 16-well array station. Eight-well chamber slides (8W10E+) were preincubated with 10 mM L-cysteine, rinsed, and incubated with 0.2% gelatin. Forty thousand HDMVEC or SVEC cells per well were plated and allowed to form a confluent monolayer overnight. On the next day, complete media was changed to serum-free media, and cells were monitored for resistance at 4000 Hz every 15 s for 4 h until a stable level of resistance was achieved. Cells were treated with inhibitors, as indicated, at the time of serum starvation and treated

with 5 μ M histamine $\sim$ 4 h into data collection. Resistance curves for individual representative experiments are presented along with data illustrating the maximal percentage decrease in resistance compiled from multiple independent experiments.

Statistical analysis

When experiments involved only two conditions, differences in means were evaluated for statistical significance using a one- or two-tailed, unpaired Student *t* test, as appropriate. For datasets involving multiple treatments and a control, data were analyzed using one-way ANOVA. In the latter case, significant differences between treatments were assessed using a Tukey test, whereas Sidak correction was used to account for multiple testing. All statistical analyses were performed using GraphPad Prism, Version 6.0 (GraphPad Software). Data are presented as mean $\pm$ SEM or as percent of control, and *p* values are shown in the figures and the figure legends.

Results

Mast cell growth and development is not affected by genetic inactivation of the MALT1 protease domain or by complete MALT1 gene disruption

To begin our evaluation of MALT1 proteolytic activity in IgE-mediated allergic response, we first assessed the impact of genetically disrupting MALT1 proteolytic activity on mast cell growth and development *in vitro*. We prepared bone marrow cell suspensions from wild-type mice ($MALT1^{WT/WT}$), from mice harboring a point mutation in the endogenous MALT1 allele that renders MALT1 catalytically inactive ($MALT1^{PD/PD}$), and from MALT1 knockout mice ($MALT1^{-/-}$). Mast cells from all three genotypes proliferated equally well *in vitro* in media containing IL-3 (Fig. 1A). By week 4–5, all three genotypes produced highly pure BMBC populations (hereafter referred to as mast cells) as evidenced by flow cytometric analysis of c-kit and Fc ϵ RI surface expression (Fig. 1B). Consistent with previous reports (9), the absence of MALT1 ($MALT1^{-/-}$) does not affect the expression of Fc ϵ RI receptors. We find that specific disruption of MALT1 proteolytic activity ($MALT1^{PD/PD}$) also does not influence Fc ϵ RI expression. Western blot analysis demonstrated that mast cells from $MALT1^{WT/WT}$ and $MALT1^{PD/PD}$ mice express MALT1 protein at equivalent levels, whereas mast cells from $MALT1^{-/-}$ mice show no detectable MALT1 protein (Fig. 1C). Expression of MALT1 in the mast cells from $MALT1^{PD/PD}$ mice was also confirmed by quantitative RT-PCR (Fig. 1D). Collectively, our data demonstrate that mast cell proliferation and differentiation are not impaired by genetic inactivation of the MALT1 protease domain.

Mast cell stimulation induces MALT1 proteolytic activity

MALT1 proteolytic activity has been demonstrated in multiple lymphocyte subtypes (10–13, 19, 25–28) but has not yet been evaluated in mast cells. Although MALT1 proteolytic activity is not required for IKK activation in lymphocytes, it is believed to play a role in fine-tuning the level of NF- κ B activation achieved after AgR stimulation by cleaving specific substrates that regulate downstream NF- κ B signaling. To evaluate whether MALT1 proteolytic activity is similarly triggered in mast cells in response to Fc ϵ RI stimulation, we sensitized mast cells from $MALT1^{WT/WT}$, $MALT1^{PD/PD}$, and $MALT1^{-/-}$ mice with anti-DNP IgE, stimulated them with DNP-HSA, and then assessed cleavage of the MALT1 substrate RelB. We found that $MALT1^{WT/WT}$ mast cells demonstrate inducible cleavage of RelB in response to Fc ϵ RI stimulation (Fig. 2A). This cleavage of RelB is absent in $MALT1^{PD/PD}$ mast cells, consistent with the fact that the C472A point mutation renders MALT1 catalytically inactive (Fig. 2A). As expected, cleavage of RelB is also absent in $MALT1^{-/-}$ mast cells. To our knowledge, together, these findings demonstrate for the first

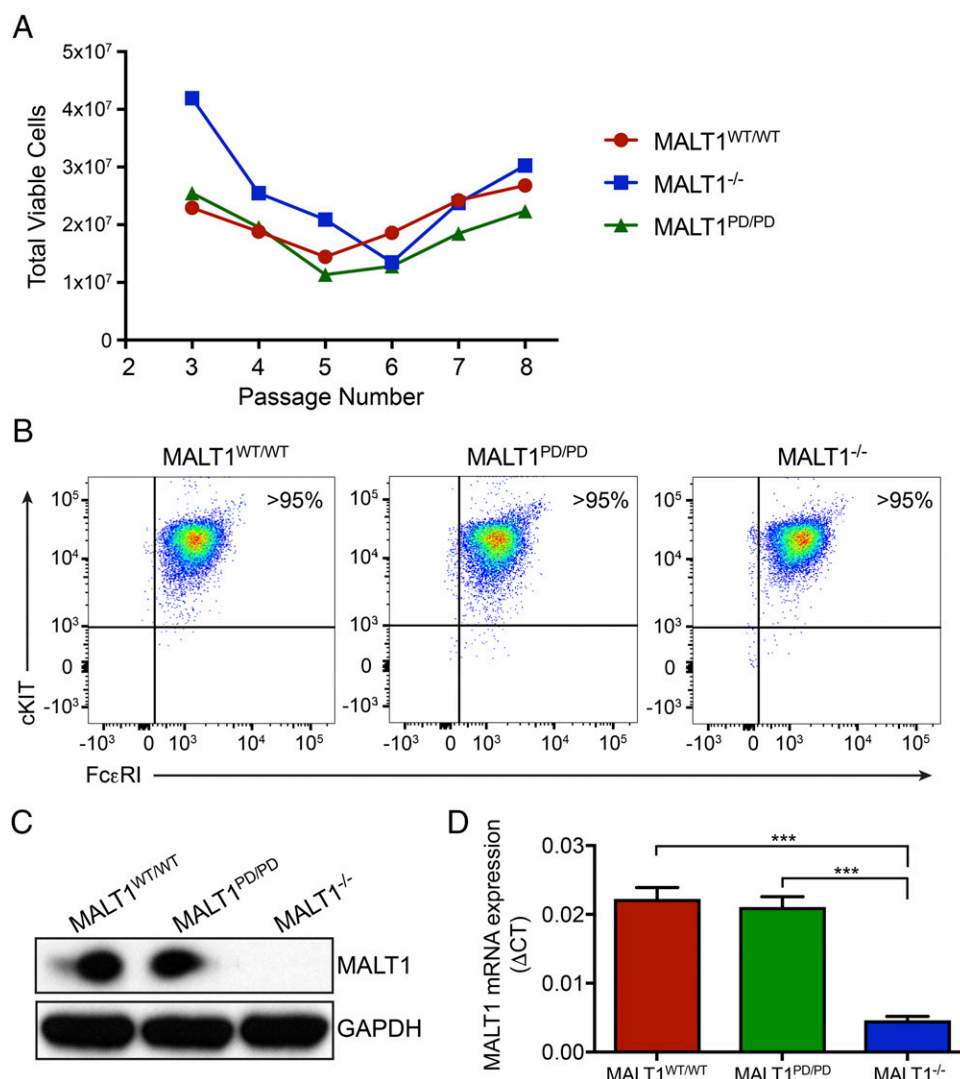


FIGURE 1. Mast cell growth and development is not affected by the absence of MALT1 protease activity. **(A)** Average number of viable cells from bone marrow isolation from MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mice. Cells were first counted at passage 3, ~2 wk postisolation. Cells differentiate, proliferate, and mature in media containing IL-3. **(B)** Flow cytometric analysis of mast cells from MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mice. Mast cells were analyzed by flow cytometry at passage 7–8, ~4 wk postisolation and were used for experiments around passage 8–11. **(C)** Western blot of MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mast cells for MALT1 protein expression. **(D)** Quantitative RT-PCR for MALT1 gene expression levels. All results are representative of three independent experiments. ****p* < 0.001.

time that MALT1 proteolytic activity is stimulated in mast cells upon FcεRI activation.

MALT1 proteolytic activity is not required for FcεRI-dependent IKK, ERK, JNK, or AKT activation, nor is it required for mast cell degranulation

In addition to activating canonical NF-κB signaling, FcεRI stimulation in mast cells results in activation of MAPK (JNK, ERK) and AKT pathways, which together contribute to both cytokine production and generation of arachidonic acid metabolites (3). Previous studies had suggested that MALT1 is not required for FcεRI-dependent ERK, JNK, or AKT activation in mast cells (9). This differs significantly from the situation in lymphocytes in which the absence of MALT1 results in loss of AgR-induced JNK activation (25–27, 29, 30). To specifically assess how disabling the MALT1 protease domain, without disrupting MALT1 scaffolding activity, might impact MAPK and AKT signaling in response to FcεRI stimulation in mast cells, we compared downstream signaling in mast cells from MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mice that were sensitized overnight with Ag-specific

IgE and activated by FcεRI cross-linking. We found that phosphorylation of IκBα, indicative of IKK activation, is rapidly induced in both wild-type and MALT1^{PD/PD} mast cells after FcεRI stimulation, but is absent in MALT1^{-/-} mast cells (Fig. 2B). These findings indicate that FcεRI-induced IKK activation is maintained in cells harboring protease-dead MALT1, presumably because of preservation of MALT1 scaffolding activity, and are consistent with the known role of MALT1 proteolytic activity in lymphocyte AgR-dependent signaling. Next, we examined the ERK, JNK, and AKT pathways. Using immunoblot analysis with specific Abs to phosphorylated ERK1/2, JNK, and AKT, we found that there is no difference in FcεRI-mediated activation of these pathways in MALT1^{PD/PD} and MALT1^{-/-} mast cells as compared with wild-type cells (Fig. 2B).

Next, we evaluated the contribution of MALT1 proteolytic activity to mast cell degranulation. MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mast cells were sensitized with Ag-specific IgE and subsequently stimulated with increasing doses of Ag to induce FcεRI-dependent degranulation. We found that mast cells from

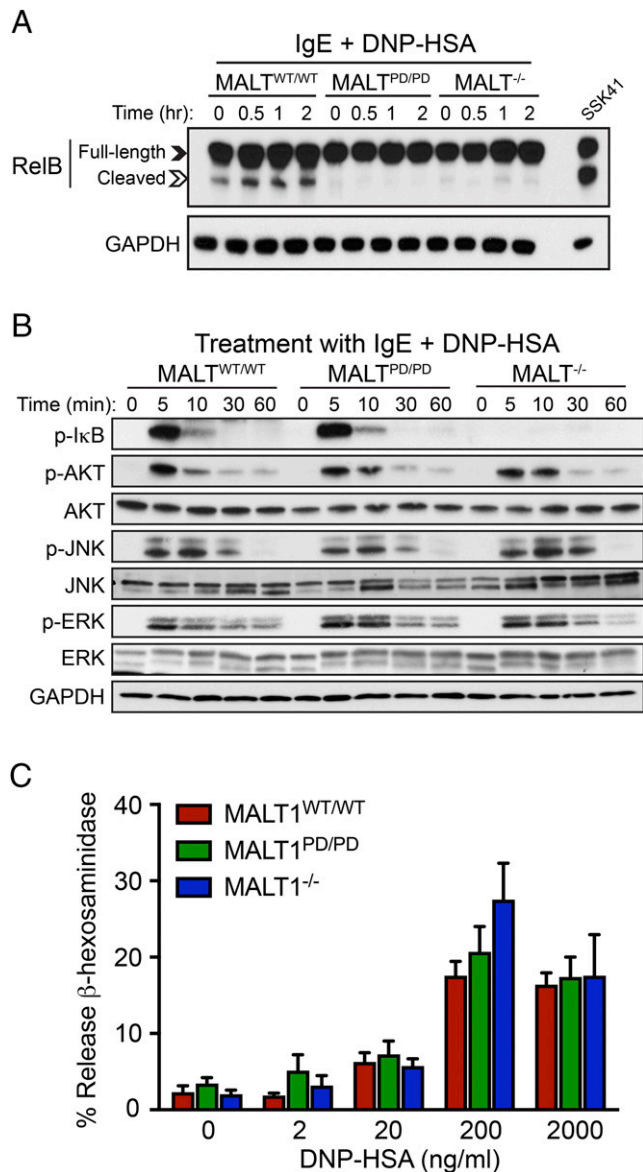


FIGURE 2. MALT1 proteolytic activity is present in mast cells but is not required for activation of specific signaling kinases or for acute mast cell degranulation. **(A)** MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mast cells were sensitized with anti-DNP IgE and then stimulated with DNP-HSA for the indicated time intervals. Experiments were performed in the presence of proteasome inhibitor MG-132, added 30 min prior to treatment. RelB was detected by Western blotting. Solid arrow indicates full-length RelB, open arrow indicates proteolytically cleaved RelB. Data are representative of three independent experiments. Lysates from SSK41 lymphoma cells, which harbor *MALT1* gene amplification and constitutive MALT1 proteolytic activity, are shown as a control for RelB cleavage. **(B)** Mast cells from MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mice were sensitized with anti-DNP IgE and stimulated with DNP-HSA for the indicated time intervals. Levels of phosphorylated and total I κ B α , AKT, JNK, and ERK1/2 were determined by Western blotting, with GAPDH serving as a loading control. Data are representative of three independent experiments. **(C)** Mast cells from MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mice were sensitized with anti-DNP IgE and then stimulated for 30 min with the indicated concentrations of DNP-HSA. As described in *Materials and Methods*, degranulation was determined by measuring the activity of β -hexosaminidase (a granule enzyme) released by mast cells. Data are displayed as mean $\pm$ SEM for triplicate samples and are representative of four independent experiments.

MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mice all release similar amounts of β -hexosaminidase, a granule component, indicating that neither MALT1 scaffolding nor proteolytic activity is required for degranulation (Fig. 2C). This finding is consistent with previous studies showing that MALT1 is not essential for degranulation (9).

Taken together, the above findings indicate that MALT1 protease activity is dispensable for a range of acute mast cell responses that include Fc ϵ RI-dependent IKK, ERK, JNK, and AKT activation as well as degranulation. MALT1 scaffolding activity, however, is critical for IKK activation.

MALT1 proteolytic activity is required for optimal Fc ϵ RI-dependent cytokine induction

In addition to stimulating degranulation, Fc ϵ RI activation promotes cytokine production in mast cells, which plays a key role in the chronic phase of allergic reactions. We therefore evaluated the contribution of MALT1 proteolytic activity to this second major function of mast cells. Specifically, we assessed the impact of MALT1 protease activity on expression of *IL6* and *TNF* because upregulation of these genes has been closely linked to NF- κ B activation in mast cells (31–36) and was previously shown to be reduced in MALT1^{-/-} mast cells (9). First, we compared the induction of *IL6* and *TNF* mRNAs in MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mast cells before and after Fc ϵ RI cross-linking using quantitative RT-PCR. In wild-type mast cells, both *IL6* and *TNF* were rapidly induced upon stimulation (Fig. 3A, 3B). Induction was significantly blunted in MALT1^{-/-} mast cells, consistent with previous studies. Interestingly, in MALT1^{PD/PD} mast cells, induction of both *IL6* and *TNF* was similarly dampened (Fig. 3A, 3B). As a control for specificity, we analyzed additional Fc ϵ RI-responsive mast cell gene products and identified several cytokines or chemokines that were not dependent on MALT1 protease activity for their induction (for example, *IL4*, *CCL5*, and *IL5*) (Supplemental Fig. 1). We speculate that expression of these factors may rely to a greater extent on MAPK, AKT, or other signaling pathways that are not regulated by MALT1 (see Fig. 2B). Finally, we compared the concentrations of IL-6 and TNF- α proteins in the supernatants of stimulated MALT1^{WT/WT}, MALT1^{PD/PD}, and MALT1^{-/-} mast cells by ELISA. Wild-type mast cells secreted IL-6 and TNF- α readily, with peak levels appearing in media around 2 h poststimulation, whereas MALT1^{PD/PD} and MALT1^{-/-} mast cells were impaired to a similar degree in the secretion of both cytokines (Fig. 3C, 3D). Taken together, our data indicate that MALT1 proteolytic activity is required for maximal Fc ϵ RI-mediated IL-6 and TNF- α cytokine expression and secretion.

MALT1 protease activity augments anaphylactic response in vivo and triggers histamine-induced vascular leakage

Fc ϵ RI is necessary for the initiation of IgE-dependent PCA (37). To evaluate the role of MALT1 protease activity in allergic response in vivo, we first performed classical Fc ϵ RI-mediated PCA experiments. Late-phase PCA response is mediated by mast cell-derived proinflammatory cytokines, particularly TNF- α (38, 39). Mice were passively sensitized by intradermal injection of anti-DNP IgE into each ear. Mice were then challenged 24 h later by i.v. injection of Ag, and ear thickness was monitored over the next 24 h (Fig. 4A). Wild-type mice demonstrated an anticipated edema response as early as 2 h postchallenge that persisted over the observed time period of 24 h. In contrast, both MALT1^{PD/PD} and MALT1^{-/-} mice showed only a minor response. Based on these findings, we conclude that MALT1 proteolytic activity is required for IgE-dependent late-phase PCA reactions in vivo. Taken together with our finding that in stimulated MALT1^{PD/PD}

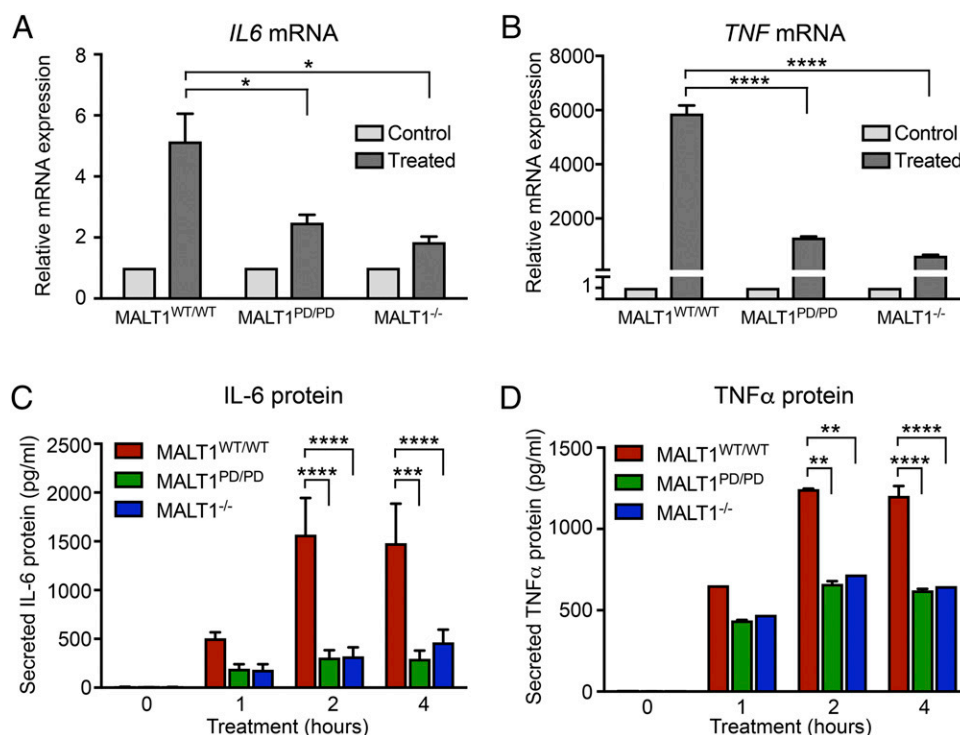


FIGURE 3. MALT1 proteolytic activity is required for optimal FcεRI-mediated cytokine induction. **(A and B)** Mast cells were sensitized with anti-DNP IgE and stimulated with DNP-HSA for 1 h. RNA was isolated, and the expression of *IL6* and *TNF* was detected by quantitative RT-PCR. Data are displayed as fold induction relative to control for each strain and represent the mean induction $\pm$ SEM for triplicate samples. Results are representative of at least three independent experiments. **(C and D)** Mast cells were sensitized and stimulated as above for the indicated time intervals. Supernatants were collected, and IL-6 and TNF- α protein concentrations were determined by ELISA. Data represent the mean $\pm$ SEM for at least three independent experiments. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

BMMCs, TNF- α and IL-6 production and release are impaired (Fig. 3), it seems likely that the defective PCA response we observed in vivo in MALT1^{PD/PD} mice reflects the requirement for MALT1 proteolytic signaling in FcεRI-mediated cytokine production.

Next, we examined the immediate-phase PCA response in MALT1^{PD/PD} mice. Again, mice were passively sensitized by intradermal injection of anti-DNP IgE into the ear and the ipsilateral flank. Mice were then challenged 24 h later by i.v. coinjection of Ag (DNP-HSA) and Evans blue dye. Extravasated Evans blue dye was monitored visually (Fig. 4B) and was quantified 60 min after Ag exposure (Fig. 4C, 4D). MALT1^{PD/PD} and MALT1^{-/-} mice showed an equally dampened response to Ag challenge with an ~2–3-fold decrease in the amount of Evans blue dye extravasated at the ear compared with what was observed with wild-type mice (Fig. 4C). MALT1^{PD/PD} mice also showed a significantly dampened response at the flank as compared with wild-type mice, with a trend ($p = 0.054$) toward a decreased response in MALT1^{-/-} mice as well (Fig. 4D).

Extravasation of Evans blue dye during the first hour of the PCA reaction is known to be dependent on degranulation of activated mast cells with rapid release of histamine and other mediators that trigger an increase in local vascular permeability (40, 41). Intriguingly, our in vitro studies had demonstrated that neither complete deficiency of MALT1 nor genetic absence of MALT1 proteolytic activity affects degranulation in FcεRI-stimulated mast cells (Fig. 2C), suggesting that the decrease in dye extravasation that we observe in MALT1^{-/-} or MALT1^{PD/PD} mice during the PCA reaction is not because of defective mast cell degranulation. Notably, our MALT1^{-/-} and MALT1^{PD/PD} mice harbor global genetic modifications such that all cells, including mast cells and endothelial cells, lack MALT1 or have absent MALT1 proteolytic

activity, respectively. We therefore hypothesized that in the MALT1^{-/-} and MALT1^{PD/PD} mice, the observed decrease in dye extravasation is due to a decrease in the endothelial permeability response to mast cell granule contents, such as histamine, and that MALT1 protease activity within the vascular endothelium is required for the immediate-phase response. Indeed, our previously published work demonstrated that MALT1 protease activity plays a critical role in endothelial cells by mediating acute vascular permeability in response to thrombin (17).

To test our hypothesis, we performed an in vivo permeability assay in which histamine is directly injected into the mice, thus bypassing the requirement for mast cell activation and degranulation. This well-established in vivo technique is referred to as the Miles Assay (24). Mice were injected i.v. with Evans blue dye, then subsequently intradermally injected with histamine in the flank. Ten minutes later, the extravasation of Evans blue dye was quantified at the site of histamine injection and normalized to the amount that extravasated in response to a control (PBS) injection in the opposite flank (Fig. 4E, 4F). Extravasation of Evans blue dye in response to 50 ng/ μ l histamine was significantly less in MALT1^{PD/PD} mice as compared with wild-type mice, consistent with the notion that MALT1 proteolytic activity within the endothelial cells is required for a maximal histamine-induced vascular permeability response. These findings are also consistent with the notion that the decreased permeability response we observed in MALT1^{PD/PD} mice during the immediate-phase PCA reaction (Fig. 4B–D) is due to a lack of MALT1 protease activation in the vascular endothelium in response to mast cell histamine release.

We next sought to directly evaluate the contribution of MALT1 protease activity to histamine-induced endothelial permeability. First, we tested for evidence of histamine-induced MALT1

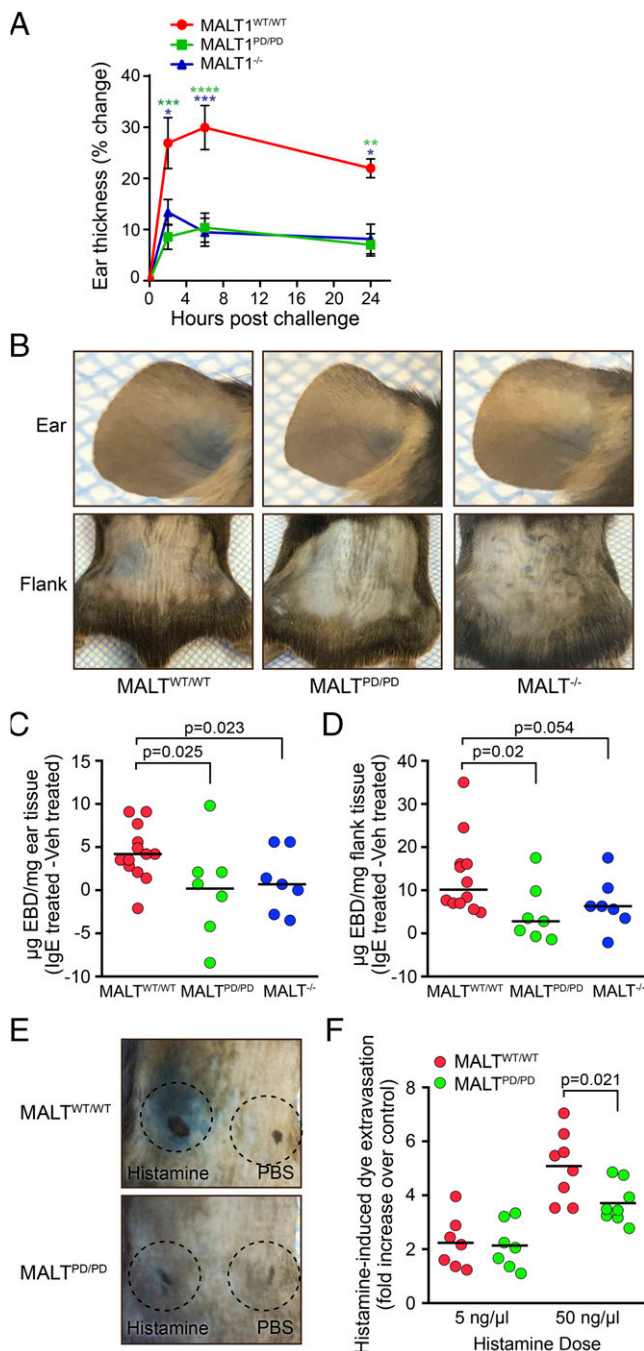


FIGURE 4. MALT1^{PD/DP} mice are resistant to late- and immediate-phase PCA and to histamine-induced vascular leakage. **(A)** Mice were passively sensitized by intradermal injection of anti-DNP IgE into both ears. Twenty-four hours later, mice were challenged by i.v. injection of Ag. Ear thickness was measured over time, as described in *Materials and Methods* ($n = 11$, 7 , and 5 MALT1^{WT/WT}, MALT1^{PD/DP}, and MALT1^{-/-} mice, respectively). **(B–D)** Mice were passively sensitized by intradermal injection of anti-DNP IgE into the left ear and left flank. The contralateral ear and flank were injected with PBS. Subsequently, mice were challenged by i.v. injection of Ag in PBS/Evans blue dye. Representative ears and flank from MALT1^{WT/WT}, MALT1^{PD/DP}, and MALT1^{-/-} mice 60 min after challenge are shown in **(B)**. The IgE-induced Evans blue extravasation was calculated as the difference in amount extravasated in the IgE-sensitized tissue and the corresponding nonsensitized tissue **(C and D)** ($n = 7–13$ for each strain). **(E and F)** MALT1^{WT/WT} and MALT1^{PD/DP} mice were injected i.v. with PBS/Evans blue dye and then subsequently injected intradermally with histamine on the left flank and vehicle (PBS) on the right flank. After 10 min, a 1 cm² area of skin/dermis surrounding each injection site was collected. Representative images of Evans blue dye extravasation

proteolytic activity in endothelial cells using pooled primary HDMVECs, which have been shown to express histamine receptors, particularly H1R, which is known to mediate a rapid permeability response (42, 43). We found that histamine treatment of HDMVECs leads to time-dependent accumulation of a RelB cleavage fragment (Fig. 5A), and pretreatment with either a cell permeable, irreversible MALT1 protease inhibitor, z-VRPR-fmk (11) (Fig. 5B) or a reversible MALT1 protease inhibitor, mepazine (44) (Supplemental Fig. 2A), abrogates RelB cleavage in these cells. z-VRPR-fmk is a peptide-based inhibitor derived from the optimal tetrapeptide substrate of the metacaspase AtmC9 (45) and has been used extensively to explore the role of MALT1 protease activity in vitro (46). The phenothiazine derivative mepazine was formerly investigated as an antipsychotic and tranquilizing agent and has more recently been found to inhibit MALT1 protease. Specifically, upon binding to MALT1, mepazine prevents rearrangement of inactive MALT1 into a proteolytically active conformation (47). As expected, inhibiting canonical NF- κ B activation with IKK-VI, a potent inhibitor of the IKK complex, prevents phosphorylation of I κ B α (Supplemental Fig. 2B). In contrast, inhibition of MALT1 protease activity does not prevent histamine-induced phosphorylation of I κ B α (Fig. 5C, 5D, Supplemental Fig. 2B), presumably because MALT1 scaffolding activity remains intact.

Next, we performed ECIS, a technique whereby changes in electrical resistance are measured in real time, across confluent monolayers of endothelial cells grown on gold-plated electrodes (43) (Fig. 6A). In this ECIS system, an increase in the permeability of the endothelial monolayer correlates with a decrease in electrical resistance. We confirmed that HDMVECs rapidly respond to histamine with a drop in electrical resistance (Fig. 6B). Cells pretreated with either z-VRPR-fmk or mepazine showed a significantly blunted response to histamine, suggesting that the acute permeability response induced by histamine is dependent on MALT1 proteolytic activity (Fig. 6B–E). In contrast to the effects of z-VRPR-fmk or mepazine, inhibition of canonical NF- κ B with IKK-VI had no effect on the permeability response (Fig. 6F, 6G). We also performed siRNA-mediated knockdown of MALT1 in SVECs, a mouse endothelial cell line that is amenable to siRNA transfection. As expected, based on our analysis with MALT1 protease inhibitors z-VRPR-fmk and mepazine, we found that MALT1 knockdown also significantly impaired the ability of histamine to induce an acute permeability response (Supplemental Fig. 3A, 3B). Together, our results demonstrate that histamine induces MALT1 protease activity in endothelial cells and that MALT1 proteolytic activity is required for a maximal histamine-induced endothelial permeability response (Fig. 7 depicts a proposed model).

Discussion

MALT1 plays a critical role as an intracellular signaling effector protein in many immune and nonimmune cells (5, 6). Its proteolytic activity has been demonstrated in lymphocytes and is noted in an increasing number of other cell types, including NK cells (25), dendritic cells (19, 25, 28), endothelial cells (17), and keratinocytes (7, 48). However, the role of MALT1 protease activity

in response to 50 ng/ μ l histamine are shown in **(E)**. Quantification of histamine-induced dye extravasation is shown in **(F)** and is expressed as the fold increase relative to the contralateral PBS control for each mouse ($n = 7–8$ for each strain). Injection sites were marked with black laboratory marker at time of injection. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

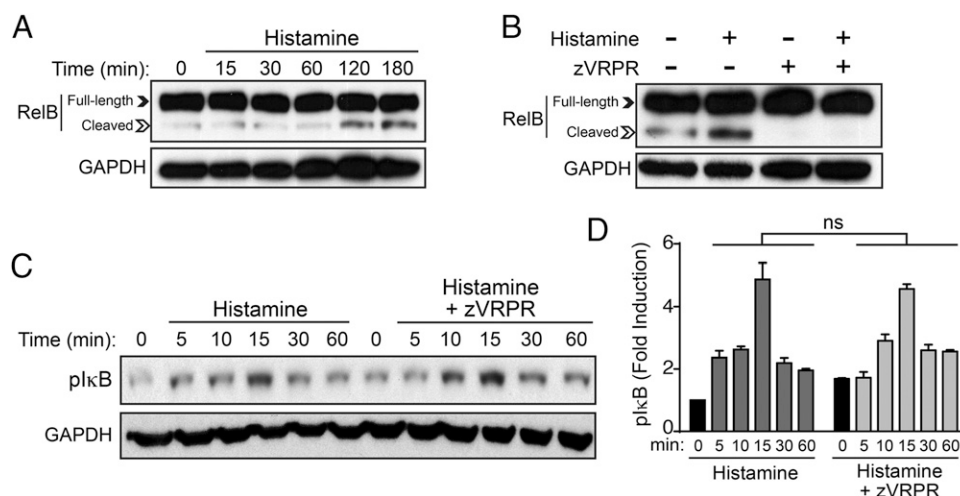


FIGURE 5. Histamine induces MALT1 proteolytic activity in endothelial cells. **(A)** HDMVECs were treated with 200 μ M histamine for the indicated time intervals. Lysates were prepared and assayed for RelB cleavage by Western blotting. **(B)** HDMVECs were pretreated with or without MALT1 inhibitor z-VRPR-fmk, followed by treatment with 200 μ M histamine for 180 min before assaying for RelB cleavage. For both **(A)** and **(B)**, solid arrows represent full-length RelB and open arrows indicate cleaved RelB fragment. **(C)** HDMVECs were treated with 200 μ M histamine for the indicated time intervals, with or without a 4-h pretreatment with z-VRPR-fmk (50 μ M). Phosphorylation of I κ B α was determined by Western blotting. **(D)** pIkB signal was quantified by densitometry, normalized to GAPDH, and expressed as fold increase compared with untreated control.

in mast cell function has not been previously evaluated. In this study, we find that stimulation of Fc ϵ RI triggers activation of the MALT1 protease, as evidenced by the cleavage of RelB, a well-known MALT1 substrate, in wild-type but not in MALT1^{PD/PD} mast cells. Furthermore, we show that MALT1 proteolytic activity is essential for Fc ϵ RI-mediated cytokine production, as measured by expression and release of IL-6 and TNF- α from mast cells. In contrast, MALT1 proteolytic activity is not required for acute Fc ϵ RI-dependent degranulation. Consistent with these *in vitro* findings, we also find that late-phase PCA reactions are severely impaired in MALT1^{PD/PD} mice. This impairment is likely because of diminished cytokine production and subsequent reduction in leukocyte infiltration. Similar to AgR-induced signaling in lymphocytes, MALT1 proteolytic activity is not required for Fc ϵ RI-dependent phosphorylation of I κ B α in mast cells. In contrast, MALT1^{-/-} lymphocytes and mast cells, which lack both MALT1 scaffolding and proteolytic activity, are completely deficient in AgR-dependent or Fc ϵ RI-dependent phosphorylation of I κ B α , respectively.

Multiple distinct substrates of the MALT1 protease have been identified in lymphocytes. In this study, we show that MALT1 proteolytically cleaves at least one of its known substrates, RelB, in mast cells. We have not yet investigated whether other known MALT1 substrates are cleaved in activated mast cells, nor have we evaluated whether cleavage of particular substrates is required for mast cells to properly respond to Fc ϵ RI stimulation. The deubiquitinase A20, another well-known MALT1 substrate, has been shown to play an important role in regulating mast cell activation (49). A20 negatively regulates NF- κ B activity in several immune cell subtypes and is critical for prevention of inflammation and autoimmunity (4). Heger et al. (49) demonstrated that in mast cells, A20 restricts NF- κ B activation downstream of IgE:Fc ϵ RI. In comparison with wild-type mast cells, A20-deficient mast cells demonstrate normal degranulation and normal phosphorylation of I κ B α , JNK, ERK, and AKT but produce significantly increased levels of the cytokine TNF- α upon activation. The authors also show that mice with A20-deficient mast cells demonstrate an increase in ear swelling during late-phase PCA as compared with control. It is tempting to speculate that elevated A20 activity may contribute to the observed suppression of cytokine production

in MALT1^{PD/PD} mast cells and the observed reduction in Fc ϵ RI-mediated PCA in MALT1^{PD/PD} mice; because the MALT1 protease is rendered inactive in and cannot cleave A20, the persistence of intact/uncleaved A20 leads to suppression of NF- κ B-dependent cytokine production and reduced PCA in comparison with MALT1^{WT/WT} controls.

Our study, to our knowledge, reveals a unique finding that both MALT1^{PD/PD} and MALT1^{-/-} mice demonstrate reduced immediate-phase IgE-mediated PCA. This is in contrast to a prior study that reported a normal immediate-phase response in MALT1-deficient mice (9). The specific reason for this discrepancy is not clear, although it is possible that the difference in genetic background between the two different MALT1^{-/-} mouse strains could be responsible (19, 29). A similar discrepancy in PCA experiments was previously seen between two groups using different lines of Bcl10^{-/-} mice (8, 9). IgE-driven PCA reactions are mediated by the rapid release of preformed mediators from mast cells, including histamine and serotonin, which then trigger increased capillary permeability (40, 41). Our current study confirms that MALT1 is not required for mast cell degranulation. Rather, we demonstrate that MALT1 proteolytic activity is required for histamine-induced endothelial permeability. Together, our findings suggest that the reduction in Evans blue dye extravasation in IgE-mediated PCA reactions in MALT1^{PD/PD} mice is not caused by reduced mast cell degranulation, but instead, is the result of a reduced permeability response of the vascular endothelium to histamine. This proposed role of MALT1 protease activity in endothelial cells during PCA is supported by our demonstration of a reduced vascular permeability response to direct histamine injection in MALT1^{PD/PD} mice and a reduced histamine-induced endothelial permeability response *in vitro* after treatment with MALT1 protease inhibitors.

MALT1 proteolytic activity can mediate a response to GPCR stimulation in endothelial cells via at least two different mechanisms. First, endothelial MALT1 promotes NF- κ B transcriptional activity in response to specific GPCRs, including the thrombin receptor (PAR1), the angiotensin II receptor (AGTR1), the IL-8 receptor (CXCR2), and the lysophosphatidic acid receptors (LPARs) (18, 50–54). The resultant NF- κ B-dependent gene reprogramming upregulates both secreted and cell surface proteins

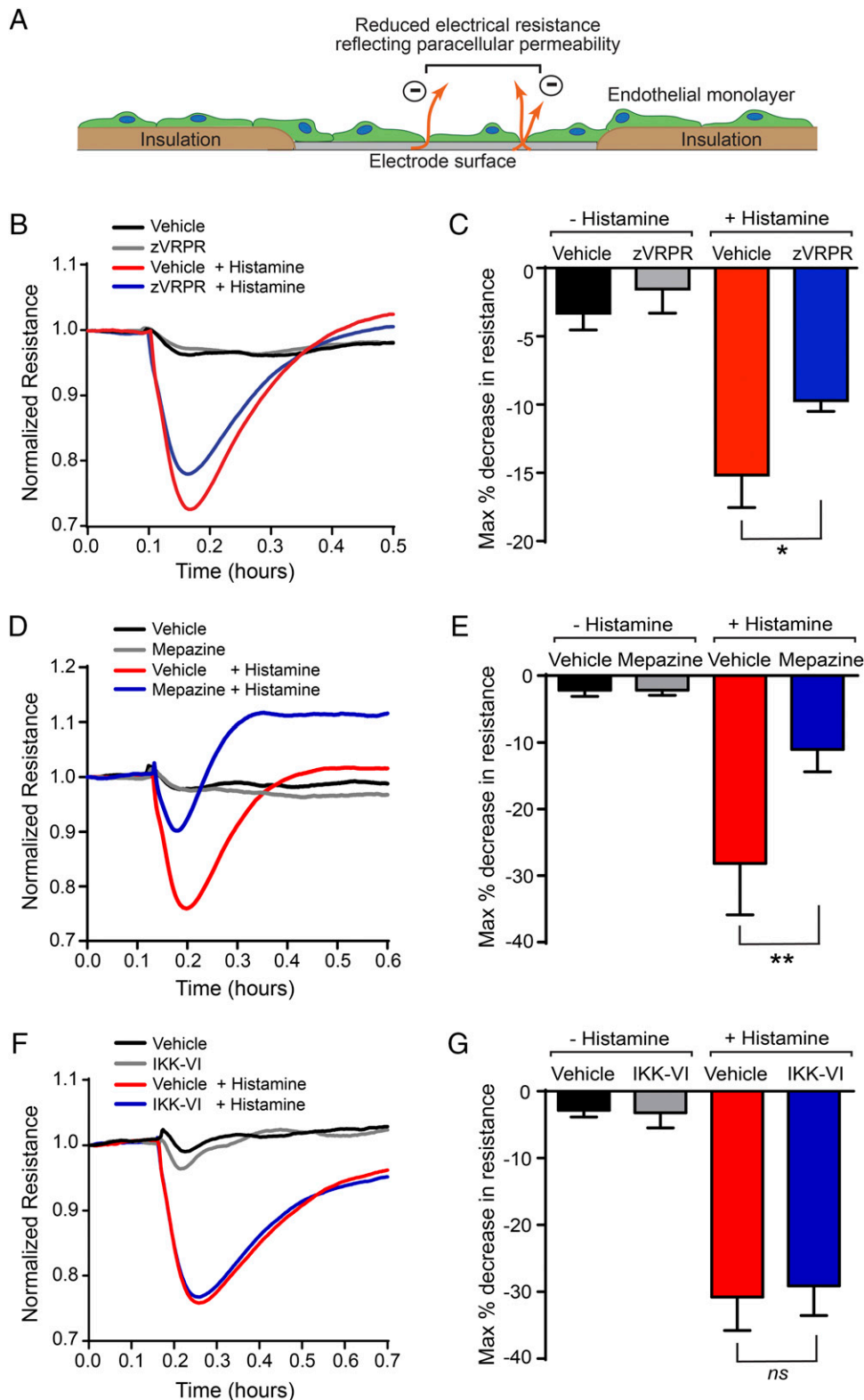


FIGURE 6. Histamine induces acute endothelial permeability in a MALT1 protease-dependent manner. **(A)** Schematic of the ECIS system. Paracellular permeability is inversely proportional to the electrical resistance measured across the endothelial monolayer. **(B and C)** HDMVECs were plated in ECIS chambers and subjected to treatment with 5 μ M histamine with or without a 4-h pretreatment with z-VRPR-fmk (50 μ M). A representative tracing is shown in **(B)**, and quantification of the percentage maximal decrease in resistance across multiple experiments is shown in **(C)**. **(D and E)** HDMVECs were plated in ECIS chambers and subjected to treatment with 5 μ M histamine with or without a 6-min pretreatment with mepazine (1 μ M). A representative tracing is shown in **(D)**, and quantification of the percentage maximal decrease in resistance across multiple experiments is shown in **(E)**. **(F and G)** HDMVECs were plated in ECIS chambers and subjected to treatment with histamine with or without a 6-min pretreatment with IKK-VI (5 μ M). A representative tracing is shown in **(F)**, and quantification of the percentage maximal decrease in resistance shown in **(G)**. Data represent the mean $\pm$ SEM for three independent experiments. * p < 0.05, ** p < 0.01.

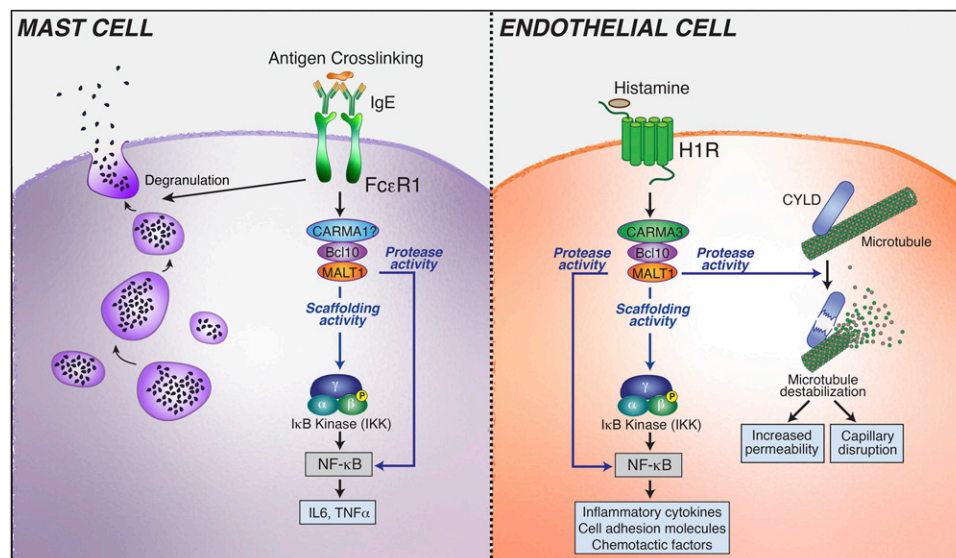


FIGURE 7. Proposed model for the dual role of MALT1 protease activity in IgE-dependent allergic response. First, in mast cells (the initiator), IgE-mediated FcεR1 receptor activation triggers downstream signaling. MALT1 functions as a scaffolding protein, leading to phosphorylation of IκB and subsequent NF-κB activation and cytokine production. MALT1 also acts as a protease, cleaving substrates that negatively regulate downstream steps in the NF-κB pathway, thereby optimizing the induction of cytokines, including IL-6 and TNF-α, which are important for the chronic phase of the allergic response. Meanwhile, histamine is released from mast cells in a MALT1-independent manner via IgE/FcεR1-mediated degranulation. The released histamine activates H1Rs on endothelial cells (the target), leading to acute changes in endothelial permeability as well as downstream inflammatory responses. MALT1 protease activity plays an important role in both of these endothelial responses. In particular, we show in this study that MALT1 protease activity is critical for histamine/H1R-mediated acute endothelial permeability, which leads to the rapid tissue edema observed in the acute phase of the allergic response. Taken together, MALT1 protease activity is a key component to both the acute and chronic stages of allergic reactions, acting in both mast cells and endothelial cells.

and thereby drives immune cell recruitment to the site of GPCR-driven tissue inflammation (18, 51, 52). Second, endothelial MALT1 proteolytic activity can also mediate GPCR-induced responses via an NF-κB-independent mechanism. Specifically, our group demonstrated that thrombin/PAR1-induced MALT1-dependent cleavage of CYLD within endothelial cells results in microtubule disruption and a cascade of events that leads to endothelial cell retraction and an acute permeability response (17). In the current manuscript, we now demonstrate, both in vitro and in vivo, that histamine drives MALT1 protease-dependent endothelial permeability. Based on our previous and current findings, it seems likely that, similar to thrombin, histamine stimulation of endothelial cells also promotes endothelial dysfunction/permeability by inducing MALT1-dependent CYLD cleavage.

Allergic disease is not limited to IgE-mediated cutaneous responses, and it is possible that MALT1 also plays an important and multifaceted role in other allergic responses such as allergic airway inflammation. Intriguingly, three recent reports show that mice with CARMA3-deficient airway epithelial cells have reduced airway inflammation and allergic response to asthma-relevant GPCR ligands that include lysophosphatidic acid and to allergens known to activate GPCRs, such as the fungus *Alternaria alternata*, and the house dust mite (55–57). Because GPCR stimulation can result in the formation of a CARMA3–Bcl10–MALT1 (CBM) complex and stimulation of MALT1 proteolytic activity (18), it seems that the MALT1 protease is also likely to mediate these responses in airway epithelial cells. Additionally, two other recent reports show CARMA1 is essential for effector and memory T cell responses in allergic airway inflammation (58, 59). Antigenic stimulation of the TCR leads to formation of a CARMA1–Bcl10–MALT1 complex, making it plausible that the MALT1 protease can also mediate T cell responses in the airway. This implies that in the lung it is possible that MALT1 plays a critical role in promoting allergic

response in at least four cell types: mast cells, endothelial cells, airway epithelial cells, and T cells.

Our findings suggest that pharmaceutical targeting of the MALT1 protease may be of great benefit in allergic disease as well as in other inflammatory states driven by mast cells. Current therapeutic approaches to allergic disease have focused on targeting particular mediators derived from mast cells, promoting generalized immunosuppression with corticosteroids, or blocking IgE directly, such as with the anti-IgE mAb omalizumab (1, 60). MALT1 proteolytic inhibition could have synergistic therapeutic benefit in both the immediate and late phase of allergic reaction by interfering with 1) the acute effects of mast cell-derived vasoactive substances on the endothelium and 2) the chronic proinflammatory effects of mast cell cytokine production.

Because of the demonstrated importance of deregulated/constitutive CBM signaling in the pathogenesis of certain subtypes of lymphoma, there is now intense interest in developing clinical-grade inhibitors of MALT1 proteolytic activity. Indeed, several MALT1 protease inhibitors have been described thus far: mepazine and related phenothiazines (44), MI-2 (61), MLT-827 (62), specific β-lapachone analogues (63), and z-VRPR-fmk derivatives, including a compound currently named “compound 3” (64). Preclinical mouse studies have shown that MALT1 protease inhibitors, including mepazine, MI-2, and compound 3 can be safely and effectively used in vivo to treat diffuse large B cell lymphomas with gain-of-function mutations that drive CBM signaling (44, 61, 64), multiple sclerosis (65), inflammatory bowel disease (66, 67), and rheumatoid arthritis (68). Our studies suggest that these or other MALT1 protease inhibitors under development may be useful as therapeutics for the prevention and treatment of allergic disease.

Overall, our findings support a novel dual role for MALT1 proteolytic activity in IgE-dependent allergic response (Fig. 7 depicts a proposed model). First, in the initiator mast cell, MALT1

protease activity is required to drive optimal NF- κ B transcriptional activation and cytokine production, thus leading to late-phase allergic reaction. Second, at an end-target organ, the endothelium, MALT1 protease activity is a required mediator of histamine/H1R-induced acute endothelial permeability and dysfunction. Previous work by our laboratory demonstrated that stimulation of the GPCR PAR1 on endothelial cells triggers MALT1-dependent proteolytic cleavage of the microtubule binding protein, CYLD. Fragmentation of CYLD then results in microtubule disruption and an acute increase in endothelial permeability (17). We speculate that MALT1-mediated cleavage of CYLD and consequent microtubule disruption may also occur after stimulation of the histamine/H1R receptor in endothelial cells, thus leading to the observed H1R-induced MALT1 protease-dependent increase in endothelial permeability.

The work described in this study takes on heightened impact and significance because of a new report that identifies single nucleotide variants (SNVs) within the MALT1 locus (most notably the rs57265082 SNV) as major risk factors for the development of peanut allergy (69). This report suggests that the top-associated MALT1 locus SNVs affect MALT1 expression and supports a relationship between MALT1 and progression to symptomatic allergy after peanut sensitization. Our findings are consistent with that notion and provide mechanistic insights into how aberrant MALT1 action could promote allergic immune pathogenesis in the setting of exposure to peanut or other allergenic substances. Future studies will be aimed at further expanding our understanding of the role of MALT1 protease activity in allergic diseases, such as anaphylaxis, mastocytosis, asthma, and others, and investigating the use of MALT1 protease inhibition as a therapeutic approach to these complex disorders.

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Disclosures

P.C.L. and L.M.M.-L. claim ownership of stock in Amgen. The other authors have no financial conflicts of interest.

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