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Tafazzin Modulates Allergen-Induced Mast Cell Inflammatory Mediator Secretion

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ABSTRACT

Allergic inflammatory diseases are a steadily growing health concern. Mast cells, a driving force behind allergic pathologies, modulate metabolic pathways to carry out various functions following IgE-FcεRI-mediated activation. Tafazzin (TAZ) is a cardiolipin transacylase that functions to remodel, and thereby mature, cardiolipin, which is important for efficient energy production through oxidative phosphorylation. In this study, we aimed to evaluate the contribution of TAZ in IgE-mediated mast cell activation. Fetal liver-derived mast cells (FLMCs) were differentiated from mice with a doxycycline (dox)-inducible TAZ short hairpin RNA (shRNA) cassette (TAZ shRNA1/1) and littermate wild-types (WTs). TAZ knockdown in FLMCs following dox treatment was confirmed by Western blotting (99.1% by day 5), whereas flow cytometry confirmed FLMC phenotype (c-kit+ FcεRI+) and retention of receptor expression post-dox. Five-day dox-treated WT and TAZ shRNA1/1 FLMCs were activated via allergen-bound IgE cross-linking of FcεRI under stem cell factor potentiation. With dox, and in response to allergen, TAZ shRNA1/1 FLMCs displayed a 25% reduction in oxygen consumption and a significant 31% reduction in mast cell degranulation compared with dox-treated WT FLMCs. Secretion of TNF, CCL1, and CCL2 were significantly reduced, with CCL9 also impaired. Notably, gene expression was not impaired for any inflammatory mediator measured. Functionally, this suggests that TAZ is a contributor to mast cell degranulation and inflammatory mediator secretion. Given unimpacted induced gene expression for mediators measured, we propose that TAZ reduction impairs mast cell exocytosis mechanisms. We thus identify a potential new contributor to immunometabolism that enhances our understanding of mast cell signaling metabolic pathway interactions during allergic inflammation. ImmunoHorizons, 2021, 5: 182–192.

INTRODUCTION

Mast cells are multifunctional immune sentinels present in vascularized tissues interfacing the external environment that drive the chronic inflammatory response that characterizes allergic pathologies (1–3). These long-lived effector cells arise from hematopoietic progenitors, and terminally differentiate within various tissues under the influence of the local cytokine milieu, including a critical role for stem cell factor (SCF) (4). Tissue-resident mast cells retain expression of the SCF receptor...
c-kit (CD117) and constitutively express the IgE receptor FcεRI.
Together, these receptors synergistically enhance the inflam-
matory response following allergen-induced IgE-FcεRI
complex cross-linkage in the presence of local SCF (2, 5).
Cross-linking of tetrameric FcεRI complexes initiates a se-
ries of intracellular biochemical events, resulting in a
biphasic mast cell response (6, 7). Early-phase reactions are
elicited within minutes of allergen exposure and are charac-
terized by the rapid degranulation of preformed proinflam-
atory mediators, cytokines, chemokines, and arachidonic
acid metabolites (1). Second, a late-phase reaction peaks
hours after allergen activation, characterized by sustained
production and release of chemokines and cytokines that
further propagate the inflammatory response (6).

Intracellular signaling upon receptor aggregation is me-
diated by ITAMs contained within the cytoplasmic domains
of the FcεRI β- and γ-subunits (8). Autophosphorylation of
the Src family kinases is initiated within the ITAMs imme-
diately following receptor cross-linking, leading to the
phosphorylation of adaptor proteins such as linker for ac-
vation of T cells (LAT) and growth factor receptor-bound
protein 2 (Grb2) (9, 10). Phosphorylated LAT functions as a
scaffold for signaling complexes, including the cytosolic
adaptor Grb2 bound to the guanosine triphosphate exchang-
er, son of sevenless (SOS), and phospholipase Cγ (PLCγ)
(10). PLCγ, in conjunction with PI3K, catalyzes the break-
down of membrane phospholipids to produce second mes-
sengers, inositol 1,4,5-triphosphate (IP3) and diacylglycerol
(DAG) (11). IP3 and DAG, respectively, result in intracellular
Ca2+ mobilization from the endoplasmic reticulum and ac-
vation of protein kinase C (11). An increase in intracellular
Ca2+ concentrations facilitates exocytosis of preformed
granules (12). Activation of Ras by the small GTPase, SOS,
results in the phosphorylation of the ERK, JNK, and p38
MAP kinase pathways and subsequent activation of gene
transcription and eicosanoid production (13, 14).

In addition to their critical role in cellular bioenergetics,
metabolic pathways substantially influence the functional re-
sponse of allergen-activated mast cells, directly contributing to
the overall inflammatory response (15). Following mast cell ac-
tivation, cellular metabolic requirements substantially increase
for optimal degranulation and proinflammatory cytokine pro-
duction (15–18). Recently, the field of immunometabolism has
established mitochondria as significant regulators of innate and
adaptive immune responses due to their ability to rapidly
switch from supporting catabolic to anabolic processes, gener-
ating ATP and metabolic intermediates required for de novo
synthesis of macromolecules (19). Furthermore, by-products of
mitochondrial respiration, known as reactive oxygen species
(ROS), have been shown to be essential signaling regulators,
targeting biochemical pathways that enhance a number of
physiological mechanisms, including mast cell degranulation,
leukotriene secretion, and cytokine production, following
IgE-mediated mast cell activation (20). As a result, inhibition
of ROS production has been targeted to suppress allergic
inflammatory responses, which has demonstrated a significant
reduction in Ca2+ mobilization and suppression of degranu-
lation and cytokine release (21).

Cardiolipin, a hallmark phospholipid predominantly located
in the inner mitochondrial membrane, allows for respiratory
supercomplex assembly through the anchoring of mitochon-
drial proteins. Immature cardiolipin undergoes remodeling, a se-
ries of decylation and reacylation reactions of the fatty acid
side chains, into mature cardiolipin, which is typically enriched
in unsaturated acyl chains such as linoleic acid (22). Tafazzin
(TAZ), a phospholipid transacylase, catalyzes cardiolipin re-
modeling, an essential process for maintenance of respiratory
chain enzyme function, and mitochondrial membrane stability
and dynamics (23, 24). Cardiolipin-deficient yeast mitochondria
have been shown to exhibit destabilized supercomplexes, com-
posed of complexes III and IV (22), and dissociation of ADP/
ATP carrier-containing complexes (23, 25), resulting in dramat-
ically impaired respiratory function. Loss of function mutations
in TAZ are the underlying cause of Barth syndrome, a metabo-
lis disorder characterized by dilated cardiomyopathy, skeletal
myopathy, and neutropenia, all attributed to mitochondrial dys-
fuction (26, 27). TAZ-knockdown models have demonstrated
decreased total mitochondrial area and size, reduced cristae
density (28), and decreased complex III and ATP synthase en-
zymatic activity (29).

Despite a well-established energy demand for optimal mast
cell degranulation and cytokine production, the consequences
of TAZ deficiency in a primary mast cell model of allergic in-
flammation, has yet to be investigated. Using a short hairpin
RNA (shRNA)–inducible TAZ-knockdown mouse model to dif-
ferrate mature primary mast cells, we demonstrate reduced
degranulation and cytokine secretion with TAZ deficiency. This
result corresponded with a reduction in oxygen consumption
following TAZ knockdown, indicating potential modulation of
mitochondrial function. Notably, although secretory mecha-
nisms were significantly attenuated following the knockdown
of TAZ, mitochondrial alteration did not affect mRNA produc-
tion of proinflammatory cytokines and chemokines. Thus, we
identify the integral role of TAZ in meeting cellular demands
for degranulation and cytokine secretion in a mast cell–medi-
ated allergic inflammatory event.

**MATERIALS AND METHODS**

**Animals**

Mice from the B6.Cg-Gt(ROSA)26Sor^{Pna77[H]/tetO-RNAi:TazArte/}
ZkhuJ (TAZ shRNA) mutant mouse strain from The Jackson
Labaratory (stock no. 014648) were maintained on a standard
diet and in normal living conditions in the Brock University
Comparative Biosciences Facility. All procedures were ap-
proved by the Animal Care Committee at Brock University.
Fetal liver isolation, genotyping, and mast cell culture generation
Mice heterozygous for the TAZ shRNA were bred to generate pups homozygous for the TAZ shRNA cassette. Fetal livers were removed from the pups and homogenized in RPMI 1640 media supplemented with 10% heat inactivated synthetic FBS (Serum Plus II; Sigma-Aldrich), 1% penicillin/streptomycin (15140-122; Life Technologies), 50 µM β-mercaptoethanol (no. M3148; Sigma-Aldrich), and 0.2 µM PGE2 (no. P5640; Sigma), as well as 30 ng/ml of SCF (no. 250-03; PeproTech) (30). Isolated fetal liver tissues were genotyped as described previously with the primers for TAZ (forward 5′-CCATGGAATTCGAACGCTGACGTC-3′; reverse 5′-TATGGGCTATGAACATAATGACCC-3′) (27). Mast cells were differentiated under the direction of IL-3, PGE2, and SCF.

Abs
Fluorochrome-conjugated Abs, including PE anti-mouse FcεRIα, PE Armenian hamster IgG isotype control, FITC anti-mouse CD117 (c-kit), and FITC rat IgG2b, were purchased from Sony Biotechnology (San Jose, CA). Custom mouse anti-TAZ Ab was described in Lu et al. (31) and secondary Ab for TAZ detection was goat anti-mouse HRP (Cell Signaling Technology).

Flow cytometry
Fetal liver-derived mast cells (FLMCs) (0.5 × 10^6 per staining condition) were collected and washed with immunofluorescence buffer (PBS with 0.2% NaN₃ and 1% BSA). Cells were stained with fluorochrome-conjugated Abs as per manufacturer guidelines (Sony Biotechnology) for 1 h on ice. Following incubation, the cells were washed with 600 µl of immunofluorescence buffer and then fixed with 400 µl of 1% formalin in PBS. Data were acquired on a Sony SH800S Cell Sorter (Sony Biotechnology).

Doxycycline-inducible TAZ knockdown and mast cell activation
TAZ knockdown was initiated with the addition of 1 µg/ml of doxycycline (dox) (Sigma-Aldrich, D9891) for 5 d. FLMCs were sensitized overnight with TNP-specific IgE harvested from TIB-141 cells (American Type Culture Collection). FLMCs were washed and resuspended in RPMI 1640 with 10% FBS and 1% penicillin/streptomycin. Cells were activated with 100 ng/ml of both TNP-BSA (Biosearch Technologies) and SCF (no. 250-03; PeproTech).

Oxygen consumption assay
A total of 5 × 10⁶ FLMCs suspended in RPMI-1640 were loaded into a Clark-type oxygen electrode (Rank Brothers, Cambridge, UK). Basal rate of oxygen consumption was measured prior to the addition of 1 µg/ml of both TNP-BSA and SCF. Stimulated respiration was measured in real-time and the change in respiratory rate was calculated over a 1- to 2-min period to obtain the slope.

Western blotting
Protein was isolated via FLMC lysis in RIPA buffer (30 µl per 5 × 10^6 cells) supplemented with protease and phosphatase inhibitors (BioBasic, Markham, ON; Sigma-Aldrich, respectively) as described previously (32). Purified lysates (20 µg of protein) were loaded onto 10% TGX FastCast acrylamide gels (161-0173; Bio-Rad Laboratories) and electrophoresed at 200 V for 35 min. Gels were transferred to polyvinylidene difluoride membranes via TransBlot Turbo (1704150; Bio-Rad Laboratories), blocked with 5% nonfat milk powder, and probed with primary mouse anti-TAZ (1:1000 in 5% BSA-TBST) and secondary goat anti-mouse HRP (1:5000 in 5% skim milk powder in TBST) Abs. Membranes were treated with Clarity Max (Bio-Rad Laboratories) and chemiluminescence was visualized on a C-Digit Blot Scanner (3600; Li-Cor Biosciences) and quantified using ImageJ software.

Quantitative PCR
RNA was isolated using a Qiagen RNeasy Plus Kit (74136; Qiagen). cDNA was generated using EcoDry RNA to cDNA Double-Primed Reverse Transcriptase Kit (no. 639549; Clontech Laboratories). Quantitative PCR assays were performed on an ABI StepOnePlus Real-Time PCR System Instrument (no. 4376592, Applied Biosystems) with KAPA SYBR FAST (KM4103; Kapa Biosystems) master mix and amplification efficiency-optimized primers (Integrated DNA Technologies). The primers used included GAPDH forward 5′-CGGTGCTGAGTATGTCGTGGAGTC-3′ reverse 5′-GGGGCTAAGCAGTTGGTGGTG-3′, CCL1 forward 5′-AGAAAGATGGGCTCCTCCTGTC-3′ reverse 5′-GCTCATCTTCAACCCCCGTAGCA-3′, CCL2 forward 5′-CCTCACACCAATGCGAGTCC-3′ reverse 5′-CAGCAAGGTAGTGGGGCGTTA-3′, CCL3 forward 5′-GGAGCTGACACCCCGAGTGC-3′ reverse 5′-GGTTTCCTCGGTCTCC-3′, CCL9 forward 5′-ACAATGGCTGTGGAATCTGGGC-3′ reverse 5′-TCAAGCCCTTCTGTCACCA-3′, IL6 forward 5′-AGACAAAAGCAGCTTCTCCAG-3′ reverse 5′-TGCTGTTCTCCTAGCCACTC-3′, and TNF forward 5′-TGAACCTTGGGTAGTGGTCTCC-3′ reverse 5′-TCAACCTTGCTTCAGAAGGA-3′ reverse 5′-TGGTCTTCTAGCCACTC-3′, and TGFβ forward 5′-TGAACCTTGGGTAGTGGTCTCC-3′ reverse 5′-TCAACCTTGCTTCAGAAGGA-3′ reverse 5′-TGGTCTTCTAGCCACTC-3′, and TGFβ forward 5′-TGAACCTTGGGTAGTGGTCTCC-3′ reverse 5′-TCAACCTTGCTTCAGAAGGA-3′ reverse 5′-TGGTCTTCTAGCCACTC-3′, and TGFβ forward 5′-TGAACCTTGGGTAGTGGTCTCC-3′ reverse 5′-TCAACCTTGCTTCAGAAGGA-3′ reverse 5′-TGGTCTTCTAGCCACTC-3′. Threshold cycle (Ct) values were recorded and analyzed using the ΔΔCt method with expression of GAPDH used as a reference gene.

ELISA
ELISAs were used to measure cytokine concentrations in cell-free supernatants from FLMCs and were conducted according to the manufacturer’s protocol. Abs and recombinant protein standards were from R&D Systems (DuoSet). TMB (tetramethylbenzidine) substrate (BD Biosciences) was applied, and absorbance values were obtained via spectrophotometry (BioTek, Synergy HT-1) at 450 nm with concentrations determined from the standard curve.

β-hexosaminidase degranulation assay
Sensitized FLMCs were resuspended in HBSS buffer (Life Technologies) at 4 × 10^6 cells per milliliter and aliquoted in...
duplicate. Cells were stimulated with 100 ng/ml of TNP-BSA and SCF. Cells were centrifuged to separate the supernatant and pellet and cells were lysed with a 1% NP-40 in HBSS buffer. Following centrifugation 50 μl of both supernatant and lysed pellet was transferred to a 96-well plate. Fifty μL of 1 mM p-NAG (4-nitrophenyl-N-acetyl-β-d-glucosaminide) solution was added and the plate was incubated at 37°C for 2 h. Two hundred μL of 0.1 M carbonate buffer was added to stop the reaction and the plate was read on a spectrophotometer (BioTek Synergy HT-1) at 405 nm.

**Statistical analysis**

Most data were analyzed using two-way ANOVA designs with post hoc t tests. A Student t test was used to compare ΔO2 consumption between TNP-BSA + SCF stimulated wild-type (WT) and TAZ shRNA+/− cells. Results were considered significant when p < 0.05.

**RESULTS**

**Generation of dox-inducible TAZ shRNA+/− fetal liver mast cell cultures**

Fetal liver-derived mast cells (FLMC) were differentiated from fetal liver tissue isolated from pups reared from dams and sires of heterozygous TAZ-knockdown mice. Homogenized liver cells were cultured in media conditioned with IL-3, PGE2, and SCF for a period of at least 4 wk. Mast cell phenotype was confirmed by flow cytometry with samples stained for both c-kit and FcRRI. Fig. 1A shows that both WT and TAZ shRNA+/− cultures expressed high levels of both c-kit and FcRRI, surface receptors characteristic of mature mast cells. FLMC cultures were genotyped to confirm the presence or absence of the TAZ shRNA cassette (Fig. 1B). Both WT and TAZ shRNA+/− cultures were treated with 1 μg/ml dox for a 5-d-time course at which point samples were analyzed using Western blot to determine the extent of TAZ protein reduction (Fig. 1C). Although dox treatment expectantly affected WT FLMC TAZ protein levels (33) as well, densitometry analysis was conducted to quantify the extent of TAZ knockdown across multiple independent cultures, revealing a 99.2% reduction in TAZ expression in TAZ shRNA+/− cells compared with their pretreatment expression levels and a 99.1% reduction when compared with WT cells also treated with 1 μg/ml of dox for 5 d (Fig. 1D). Treatment with dox did not lead to a difference in the expression of key functional mast cell surface receptors c-kit and FcRRI between WT and TAZ shRNA+/− cells after 5 d (Fig. 1E). Receptor expression pre- and post-dox treatment was also analyzed by flow cytometry. An increase in c-kit expression following dox treatment was detected in both the WT and the TAZ shRNA+/− FLMCs (Fig. 1F). Importantly however, there was no significant difference in receptor expression between the WT and TAZ shRNA+/− FLMCs in the same dox treatment condition. To account for potential impacts of the dox treatment or shRNA on cell viability, protein and RNA concentrations following dox treatment were examined. No difference in yields were detected suggesting that cell survival was not negatively impacted (Supplemental Fig. 1). Altogether, these results indicate the establishment of primary mast cell culture model of TAZ deficiency.

**Quantification of allergen/SCF-dependent mast cell respiration**

To determine whether the dox-induced reduction in TAZ resulted in a decrease in mast cell respiration, a Clark-type electrode was used to measure the amount of oxygen consumed by the cells following activation. Both WT and TAZ shRNA+/− FLMCs were treated with 1 μg/ml dox for 5 d and sensitized with TNP-specific Ab during the final 24 h. Five million IgE-sensitized FLMCs were loaded into the chamber of the Clark-type electrode where basal respiration for each culture was measured. Following stimulation with a synthetic allergen (TNP-BSA) under SCF potentiation, respiration rapidly and significantly increased (p < 0.0001) in both WT and TAZ shRNA+/− FLMCs (Fig. 2A). Although there was no significant interaction, the change in respiration in response to TNP-BSA + SCF treatment was blunted in the TAZ shRNA+/− FLMCs compared with WT FLMCs, albeit trending toward significance (p = 0.06) (Fig. 2B). These results may indicate that TAZ deficiency impairs the recruitment/activation of aerobic energy production in response to allergen stimulation in FLMCs.

**TAZ is required for normal allergen/SCF-dependent mast cell degranulation**

Given our hypothesis that a reduction in TAZ protein content would result in a decrease in energy production in the cell, it was further hypothesized that this would also result in an impairment in mast cell degranulation. To assess the contribution of TAZ to the characteristic early-phase allergic degranulation response, rapid activation-dependent secretion was measured. Sensitized mast cells were activated by TNP-BSA and SCF. As shown in Fig. 3, dox-induced TAZ deficiency resulted in a significant 31.4% reduction in degranulation compared with dox-treated WT cells. These results suggest that TAZ contributes to the rapid biochemical changes supporting mast cell degranulation in the early phase of allergic inflammation.

**TAZ is required for secretion of select late-phase inflammatory mediators following IgE/c-kit–dependent mast cell activation**

Impaired early-phase secretion, indicative of an impact on the early phase of the allergic response, prompted an investigation into the release of de novo synthesized inflammatory mediators (another exocytosis mediated function of mast cells) during the late phase of the allergic response. Five-day dox-treated, IgE-sensitized FLMCs were activated with TNP-BSA and SCF over a 24-h period, and cell-free supernatants were collected and analyzed for a variety of proinflammatory mediators. As shown in Fig. 4, there was a significant reduction in the secretion of...
CCL1, CCL2, and TNF, a nonsignificant decrease in CCL9 secretion ($p = 0.10$), and no significant differences in the secretion levels of CCL3 and IL-6 in TAZ shRNA$^{+/+}$ cells compared with WT. These results suggest that TAZ contributes to mechanisms supporting secretion of select mediators in allergen/SCF-activated mast cells.

**Inflammatory mediator gene expression does not require TAZ**

Considering the results that both degranulation and the secretion of select mediators was impacted by the reduction in TAZ protein content, gene expression was analyzed to determine whether the differences in secretion could be attributed to a deficit in...
the secretion mechanism, or if there was a difference in gene expression that was also playing a role in the decreased secretion levels detected in the ELISA assays. To answer this question, quantitative PCR assays were conducted to analyze changes in induced gene expression. WT and TAZ shRNA \(^{+/+}\) FLMCs were exposed to 1 \(\mu\)g/ml of dox for 5 d and then sensitized and activated with TNP-BSA and SCF over a 5-h period. As shown in Fig. 5, there was no change in gene expression for mediators examined in this study in TAZ shRNA \(^{+/+}\) FLMCs when compared with WT following the 5-d dox treatment. This data, when considered with the results from the \(\beta\)-hexosaminidase and ELISA assays, suggest that the decrease in the intensity of the allergic response in FLMCs that have a reduced level of TAZ protein may be due to impairments in the exocytosis machinery of the cell and not the modulation of gene expression.

**DISCUSSION**

Mast cells are immune sentinels that differentiate in peripheral tissues and are positioned and enriched at environmental interfaces in contribution to immunological defense and tissue homeostasis. However, they are most well characterized for their pathology-inducing activation by innocuous environmental stimulants, or allergens. Following recognition by IgE Abs bound to the high-affinity Fc\(\varepsilon\)RI receptor on the mast cell surface, these allergens drive an often chronic, inappropriate inflammatory response supporting the development of pathology (e.g., asthma). The emergent field of immunometabolism has brought recognition to the potential of targeting immune cell metabolic processes as an approach to altering their functional outcomes; however, only limited work has examined mast cells in this context (17, 34). In this study, we have identified the mitochondrial transacylase, TAZ, as an important contributor to allergen-mediated mast cell secretory functions. Our model of TAZ deficiency displayed impaired early-phase degranulation and late-phase secretion of critical proinflammatory mediators, whereas induced gene expression remained intact. To our knowledge, this is the first demonstration of the potential role of mitochondrial-mediated metabolism in mast cells contributing to allergic inflammation (Fig. 6).

With \(\sim\)15% of gene modifications being embryonic lethal (35), extensive work has been carried out to develop creative methods of working around this. In the case of mast cell biology, where the majority of mast cells are either harvested from tissue or generated from the bone marrow, other sites of
hematopoiesis (serving as a source of the hematopoietic stem cells and progenitors needed for the generation of mast cells) have been developed. The murine fetal liver is a source of hematopoietic stem cells (36) in which 67–77% of isolated hematopoietic stem cells exhibit long-term progenitor activity (37).

In 2013, a study by Fukuishi et al. (38) looked to characterize the differences between FLMCs and bone marrow–derived mast cells (BMMCs). Gene transcription was identical between FLMCs and BMMCs for 98.6% of all transcripts and granule content and number were identical in both cell types, whereas secreted cytokines varied slightly. BMMC cultures express a higher amount of FcεRI, but both BMMC and FLMC cultures have greater than 90% of cells that express both FcεRI and c-kit receptors, further supported by our findings in this study (Fig. 1A). Overall, FLMCs are considered a powerful tool to study mast cell function (38).

Our work to establish a dox-inducible TAZ-knockdown FLMC model permitting investigation of TAZ in FcεRI/c-kit-mediated mast cell activation further underscores this utility.

Considering the role of TAZ in cardiolipin remodeling and the close relationship of this process to optimal electron transport chain activity, we sought to determine the contribution of TAZ to activation-induced mast cell respiration. TAZ function has been extensively studied in muscle and isolated mitochondria where it has been shown to function in cardiolipin maturation and stimulated oxidative phosphorylation (39, 40). We did not observe a difference in basal respiration levels between TAZ(shRNA)/WT FLMCs; however, it is important to note that our respiration measures were conducted in intact mast cells and not isolated mitochondria. We chose this preparation to determine the effects of an artificial allergen on intact mast cell respiration. Specifically, we employed TNP-BSA to explore the role of TAZ in a new context by activating anti-TNP IgE-sensitized FLMCs under SCF potentiation, initiating a change in oxygen consumption. This dual stimulation models the mast cell allergic inflammatory environment and induced a rapid increase in oxygen consumption in both the TAZ

**FIGURE 4. TAZ is required for secretion of de novo synthesized inflammatory cytokines and chemokines TNF, CCL1, and CCL2 in response to allergen.**

ELISA quantification of protein concentration in cell-free supernatants collected from dox-treated and IgE-sensitized FLMCs stimulated with TNP-BSA and SCF over a time course up to 24 h. Data for CCL1, CCL2, CCL3, CCL9, TNF, and IL-6 ([A]–[F], respectively) are expressed as mean cytokine concentration (pg/ml) ± SEM, n = 6 independent cultures. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.
shRNA<sup>+/+</sup> and WT FLMCs as indicated by a significant main effect. Although we did not observe a significant interaction between mast cell activation and genotype (Fig. 2), there was an approximate 25% reduction in the change in respiration observed in TAZ shRNA<sup>+/+</sup> FLMCs compared with WT that trended toward significance (<i>p</i> = 0.06). This could suggest that TAZ deficiency blunts the ability to increase respiration and thus recruit aerobic energy production in response to an allergen. Carbonyl cyanide-4-((trifluoromethoxy)phenylhydrazone (FCCP), a compound commonly used in the study of mitochondrial function, works to uncouple oxidation from phosphorylation allowing for maximal consumption of oxygen (41). However, in our model we attempted to mimic a natural event where oxidation and phosphorylation remain coupled to see the effects of the TAZ knockdown in a setting that mimics naturally occurring events in mast cells.

To understand the extent of the impact of TAZ on mast cell biology it is important to look at functional measures. To this end, degranulation was measured through the release of β-hexosaminidase, an enzyme that is found in a subset of preformed granules in mast cells (42) and is commonly used as a marker of degranulation because of its ability to cleave a synthetic substrate that allows for quantification of degranulation (43, 44). We detected a decrease in degranulation in FLMCs with TAZ deficiency (Fig. 3). Understanding that the process of degranulation is energy dependent (45-47), with work suggesting that the majority of this energy comes from mitochondrial ATP (34), and that a reduction in TAZ protein content in a variety of cell types has been shown to impact energy production (29, 48-50), we attribute this impairment to TAZ-dependent metabolic considerations. Indeed, our results show that TAZ deficiency lowered the aerobic response of FLMCs to TNP-BSA + SCF and, although statistical

**FIGURE 5.** Allergen-mediated mast cell inflammatory cytokine and chemokine gene expression does not require TAZ.
Dox-treated and IgE-sensitized FLMCs were stimulated with TNP-BSA under SCF potentiation and RNA was isolated across a time course. Quantitative PCR was used to measure proinflammatory mediator mRNA expression in WT and TAZ shRNA<sup>+/+</sup> FLMCs. Data for CCL1, CCL2, CCL3, CCL9, TNF, and IL6 ([A]–[F], respectively) are expressed as fold change ± SEM, n = 4 independent cultures.
significance was not quite met, the reduction in aerobic respiration corresponds to a biologically relevant and statistically significant effect of lowering degranulation. Notably, although there was a significant reduction in degranulation, it was certainly not abolished in the TAZ shRNA$^{-/-}$ mast cells. This suggests that the loss of TAZ is not sufficient to completely abrogate degranulation in FcεRI/c-kit-mediated mast cell activation. This is most likely because of incomplete impairment of mitochondrial oxidative phosphorylation, as our data suggest, metabolic pathways that occur outside of the mitochondria (hence are unimpacted by the reduction in TAZ) or even differential kinetics between TAZ depletion and cardiolipin depletion, all ultimately contributing sufficient energy to carry out partial degranulation (51).

Following up on the deficiencies in secretion detected during the early phase of the allergic response, we sought to assess whether TAZ contributes to secretion of de novo synthesized mediators that contribute to the late phase of allergic inflammation. The measured reduction in secretion of only a subset of select proinflammatory mediators (Fig. 4) led us to question whether the secreted mediator impairments measured in the supernatants by ELISA were due to a defect in secretory mechanisms, or if there was an impact on the induced transcription of the corresponding genes. Interestingly, we found that mRNA levels were unchanged following the TAZ knockdown suggesting that upstream signaling events are unaffected (Fig. 5). Unchanged mRNA levels do not directly indicate that the reduction in proinflammatory mediator release is due to a deficit in secretory mechanisms, as there could be impacts at the translational level, with mRNA translation being one of highest energy demanding processes in the cell, accounting for ~20% of total respiration (52). However, taken in tandem with the degranulation results, a deficit in the release of proinflammatory mediators could be attributed to deficits in secretory machinery dependent on TAZ.

In conclusion, we established and validated a novel, to our knowledge, model of TAZ deficiency employing FLMCs and interrogated a role for TAZ in a model of allergen-mediated, SCF-potentiated mast cell activation. Detected impairments in early-phase degranulation and late-phase mediator secretion, coupled with intact induced gene expression, suggest a significant contribution to proinflammatory mediator secretion and establish TAZ as a potential novel immunometabolic modulator of consequential mast cell functions in allergy.

**DISCLOSURES**

The authors have no financial conflicts of interest.

**REFERENCES**


