The Balance of Tissue Inhibitor of Metalloproteinase-1 and Matrix Metalloproteinase-9 in the Autoimmune Inner Ear Disease Patients

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Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a protein implicated in the control of inflammation in a number of autoimmune diseases. We hypothesized that the balance of TIMP-1 and matrix metalloproteinase-9 (MMP-9) may influence the control or perpetuation of inflammation in corticosteroid-responsive (RES) and corticosteroid-resistant (NR) autoimmune inner ear disease (AIED) patients. In the present study, we observed that plasma from AIED patients exhibited greater levels of TIMP-1 values compared with normal healthy controls. TIMP-1 abrogates lipopolysaccharide-mediated interleukin (IL)-1 β release from peripheral blood mononuclear cells in a dose-dependent manner. RES AIED patients have higher basal TIMP-1 levels and produce more TIMP-1 in response to IL-1 β . Conversely, consistent with our previous studies, we found that NR patients have higher basal MMP-9 levels and produce more MMP-9 levels in response to IL-1 β .

Keywords: tissue inhibitor of metalloproteinase-1 (TIMP-1), matrix metalloproteinase-9 (MMP-9), autoimmune inner ear disease (AIED)

Introduction

TREATMENT OF AUTOIMMUNE INNER EAR DISEASE (AIED) is with timely corticosteroid administration, and although 70% initially respond, this response is lost over time (Broughton and others 2004). Overexpression of inflammatory cytokines has been observed in the plasma of patients with AIED, including interleukin (IL)-1 β (Pathak and others 2011). The role of IL-1 β as master regulator of the innate immune system has become evident in the last couple of years (Sims and Smith 2010; Dinarello 2011). IL-1 β initiates pro-inflammatory immunologic responses, and, as a result, can mediate a large number of inflammatory diseases (Dinarello 2009; Akdis and others 2011; Dinarello and others 2012). Our previous studies showed that IL-1 β and matrix metalloproteinase-9 (MMP-9) are overexpressed in the plasma of corticosteroid-resistant (NR) AIED patients (Pathak and others 2011) and that inhibition of IL-1 β results in clinical hearing improvement in these patients (Vambutas and others 2014). IL-1 β dysregulation is the hallmark of

Muckle–Wells Syndrome, a rare, autosomal dominant disease characterized by uveitis, fever, skin rash, and sensorineural hearing loss (SNHL) (Agostini and others 2004; Kuemmerle-Deschner and others 2013), thereby further solidifying the role of IL-1 β in the development of SNHL.

MMP-9 is a member of family of enzymes that belong to the zinc dependent metalloproteinases, playing a role in degradation of the extracellular matrix. Matrix metalloproteinases (MMPs) are downstream regulators of IL-1 β signaling (Eberhardt and others 2000; Lin and others 2009; Cheng and others 2010). MMP-9 is specifically overexpressed by AIED patients that secrete high levels of IL-1 β , as shown by our group (Pathak and others 2011). In normal healthy adults, MMPs are expressed at low levels and their upregulation appears to play a central role in numerous pathological processes. The MMP family member MMP-9 is implicated in the progression of diseases like chronic obstructive pulmonary disease (Churg and others 2012), multiple sclerosis (MS) (Rosenberg 2002), rheumatoid arthritis (RA) (Ahrens and others 1996), and other autoimmune diseases (Ram and others 2006).

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One mechanism by which MMPs may induce SNHL is potentially to disrupt the blood–labyrinthine barrier, as MMPs have been characterized to disrupt the blood–brain barrier (Mun-Bryce and Rosenberg 1998). Disruption of the blood–brain barrier is seen in autoimmune diseases that target the brain (Diamond and others 2013). MMP-9 is known to disrupt the blood–brain barrier in MS patients. Levels of MMP-9 are found to be elevated in cerebrospinal fluid (Brew and others 2000; Yong and others 2001). Similarly, infectious diseases also may compromise the blood–brain barrier and the blood–labyrinthine barrier. In a rat model of bacterial meningitis, MMP-9 is upregulated following streptococcal pneumoniae administration which could be abrogated by the coadministration of antibiotic and dexamethasone (Liu and others 2008). Bacterial meningitis is known to induce SNHL (Du and others 2006). MMP expression has been characterized in the cochlea. MMP-9 expression has been observed in spiral ganglion cells, inner hair cells, and supporting cells. Treatment with the ototoxic medication gentamycin resulted in upregulation of MMP-9 (Setz and others 2011). Furthermore, MMP-9 inhibition had a protective effect in lipopolysaccharide (LPS)-induced lateral wall damage of cochlea in guinea pigs (Jang and others 2014).

MMP activity is tightly regulated at the gene transcription level by proteolytic activation of the inactive proenzyme and by suppression of the active form of enzyme by tissue inhibitors of metalloproteinases (TIMPs). TIMPs are specific inhibitors of MMPs that are involved in modulating the activities of MMPs (Gomez and others 1997; Brew and others 2000; Brew and Nagase 2010). MMPs are co-secreted with TIMP-1 in 1:1 ratio in normal healthy controls (Gomis-Rüth and others 1997). Reduction of TIMP-1 has been associated with sepsis (Mühl and others 2011). Plasma TIMP-1 levels were significantly higher in patients with ANCA-associated vasculitis (Sanders and others 2007), and levels of TIMP-1 were also considerably higher in Kawasaki disease than in controls (Senzaki 2006). Disparity in the MMP/TIMP ratio results in the development of fibrosis, cancer cell invasion, metastasis, and arthritis (Frederick Woessner 1994; Nagase 1996).

In RA, high levels of TIMP-1 and MMP-9 were detected in the synovial fluids of patients with the disease, with evidence of MMP/TIMP molar imbalance in favor of MMPs with disease progression (Yoshihara and others 2000). In MS, elevated serum MMP-9 and reduced TIMP-1 were associated with development of new brain lesions as seen by MRI (Waubant and others 1999). In experimental autoimmune encephalomyelitis, the animal model of MS, dexamethasone was demonstrated to reduce the MMP-9/TIMP-1 ratio by the upregulation of TIMP-1 (Förster and others 2007). In a study involving lung parenchymal remodeling in sarcoidosis and Crohn's disease patients, the MMP-9/TIMP-1 molar ratio was significantly higher in both groups of patients compared to controls (Fireman and others 2002). In asthma, corticosteroid therapy reduced MMP-9 levels and increased TIMP-1, thereby suggesting that corticosteroid responsiveness may be influenced by the MMP-9/TIMP-1 balance (Hoshino and others 1999). IL-1 β acts upstream of TIMP-1 and affects TIMP-1 levels. Human astrocytes and orbital fibroblasts produce high levels of TIMP-1 when stimulated with IL-1 β (Han and Smith 2005). These studies suggest that in areas of critical function, such as the brain and the eye, mechanisms exist to dampen untoward inflammation to mitigate organ destruction.

Given our prior observations that NR AIED patients have high IL-1 β levels, and IL-1 β induces the expression of MMP-9 in these patients, we hypothesized that IL-1 β induces MMP-9 preferentially over TIMP-1. Since MMP-9 is stoichiometrically inhibited by TIMP-1 (Lambert and others 2004), we hypothesized that an unfavorable TIMP-1/MMP-9 molar ratio would correlate with the clinically unfavorable phenotype of NR AIED, and a greater ratio would clinically correlate with a corticosteroid-responsive (RES) AIED or a more protective phenotype. Therefore, if our hypothesis was correct, higher TIMP-1 levels in plasma could be a protective mechanism to keep the inflammation under control. In support of this hypothesis, in a study involving acoustic trauma in mice, where 4 different MMPs are initially upregulated for several hours after noise exposure, TIMP-1 remained upregulated for greater than 24 h, which demonstrates the ability to control inflammation in sensory organs (Hu and others 2012).

Materials and Methods

Study design and patient recruitment

In this Northwell Health System IRB approved study, several Neurologists recruited patients with AIED. In total, 36 AIED patients and 13 control subjects (CTRL) were recruited. Control subjects (CTRL) were age- and gender matched and had no hearing problems or autoimmune/inflammatory diseases. The clinical characteristics of these patients, including corticosteroid responsiveness, are shown in Table 1. In all cases, informed consent was obtained and clinical corticosteroid responsiveness documented. The inclusion criteria for this study and metrics for corticosteroid responsiveness were previously described (Niparko and others 2005). Genetic testing for Muckle-Wells syndrome was performed commercially at GeneDx when clinically indicated for patients with clinical signs that could be consistent with that disease.

Peripheral blood mononuclear cell isolation and stimulation

Peripheral blood mononuclear cells (PBMCs) were prepared from heparinized peripheral blood by density-gradient centrifugation for 30 min at 400 g using Ficoll-Paque PLUS (GE Healthcare Bio-Sciences, Uppsala, Sweden) as previously described (Pathak and others 2011). Two washes in 1 $\times$ RPMI 1640 (Thermo Fisher Scientific, Waltham, MA) were performed, and then the PBMCs were counted in a Z2 Coulter™ Particle Count and Size Analyzer (Beckman Coulter, Fullerton, CA). RPMI 1640 supplemented with 4.1 mM L-glutamine, 100 U/mL penicillin, 100 μ g/mL streptomycin (All from Thermo Fisher Scientific, Waltham, MA), and 10% fetal bovine serum (FBS) (Atlanta Biologicals, Atlanta, GA) was used to dilute the PBMC. To prevent the possibility of fluctuating cortisol levels, all PBMC preparations utilized the same FBS lot.

The PBMCs were plated in 24-well plate (Costar, Corning, NY) with cell density 1×10^6 per 1 mL and were left untreated, exposed to 4 μ g/mL dexamethasone (Fresenius Kabi USA, Lake Zurich, IL), 20 ng/mL human rIL-1 β , TIMP-1 at different concentrations (both from PeproTech, Rocky Hill, NJ), or 100 ng/mL LPS (Sigma-Aldrich, St. Louis, MO) alone or in combination as needed. The plates were kept for incubation at 37°C in 5% CO₂ for 16 h.

TABLE 1. CLINICAL FEATURES OF PATIENTS WITH AUTOIMMUNE INNER EAR DISEASE

	Gender	Age	Race	Steroid response	Other autoimmune or significant medical disease
1	Female	21	Caucasian	RES	
2	Male	21	Caucasian	RES ^a	
3	Female	50	Caucasian	RES	Parkinson's
4	Female	74	Caucasian	RES	
5	Female	62	African American	RES	
6	Male	58	African American	RES ^a	
7	Female	75	Caucasian	RES	
8	Female	59	Caucasian	RES	
9	Male	79	Caucasian	RES	Thyroid goiter, Gout
10	Female	61	Hispanic	RES	
11	Female	58	Caucasian	RES	Sjogren's, hyperthyroidism
12	Female	67	Unknown	RES	
13	Female	57	Caucasian	RES	
14	Female	66	Unknown	RES	Hashimoto's thyroiditis
15	Female	64	Caucasian	RES	
16	Female	67	Caucasian	RES ^a	
17	Male	86	Caucasian	RES	Vestibular schwannoma in contralateral ear
18	Female	29	Caucasian	RES	Celiac disease
19	Female	81	Caucasian	NR	
20	Female	65	Caucasian	NR	Hypothyroidism
21	Male	18	Caucasian	NR	Cogan's syndrome, MWS screen negative
22	Male	13	Hispanic	NR	Relative has MS, MRI—possible demyelination
23	Female	41	Caucasian	NR	uveitis, MWS screen negative
24	Male	50	Caucasian	NR	
25	Male	56	Hispanic	NR	
26	Female	56	Caucasian	NR	
27	Male	68	African American	NR	
28	Male	40	Hispanic	NR	
29	Male	55	Caucasian	NR	
30	Female	31	Caucasian	NR	Fibromyalgia, Familial dysautonomia,
31	Male	66	Caucasian	NR	Psoriasis
32	Male	75	Caucasian	NR	
33	Male	13	Caucasian	NR	Psoriasis
34	Female	64	Caucasian	NR	
35	Female	42	Caucasian	NR	Hypothyroidism, psoriasis, Raynaud's, FMF, MWS screen negative
36	Male	44	Caucasian	NR	

^aSteroid dependent patient where tapering of corticosteroids results in worsening of hearing loss.

FMF, Familial Mediterranean fever; MS, multiple sclerosis; MWS, Muckle-Wells syndrome; NR, corticosteroid-resistant; RES, corticosteroid-responsive.

Trypan blue exclusion was performed to check viability by mixing cultured cells in a 1:1 ratio with trypan blue solution (0.4%; Thermo Fisher Scientific, Waltham, MA) using Cellometer (Nexcelom Bioscience, MA). Cell viability exceeded 80% in all cultures. The samples were centrifuged at 400 g for 15 min, and conditioned supernatants were stored at -20°C in multiple aliquots until experimentation.

TIMP-1 and MMP-9 enzyme-linked immunosorbent assay

TIMP-1 and MMP-9 enzyme-linked immunosorbent assays (ELISAs) were performed on conditioned supernatant, as well as plasma samples, as per the manufacturer's protocol (R&D Systems, Minneapolis, MN). Patient and control subject samples were collected and immediately stored at -20°C until use. All samples were run in duplicate from same aliquot and on same day. The variance between replicate sets was 0.0002. A Four Parameter Logistic Fit (4PL) was constructed for quantitative analysis of data.

Calculation of molar ratio

The calculation of molar ratio of TIMP-1 to MMP-9 (TIMP-1/MMP-9) was based on their respective molecular weights 28.5 and 92 kD and the quantity of TIMP-1 and MMP-9 detected by ELISA (Kelly 2001) (Mao and others 2003) from plasma and cultured supernatants. Specifically we tested culture supernatants from PBMCs of RES (*N*=8) and NR (*N*=12) patients stimulated with either dexamethasone or IL-1 β and plasma samples of RES (*N*=6) and NR (*N*=11) patients.

IL-1 β ELISA

IL-1 β concentrations in conditioned supernatant were determined by quantitative sandwich enzyme immunoassay according to the manufacturer's instructions (R&D Systems, Minneapolis, MN). All samples were run in duplicate. The variance between replicate sets was 0.002. A 4PL was constructed for quantitative analysis of data.

Statistical analysis

GraphPad Prism version 5.00 for Windows was used for performing statistical analysis (San Diego, CA; www.graphpad.com). On all graphs, bars representing the mean are shown with the SEM. A value of $P < 0.05$ was considered significant.

Results

TIMP-1 is overexpressed in AIED patients

To observe if TIMP-1 is differentially expressed in the plasma of AIED patients, a TIMP-1 ELISA was performed on plasma collected from AIED patients ($N=17$) and healthy control subjects (CTRL) without hearing loss ($N=9$) (Fig. 1). AIED patients had higher circulating TIMP-1 plasma levels compared to the TIMP-1 plasma levels of healthy control subjects (CTRL) (106.4 ± 6.3 vs. 81.8 ± 8.2 ng/mL, error bars show $\pm$ SEM). This difference was statistically significant, $P=0.027$, by a Mann–Whitney U test. When we segregated them into 2 different groups (steroid sensitive or resistant) the difference was not statistically significant.

MMP-9 is overexpressed in AIED patients

We previously reported that AIED patients had elevated levels of MMP-9 (Pathak and others 2011). To determine the patient-specific effect of MMP-9 on TIMP-1 expression, we analyzed MMP-9 expression in a new cohort of AIED patients for which we also analyzed TIMP-1 expression. Elevated MMP-9 expression in AIED patients was confirmed by performing a MMP-9 ELISA on plasma collected from the same cohort AIED patients as for TIMP-1 ($N=17$)

FIG. 1. Circulating TIMP-1 plasma levels in control subjects (CTRL) and AIED patients. A TIMP-1 ELISA was performed on plasma collected from control subjects (CTRL) ($N=9$) and AIED patients ($N=17$). AIED patients have significantly higher plasma levels of TIMP-1 compared to normal healthy control subjects (CTRL). The difference between the 2 groups was statistically significant ($P=0.027$) (Mann–Whitney U test with a 95% confidence interval). Error bars show $\pm$ SEM. AIED, autoimmune inner ear disease; ELISA, enzyme-linked immunosorbent assay; TIMP-1, tissue inhibitor of metalloproteinase-1.

FIG. 2. MMP-9 is overexpressed in AIED patients. MMP-9 ELISA done on plasma collected from AIED patients ($N=17$) and healthy control subjects (CTRL) ($N=9$). AIED patients had higher MMP-9 plasma levels compared to healthy control subjects. Statistical significance was calculated using a Mann–Whitney U test ($P=0.005$). Error bars show $\pm$ SEM. MMP-9, matrix metalloproteinase-9.

and the same cohort of healthy control subjects (CTRL) without hearing loss ($N=9$) (Fig. 2). Consistent with our prior studies, AIED patients had significantly higher MMP-9 plasma levels compared to healthy control subjects (CTRL) in this new cohort (Mann–Whitney U test, $P=0.005$).

TIMP-1 expression counterbalances MMP-9 elevation: a protective microenvironment

Given that TIMP-1 is known to counterbalance MMP-9, and MMP-9 is active at sites of inflammation, we hypothesized that TIMP-1 could mitigate MMP-9 induced inflammation in a dose-dependent manner. To test whether TIMP-1 would be able to reduce LPS-induced IL-1 β expression, we isolated PBMC from normal healthy controls ($N=4$) and then treated them with increasing concentration of TIMP-1 [20–80 nM] with and without LPS. TIMP-1 was able to reduce IL-1 β release into the culture supernatant in a dose-dependent manner (Fig. 3). Error bars show $\pm$ SEM.

RES patients have higher basal TIMP-1 levels and produce more TIMP-1 levels in response to IL-1 β

Given the TIMP-1 overexpression observed in AIED patients, we queried whether the balance of TIMP-1 and MMP-9 expression correlates with clinical corticosteroid responsiveness. A TIMP-1 ELISA was performed on conditioned supernatant samples from PBMC of AIED RES ($N=8$) and NR patients ($N=12$). The samples were treated with either dexamethasone, or IL-1 β , and compared to untreated samples (Fig. 4). PBMC from RES patients showed more basal TIMP-1 compared to NR patients (5.2 ± 0.61 vs. 3.6 ± 0.41 ng/mL, error bars show $\pm$ SEM), and their PBMC produced more TIMP-1 when stimulated with recombinant IL-1 β (nonsignificant).

FIG. 3. TIMP-1 inhibits IL-1 β release in dose-dependent manner. PBMCs from healthy controls ($N=4$) were isolated and then treated with increasing concentration of TIMP-1 [20–80 nM] with and without 100 ng/mL LPS. TIMP-1 was able to reduce IL-1 β in a dose-dependent manner in LPS-treated samples. Error bars show $\pm$ SEM. IL, interleukin; LPS, lipopolysaccharide; PBMCs, peripheral blood mononuclear cells.

NR patients have higher basal MMP-9 levels and produce more MMP-9 levels in response to IL-1 β

Consistent with our prior observations (Pathak and others 2011), MMP-9 release was observed in PBMC treated with recombinant IL-1 β and inhibited by dexamethasone. Simi-

FIG. 4. TIMP-1 release in AIED corticosteroid-responsive (RES) and corticosteroid-resistant (NR) patients. TIMP-1 ELISA was done on the conditioned supernatant samples collected from PBMC of AIED RES ($N=8$) and AIED NR ($N=12$) patients. PBMC samples from each patient were either left untreated (UNTX) or treated with dexamethasone (DEX) or treated with IL-1 β . The bars show the mean protein release in ng/mL with $\pm$ SEM. Comparison between untreated samples ($P=0.037$) and dexamethasone-treated samples ($P=0.049$) of RES patients to NR patients were found to be statistically significant by Mann–Whitney U test with a 95% confidence interval. Error bars show $\pm$ SEM.

larly, in PBMC from NR patients ($N=12$), basal MMP-9 expression was almost 4-fold greater compared to PBMC from RES patients ($N=8$). IL-1 β seems to induce more MMP-9 in PBMC from NR patients (20.51 ± 6.2 ng/mL) compared to corticosteroid-responsive patients (11.6 ± 2.2 ng/mL, error bars show $\pm$ SEM). Again, consistent with our prior publication (Pathak and others 2011), comparison between basal expression of RES patients to NR patients was found to be statistically significant ($P=0.036$). These data represent a new cohort of patients separate from our 2011 publication (Pathak and others 2011) and are being shown for comparison to TIMP-1 (Fig. 5).

The ratio between TIMP-1/MMP-9 mitigates or perpetuates inflammation

The TIMP-1/MMP-9 molar ratios for untreated, dexamethasone-treated, and IL-1 β -treated PBMCs were 9.60, 7.21, and 3.10, respectively, for RES patients and 1.21, 1.59, and 1.52, respectively, for NR patients (Fig. 6). We also calculated the molar ratio of TIMP-1/MMP-9 in plasma samples of RES and NR patients and observed a slight difference. For RES patients, the molar ratio was 1.73, and for NR patients molar ratio was 0.96 (Fig. 7).

Discussion

The mechanisms that regulate the ability to respond to corticosteroids in AIED patients are presently not well characterized; however, we have shown that IL-1 β inhibition clinically reduces hearing loss in NR AIED patients in an early phase clinical trial (Vambutas and others 2014). Because of the putative role of IL-1 β in these patients, and our previous characterization of MMP-9 plasma overexpression in AIED patients that secrete high levels of high IL-1 β (Pathak and others 2011), we hypothesized that endogenous protective

FIG. 5. NR patients have higher basal MMP-9 levels and produce more MMP-9 levels in response to IL-1 β . MMP-9 ELISA was performed on conditioned supernatant samples from PBMC of AIED RES ($N=8$) and NR patients ($N=12$). The samples were kept untreated (UNTX), dexamethasone treated (DEX), or IL-1 β treated. Comparison between basal expression of responders to NR patients was found to be statistically significant ($P=0.036$). Consistent with our prior observations, these data represent a new cohort of patients separate from our 2011 publication and is being shown for comparison to TIMP-1. Error bars show $\pm$ SEM.

FIG. 6. Comparison of the TIMP-1/MMP-9 molar ratio in RES and NR patients by ELISA on conditioned supernatant. TIMP-1 and MMP-9 release from PBMC in response to dexamethasone (DEX), IL-1 β , or untreated (UNTX) was compared as a molar ratio of TIMP-1/MMP-9 in RES and NR AIED patients. Comparison between the untreated samples of RES and NR was found to be statistically significant ($P=0.0015$) by Mann-Whitney U test with a 95% confidence interval. Error bars show $\pm$ SEM.

mechanism(s) must exist to harness this untoward inflammation. We have previously identified Interleukin-1 Receptor type 2 (IL-1R2) decoy receptor expression as one mechanism of endogenous sequestration of inflammation to reduce IL-1 availability (Vambutas and others 2009), which was similarly observed by others (Rubinstein and others 1998; Shimizu and others 2015). However, we anticipated that multiple such mechanisms must exist to maintain organ preservation. Consistent with this hypothesis, we observed that plasma levels of TIMP-1 were greater in AIED patients than controls. To exclude confounding inflammatory proteins participating in the regulation of IL-1 β by TIMP-1 in PBMC, we treated PBMCs

FIG. 7. Comparison of molar ratio of TIMP-1/MMP-9 in the plasma of RES and NR patients. Molar ratios of TIMP-1/MMP-9 plasma levels were compared in RES ($N=6$) and NR ($N=11$) AIED patients which were 1.73 and 0.96, respectively. Error bars show $\pm$ SEM. No statistically significant differences were seen between the 2 groups ($P=0.21$).

of control subjects (CTRL) with increasing concentration of recombinant TIMP-1 with and without LPS (Fig. 3). In this study, TIMP-1 abrogated LPS-mediated IL-1 β expression in a dose-dependent manner in control PBMCs.

The molar ratio of TIMP-1/MMP-9 seems to correlate with clinical corticosteroid responsiveness as the ratios of untreated, dexamethasone treated, and recombinant IL-1 β treated were much higher in RES patients than NR AIED patients. As anticipated, a greater TIMP-1/MMP-9 molar ratio was also observed in the plasma of clinical RES patients. Furthermore, the abrogation of LPS-mediated inflammation by TIMP-1 treated healthy control PBMCs supports our hypothesis that the dominating molar excess of the TIMP-1 can effectively counteract MMP-9 activation in corticosteroid-responsive patients. Others have similarly observed that the molar ratio of TIMP-1/MMP-9 may affect disease progression (Mao and others 2003) (Dohi and others 2015).

In our AIED patients, a TIMP-1/MMP-9 molar ratio of ≥ 3 of PBMC-secreted cytokines correlated with corticosteroid responsiveness and was, therefore, hypothesized to be protective. In contrast, a molar ratio of <2 was unfavorable and was observed in NR AIED patients. Unstimulated PBMC demonstrated the greatest difference between RES and NR patients (9:1) compared with dexamethasone stimulated (7:1) and IL-1 β stimulated samples (3:1). Given that IL-1 β resulted in the closest difference between responsive and unresponsive patients, we would infer that TIMP-1 is not limitless in its ability to control MMP-9 induced inflammation. In plasma samples, RES patients exhibited a ratio of 1.73 compared with 0.96 in NR patients, and although trended with the results from cultured supernatants, the difference was not statistically significant ($P=0.21$).

We have previously shown that IL-1 β expression results in increased MMP-9 expression (Pathak and others 2011). In this study, we have identified that TIMP-1 is an important counter-regulatory protein that serves to mitigate the effect of MMP-9 in AIED patients. TIMP-1 is differentially expressed in these AIED patients. In RES patients, the TIMP-1/MMP-9 molar ratio in response to both dexamethasone and IL-1 β is skewed toward control of inflammation, whereas the TIMP-1/MMP-9 molar ratio in NR patients is skewed toward perpetuation of inflammation. Therefore, understanding how to enhance expression of TIMP-1 in these patients may confer a protective phenotype which correlates with clinical corticosteroid responsiveness. Future studies characterizing TIMP-1 regulation, downstream signaling, and its role in regulation of MMPs and other molecular targets may assist in identifying other novel therapeutic targets for these patients.

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Authors' Contributions

Conceived and designed the experiments: A.V. and S.P. Performed the experiments: S.P. and L.E. Analyzed the data: S.P. and A.V. Contributed reagents/materials/analysis tools: A.V. Wrote the article: S.P., A.V., and L.E.

Author Disclosure Statement

No competing financial interests exist.

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