

ORIGINAL ARTICLE

Serum metalloproteinase leukolysin (MMP-25/MT-6): a potential metabolic marker for atopy-associated inflammation

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Summary

Background Leukolysin is a novel matrix metalloproteinase (MMP-25/MT-6) released mainly by granulocytic cells, primarily neutrophils, which are implicated in chronic airways inflammation.**Objective** To determine if leukolysin might be a serum marker for atopic asthma or chronic obstructive pulmonary disease (COPD).**Methods** Three study populations were evaluated: (1) nuclear families with medical history of atopic asthma ($N = 337$), (2) married-in individuals from an independent study of asthma genetics ($N = 122$) and (3) randomly selected males with diagnosis of COPD ($N = 100$). Each person was screened for asthma or COPD symptoms, respiratory function by standardized spirometry and serum total IgE and leukolysin and anti-IL1 levels by immunoassay. Study groups (1 and 2) were also screened by skin prick test using a battery of 14 common aeroallergens. Heritability estimates for leukolysin and total IgE were made by variance components analysis.**Results** For those without asthma or who had asthma defined as having symptoms, a physician's diagnosis and bronchial hyper-reactivity as demonstrated by reversibility in response to albuterol and/or bronchial reactivity as measured by a methacholine challenge, serum leukolysin levels were found to be higher for those with any positive skin test result. This paralleled trends for serum total IgE. In the nuclear families and COPD patients, serum leukolysin levels were significantly elevated for those who also had elevated total IgE levels ($\log[\text{IgE}] > 2.0$) compared with those with lower IgE ($\log[\text{IgE}] < 2.0$). Serum IL-1 levels correlated with the leukolysin levels. In contrast to IgE, leukolysin showed no apparent inherited component.**Conclusion** Among individuals with history of chronic airways inflammation (asthma and COPD) serum leukolysin may be a metabolic marker associated with chronic atopy-associated respiratory inflammation. Common factors may stimulate increased production or release of both leukolysin from myeloid cells and IgE from lymphoid cells.**Keywords** asthma, atopy, COPD, IgE, leukolysin, matrix metalloproteinase

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Introduction

Matrix metalloproteinases (MMP) are ubiquitously distributed enzymes that play vital roles in regulating development, normal tissue architecture and host defence [1, 2]. These enzymes are primarily found within intracellular granules of a wide range of cells, six are a membrane-type (MT-MMP), two of which are attached to plasma mem-

branes by glycosyl-phosphatidylinositol (GPI)-anchors [3]. Upon cell activation, MMPs may be released by degranulation or, in the case of the GPI-anchored MT-MMPs, shedding involving the cleavage of the GPI-anchor.

All MMPs are regulated at one of three levels: (1) transcription that regulates the level of production, (2) conversion of a latent pro-enzyme form into an enzymatically active form and (3) inhibition of enzyme activity by

tissue inhibitors of matrix proteinases (TIMPs) [1, 2]. These regulatory mechanisms are important as the MMPs act upon a wide range of substrates, including extracellular matrix (ECM) components such as fibronectin, collagen and cell membranes. In the absence of appropriate regulatory controls, MMP activity has been implicated in a number of pathogenetic mechanisms such as chronic respiratory inflammatory states, involving ECM restructuring and other damaging results [4–6].

Leukolysin (MMP-25/MT-6) is a MT-MMP with a GPI-anchor [7]. While many MMPs show a wide range of distribution in a variety of cells and tissues, leukolysin is novel in that it appears to be expressed only in granulocytic cells of the myeloid series, primarily neutrophils. Leukolysin can be released both by degranulation and shedding after stimulation with pro-inflammatory factors like IL-1 and IL-8 [8]. It acts upon a variety of ECM components, including type-IV collagen, fibronectin and others, and it can be inhibited by several TIMPs, including TIMP-1, -2 and -3 [9].

The most stricken feature of MT5-MMP may be its tendency to shed from the cell surface efficiently. A model suggested is that MT5-MMP protein is synthesized, packaged and delivered to cell surface where proteolytic cleavage on the stem region release soluble MT5-MMP species into the extracellular space. Pei D [7] demonstrated an antibody against human MT5-MMP which could be raised in rabbits using GST-hMT5-MMP. These as well as other preliminary studies suggested that the use of ELISA technique to measure leukolysin in the serum may be informative.

Clearly mechanistic studies are important to understand where to start investigating disease-associated molecules. Most work today has been derived from *in vitro* studies that detail the regulation of MMPs substrate specificities. MMPs have overlapping cellular and tissue distribution in addition to redundancy of function and specificities. Thus, attempting to make a clear distinction between a normal physiological and pathological role is largely speculative. MMP leukolysin (MMP-25/MT-6) may be an ideal candidate for elaborating the *in vivo* regulation of these enzymes [7, 10]. It appears to be a novel MMP in that its cellular distribution is limited primarily to cells of the granulocytic myeloid series. While these cells play vital roles in host defence, their enhanced or prolonged activation is implicated in chronic inflammatory conditions, including atopic asthma [11]. One of the many questions raised is whether neutrophils of asthmatics have a higher frequency to release free MMP-25 *in vivo* and *ex vivo*.

Considerable effort has been made to elaborate the biochemical characteristics and biological activities of leukolysin, at least for *in vitro* analysis. Leukolysin is stored within intracellular granules as well as a membrane bound form attached via a non-transmembrane GPI-

anchor [8]. Its release can be induced by agents that activate leucocytes, such as IL-1 and IL-8 [8]. In the case of the known neutrophil chemoattractant IL-8, leukolysin is implicated in aiding cell migration across or through ECM components. This is supported, in part, by findings that the ECM specificity of leukolysin includes type-IV collagen and fibronectin [9].

Because of this limited distribution in granulocytic cells, leukolysin may play a role in chronic inflammatory conditions, including atopic asthma and chronic obstructive pulmonary disease (COPD) that involves these cell types in their pathogenesis. However, to our knowledge, this possibility has not been explored. The aim of this study was to make basic determinations of the statistical distributions of serum leukolysin in people with and without histories of atopic asthma and COPD.

In this study, we screened three human populations for serum leukolysin levels. One comprised small nuclear families with history of atopic asthma. A second group comprised married-in individuals from previous asthma genetics studies, used as a surrogate for the at-large population. A third group was randomly selected patients with diagnosis of COPD. We compared 'trends' in serum leukolysin levels with those of serum total IgE whose values are known to vary with clinical atopic or asthmatic status.

Material and methods

Study populations

Nuclear families with history of atopic asthma. Caucasian families residing in the Minneapolis/St Paul, Minnesota metropolitan area that included two children with a physician's diagnosis of asthma were recruited through media advertisements as part of studies for the genetics of asthma [Collaborative Study of the Genetics of Asthma (CSGA)] [12]. Each person, or legal guardian, provided written, informed consent according to the guidelines of the Institutional Review Board of the University of Minnesota.

Married-in individuals. An additional 122 Caucasians living in the same metropolitan region were recruited as part of the former CSGA [12]. These individuals were married-in to the families previously ascertained for asthma genetics studies. Although they shared common environmental influences, they presumably did not share the same genetic predisposition for atopic disorders as the families recruited for asthma, and were used as representatives of the at-large human population. After obtaining written informed consent, these individuals were given the same battery of screens and tests as the nuclear atopic families.

Asthma diagnosis (study groups A and B)

Following the former CSGA ascertainment criteria [12], subjects were categorized as positive for asthma if they had characteristic asthma symptoms, a physician's diagnosis of asthma and positive results for bronchial hyper-reactivity and/or bronchial reversibility.

Asthma and bronchial hyper-reactivity screens (study groups A and B)

Each subject was given a nurse-administered, standardized questionnaire, prepared according to American Thoracic Society (ATS) guidelines, regarding history of asthma and other respiratory diseases as described previously [12]. Baseline spirometry measures for forced expiratory volume in 1 s (FEV₁) were made according to ATS guidelines, followed by reversibility and or a provocative methacholine challenges [12]. Bronchial hyper-reactivity (BHR) was recorded if there was a decrease of 20% or more in FEV₁ after methacholine challenge (≤ 25 mg/mL maximum dosage), or reversibility of 15% or greater after administration of albuterol. We used either BHR and/or bronchial reversibility as part of the criteria of asthma as this is what the CSGA decided. We wanted to keep this definition constant.

Chronic obstructive pulmonary disease patients. This group comprised 100 Caucasian males randomly selected from an on-going lung health survey (LHS) who had evidence of spirometric signs for early COPD [13]. Written informed consent was also obtained from this group.

Chronic obstructive pulmonary disease diagnosis (study group C)

Participants were cigarette smokers, 35–60 years of age at time of entry into LHS, with borderline to moderate airflow obstruction defined as a ratio of 1-s forced expiratory volume to forced vital capacity of <0.7 , together with a baseline 1-s forced expiratory volume that was 55–90% of the value predicted for the participant's age, sex, height and race.

Immunoglobulin E screens

Serum total immunoglobulin E tests. Serum total IgE tests were done using the ACCESS Immunoassay System[®] (Beckman Coulter, Chaska, MN, USA), according to manufacturer's specifications. All IgE results are reported as IU/mL (1 IU = 2.4 ng).

Skin prick tests. Skin prick testing (SPT) was done as described previously [12] using 14 standardized allergen extracts for dust mites [2], ragweed, moulds [3], trees [4],

grasses [2] and animal danders [2]. A positive SPT result was recorded for a weal area ≥ 9 mm² above negative saline control.

Enzyme linked immunosorbent assay for serum leukolysin

Serum leukolysin was measured using modifications of 'reverse sandwich' ELISA. Anti-leukolysin monoclonal antibodies (mAb) were developed in guinea-pigs and rabbits as described previously, as were leukolysin standards [7, 8]. Briefly, a 'capture antibody' was guinea-pig IgG anti-leukolysin that was prepared at a concentration of 0.5 µg/mL in 50 mM carbonate buffer, pH 9.6. The wells of 384-well opaque microtest plates (Costar #3705, Corning, Corning, NY, USA) were coated with this mAb and incubated for 5 h at 37 °C, followed by thorough washing in PBS with 2% Tween-20. Non-specific binding sites were blocked for 2 h at 37 °C with 2% BSA. Serum or serially diluted standard was added, and plates incubated for 2 h at 37 °C. 'Detection antibody' was rabbit anti-leukolysin that was added for 2 h at 37 °C. To generate a response signal, goat anti-rabbit antibody conjugated with alkaline phosphatase (Sigma-Aldrich, St Louis, MO, USA) was added for 1 h at 37 °C. After washing, a chemiluminescent substrate was added (Lumi-Phos 550[®], Beckman Coulter, Chaska, MN, USA), and the chemiluminescent signals were read with a Tropic TR717 luminometer (Tropic Inc., Bedford, MA, USA). On a log-log scale, this assay gave a linear response of 0.05–100 ng/mL.

Enzyme linked immunosorbent assay for serum interleukin-1 levels

ELISA for measurement for IL-1 was performed in a similar fashion as used for serum leukolysin levels but we used an anti-IL-1 mAb and assay obtained from R&D systems.

Statistics

Comparisons between two group means were made by two-sided *t*-tests (significance level = 0.05). Multiple group comparisons used analysis of variance (ANOVA) with a 2×2 factorial design. Age effects for leukolysin were assessed by simple linear regression (SLR) and evaluated by ANOVA. Statistical analyses used commercial software: Microsoft Excel[®] (Redmond, WA, USA) or SPSS[®] version 10.0 for Windows[®] (SPSS Inc., Chicago, IL, USA).

Variance components analysis was used to partition total phenotypic variance (V_P) into additive genetic (V_A) and non-genetic, environmental components (V_E): $V_P = V_A + V_E$. The environmental component was further partitioned into two components for common family environment, V_{CE} , and common sibling effects, V_{CS} . The

narrow-sense heritability (h_N^2) was defined as the ratio of the variance due to additive genetic effects to all sources of phenotype variance: $h_N^2 = V_A / (V_A + V_{CE} + V_{CS})$.

As described previously [14], the variance components were determined from calculations of the covariances (COV) about common mean values for quantitative traits for three kinship pairings (spouse–spouse, parent–child and sib–sib). This results in three algebraic equations that can be simultaneously solved for three unknown values, namely

$$\text{COV (spouse–spouse)} = V_{CE}, \quad (1)$$

$$\text{COV (parent–child)} = 1/2V_A + V_{CE}, \quad (2)$$

$$\text{COV (sibling–sibling)} = 1/2V_A + V_{CE} + V_{CS}. \quad (3)$$

The COV determinations were made using standard statistical software (Microsoft Excel[®] and SPSS[®]).

Results

Study population leukolysin distributions

We screened three separate study groups, including (1) 337 people from 94 nuclear families with history of atopic asthma, (2) 122 married-in individuals from previous asthma genetics studies and (3) 100 randomly selected patients with diagnosis of COPD. Each person was tested for respiratory disease history, respiratory function by standardized spirometry and serum levels of the MMP leukolysin (MMP-25/MT-6) and total IgE. The nuclear families and the married-in group were also screened for sensitivity to allergens by standardized SPT using a battery of 14 common aeroallergens. The demographic characteristics for these two study groups (1 and 2) are given in Table 1.

For all people screened, without regard for clinical status, serum leukolysin values were approximately normally distributed on a logarithmic scale. Overall mean log-value = 1.02 ng/mL (standard deviation = 0.40; $N = 559$).

There were no differences in mean log[leukolysin] levels for gender. Females' mean = 1.04 (standard

deviation = 0.41; $N = 213$). Males' mean = 1.03 (standard deviation = 0.40; $N = 346$). t -value = 0.22; P -value = 0.41.

In the atopic nuclear families, subjects ranged in age from 6 to 75 years. By SLR, there was a slight, although statistically significant, decline with age in log(leukolysin) values: $\log[\text{leukolysin}] = 0.85 - 0.008 \times (\text{age})$. $F(1, 335) = 38.5$; $P < 0.001$. (On average, children's leukolysin levels were higher than those of the parents in this study group.) There was no significant age effect in the COPD patient or married-in groups.

Overall, serum leukolysin levels were roughly equally distributed in the three study groups, with no detectable differences due to gender and only a minimal decline with age in the nuclear families.

Leukolysin and interleukin-1 levels

As *in vitro* studies suggest that cytokine IL-1 has the ability to activated leucocytes. We checked for the correlation between IL-1 and leukolysin. We found that the regression curve was excellent with a R^2 of 0.987.

Leukolysin levels and atopic outcomes

In the nuclear families and the married-in group, we categorized subjects according to the results of their asthma symptoms, spirometry and atopy screens. This resulted in four categories based upon negative or positive responses for asthma symptoms with BHR (defined in 'Material and methods') and Atopy based upon results of standardized percutaneous SPT results. Part A of Table 2 gives the comparisons of mean log(leukolysin) values based upon these categories for these two groups. As a comparison, part B of Table 2 shows the results for serum log(total IgE), a trait whose values are known to vary by these clinical categorizations.

We analysed possible differences in group mean values by ANOVA using a 2×2 factorial design in order to determine main effects (SPT results or asthma with BHR) and a possible interaction effect. As shown in part A of Table 2 for log[leukolysin] values, only SPT [+] results showed a significant effect. Log[leukolysin] values were not significantly influenced by asthma with BHR, and there was no interaction effect between SPT results and asthma/BHR.

Part B of Table 2 for log[total IgE] values shows that there were main effects for both SPT results and asthma with BHR, and there was a significant interaction among these factors. Those with any SPT [+] result had higher log[total IgE] values than those who were SPT [–], and values were highest among those with both SPT [+] and positive for asthma with BHR results.

Among those who were SPT [+], those who had positive responses for asthma symptoms+BHR had increased levels of both serum leukolysin and total IgE. This was due, in part, to an increased average number of SPT [+]

Table 1. Nuclear family study population

Dermographics	Nuclear families	Asthma study 'married-in'
Total N	337	122
Female	161	52
Male	176	70
Mean age [range]	28.1 years [6–75]	48.8 years [23–74]
% Asthma symptoms	79.3%	53.3%
% BHR [+]	61.0%	19.7%
% Asthma+BHR [+]	58.6%	14.8%
% Any SPT [+]	63.7%	52.5%

BHR, bronchial hyperreactivity; SPT, (percutaneous) skin prick test.

Table 2. Serum leukolysin and IgE levels by atopic status*

Group	Asthma and BHR [+]	SPT [+]	Mean (ng/mL)	Standard deviation	N
<i>(A) Log[leukolysin]</i>					
A	No	No	0.94	0.47	101
B	Yes	No	0.94	0.41	54
C	No	Yes	1.00	0.40	119
D	Yes	Yes	1.13	0.12	185
<i>ANOVA results[†]</i>					
(1) SPT effect	$F(1, 455) = 10.1, P = 0.002$				
(2) Asthma and BHR effect	$F(1, 455) = 2.4, P = 0.13$				
(3) Interaction effect	$F(1, 455) = 2.4, P = 0.13$				
<i>(B) Log[total IgE]</i>					
A	No	No	1.24	0.30	101
B	Yes	No	1.36	0.43	54
C	No	Yes	1.81	0.40	119
D	Yes	Yes	2.25	0.36	185
<i>ANOVA results[†]</i>					
(1) SPT effect	$F(1, 455) = 136.1, P < 0.001$				
(2) Asthma and BHR effect	$F(1, 455) = 20.2, P < 0.001$				
(3) Interaction effect	$F(1, 455) = 6.3, P = 0.012$				

*Subjects from nuclear families and married-in group.

[†]ANOVA main effects and interaction effect for 2×2 factorial design.

results. Asthma and BHR [−]: mean # SPT [+] = 3.8 (standard deviation = 2.35). Asthma and BHR [+]: mean # SPT [+] = 4.9 (standard deviation = 3.12). $t(302) = 2.51$; $P = 0.006$. However, this was a generalized phenomenon and not 'pathway-specific' as there was no relationship between serum leukolysin levels and the # SPT [+] results as assessed by SLR.

$$\log[\text{leukolysin}] = 1.04 + 0.009 \times [\# \text{SPT}(+)] ; F(1, 302) = 1.56 ; P = 0.21.$$

Taken together, these results suggest that factors common to sensitivity to allergens (any SPT [+] result) and predisposition to asthma (asthma symptoms+BHR [+]) enhance the levels of serum leukolysin comparable to what is observed for total IgE values.

Leukolysin levels and pulmonary function test 'Indices of Asthma Severity'

We next sought to determine if there were any associations between serum leukolysin levels and various indices of pulmonary function test (PFT) parameters associated with 'asthma severity'. We used the results for those with characteristic asthma symptoms who also had BHR [+] results after methacholine challenges. We made comparisons of three PFT indices to serum leukolysin levels by SLR.

$$\% \text{ Predicted FEV}_1 = 86.1 + 0.21 \times [\text{leukolysin}] ; F(1, 228) = 0.66 ; P = 0.42.$$

$$\text{PD}_{20} = 9.74 + 0.199 \times [\text{leukolysin}] ; F(1, 228) = 1.63 ; P = 0.20.$$

$$\begin{aligned} \text{M.C. PFT Slope} &= 15.39 + 0.069 \\ &\times [\text{leukolysin}] ; F(1, 228) \\ &= 0.05 ; P = 0.85. \end{aligned}$$

There was no evidence of associations between serum leukolysin values and PFT parameters for asthma with BHR [+] responses.

Possible heritability of serum leukolysin levels

Serum total IgE levels have been shown to be influenced by both inherited and environmental components. To determine if there was any inherited component to serum leukolysin values, we made basic determinations for variance components analysis. For this we calculated the COV about common mean leukolysin values for three kinship groups in the nuclear families: spouse-spouse, parent-child and sibling-sibling. These COV determinations can be used to dissect genetic and environmental variance components for quantitative traits [14].

The following estimates were made for the atopic nuclear families' serum leukolysin levels: (1) spouse-spouse COV = 0.027, (2) parent-child COV = 0.027 and (3) sibling-sibling COV = 0.034. Simple algebraic rearrangement of these values and their expected theoretical values shows that $h^2 \approx 0.0$.

For comparison, estimates were also obtained for serum total IgE in this study population: (1) spouse-spouse COV = 0.027, (2) parent-child COV = 0.092 and (3)

sibling-sibling COV = 0.209. Based upon these values, the estimated heritability for serum total IgE in these families was: $h^2 \approx 47.8\%$. (This estimate was not adjusted for age or gender effects.)

Although there was heritability for serum IgE level there is no apparent heritability to serum leukolysin levels in these nuclear atopic asthmatic families. The COV results can be attributed to environmental factors, which in this case could include the presence of atopic allergen sensitization and/or asthma.

Serum leukolysin and total immunoglobulin E levels in atopic asthma and chronic obstructive pulmonary disease

Leukolysin is produced in and released from granulocytic myeloid series cells, primarily neutrophils, while IgE is produced by lymphoid series cells (B cells, plasma cells). Thus, it seems unlikely that these molecules would share common metabolic pathways for their *de novo* production. Yet, the results in Table 2 suggest that they may share common elements that either enhances their production or their release.

We compared serum leukolysin and total IgE levels in the three study groups (nuclear families, COPD patients and married-in group) as, from the results of Table 2, a possible association between serum leukolysin and IgE levels was apparent. In particular, those people with asthmatic respiratory involvement and elevated total IgE levels also had elevated serum leukolysin.

Because all of the COPD patients had history of cigarette smoking, we first had to rule out a possible effect of smoking on leukolysin levels. As shown in Table 2 for the nuclear family and married-in groups, leukolysin levels were elevated for those who had any SPT [+] result compared with those who were SPT [–]. We subdivided these people into four groups based upon SPT [–] and SPT [+] results, and those with and without smoking history (>1 pack-year). Using a 2×2 factorial design, ANOVA showed no significant main effect for smoking: $F(1, 442) = 2.5$; $P = 0.11$.

Because the COPD patients were not screened for sensitivity to allergens by skin testing, we categorized subjects in the study groups has being 'low' IgE or 'high' IgE producers using an arbitrary cut-off point of 100 IU/mL ($\log[\text{total IgE}] = 2.0$), as it was most likely that people with total IgE values greater than this were also sensitized to one or more allergens. The results for these categorizations are shown in Table 3.

In part A of Table 3 ('low' IgE) and part B ('high' IgE), there was no difference in mean leukolysin values comparing the three study groups against one another as assessed by ANOVA. For each of the three study groups, we next compared mean $\log[\text{leukolysin}]$ values for 'low' and 'high' IgE producers by two-sided *t*-tests. These results are shown in part C of Table 3.

Table 3. Log[leukolysin] values in study groups

Group	Mean (ng/mL)	Standard deviation	N
<i>(A) Low log[total IgE]*</i>			
Nuclear families	0.97	0.39	178
COPD patients	0.94	0.42	69
Married-in	0.99	0.20	93
$F(2, 337) = 0.29^{\dagger}$, P -value = 0.75			
<i>(B) High log[total IgE]*</i>			
Nuclear families	1.12	0.36	159
COPD patients	1.09	0.31	31
Married-in	1.04	0.42	29
$F(2, 216) = 0.59^{\dagger}$, P -value = 0.55			
Group	t -Test value (d.f.)		P -value
<i>(C) Log[leukolysin] t-test comparisons[‡]</i>			
Nuclear families	3.70 (335)		0.0001
COPD patients	2.01 (69)		0.024
Married-in	0.63 (50)		0.27

*'Low' total IgE: $\log[\text{IgE}] < 2.0$; 'high' total IgE: $\log[\text{IgE}] > 2.0$.

[†]*F*-test ratio for group comparisons by ANOVA.

[‡]Comparison of mean values for 'low' vs. 'high' total IgE by two-sided *t*-tests assuming unequal group variances.

Surprisingly, changes in serum leukolysin levels by clinical category closely paralleled those of total IgE in both those with either atopic asthma or COPD. In both the nuclear families and the COPD patients mean serum leukolysin levels were higher among those people who also had elevated (high) total IgE values. Overall, elevated serum leukolysin levels parallel those of total IgE among people with history of atopic asthma or COPD. These results suggest that among people with chronic airways inflammation, there are common factors that stimulate and activate cells of both the myeloid and lymphoid series. Further, elevated serum leukolysin levels may be a metabolic marker associated with chronic atopy-associated airways inflammation.

Discussion

The results of this study are, to the best of our knowledge, the first report of the presence of a novel MMP of the membrane-type, designated leukolysin (MMP-25/MT-6) [7, 8] associated with chronic human respiratory diseases. The positive association between serum leukolysin levels and a marker known to be elevated in human atopic asthma (total IgE) indicates, at the least, that there are common factors in this inflammatory condition inducing elevated production and/or release of these metabolites. If these observations are borne out in subsequent studies, leukolysin may prove to be a metabolic marker associated with atopic asthma, as well as COPD patients who are also atopically sensitized, that can be measured in human serum.

MMP are Zn^{2+} -dependent endopeptidases ubiquitously distributed in tissues and cells that operate at neutral pH. While their initial discovery and characterization was based upon their catalytic activities against ECM components, their biological roles encompass a wide spectrum of activities, including embryogenesis, maintenance of tissue architecture, wound healing, host defence and others [1, 2]. Their production release and catalytic activities are tightly regulated as, although they play vital roles in maintaining normal homeostatic balances in a wide range of settings, it is thought that disruption of regulatory controls can lead to tissue damage or pathological restructuring, such as what occurs in chronic respiratory inflammatory states [4–6].

Additionally, as with other MMPs, leukolysin can alter the activities of other enzymes by cleavage of a latent pro-enzyme form of MMP-2 [15], or inactivation of proteinase inhibitors [16]. Countering its regulatory function(s), leukolysin itself is subject to regulation by members of a class of tissue inhibitors of metalloproteinases TIMP-1, -2 and -3 [9]. Again, as with other MMPs, little is known of its activities or regulation *in vivo*.

As shown in Table 2, among members of families with positive history of atopic asthma, serum leukolysin levels are elevated in those with any evidence of sensitivity to common aeroallergens (any SPT [+] result), and are highest among those with both atopy (SPT [+]) and a positive screen for atopic asthma (asthma symptoms plus BHR [+] response). In these nuclear atopic families, these trends parallel those of serum total IgE, a marker known to be elevated in atopic asthmatic pathology.

This trend was due, in part, to greater sensitivity to allergens among some people. Among those with any SPT [+] result, serum leukolysin levels were higher among those with asthma symptoms and BHR [+] results, compared with those with asthma symptoms but who were BHR [–]. The average # SPT [+] results for those who were asthma/BHR [+] were higher, although there was no relationship between serum leukolysin levels and the # SPT [+] results.

It is realized that the first group of patients consisted of nuclear families with atopic asthma had patients between 6 and 75 years of age. Important differences may exist between asthma in children and adults. The phenotype of asthma and atopy may change with age. In addition the COPD group consisted of only adult males who smoked. We used the married-in individuals as surrogate for the at large population. Interpretation of our results may be influenced by many variables including the ages, sexes and smoking history of the participants.

Serum leukolysin levels did not provide evidence that it is an inherited trait, as an estimate of its narrow-sense heritability was $\approx 0\%$. In contrast, in the nuclear asthmatic families, a rough estimate of the narrow-sense heritability for total IgE was $\approx 48\%$. In conjunction with

the other experimental findings, this suggests that leukolysin is released due to conditions associated with atopic asthma, but is not a predisposing factor (i.e. its release is a consequence, not a cause). This is also borne out, in part, by a lack of association between serum leukolysin levels and pulmonary test results reflective of 'asthma severity', like percent predicted FEV₁ or methacholine PD20 values.

Because leukolysin is produced in cells of the granulocytic myeloid series (e.g. neutrophils) while IgE is produced by lymphoid series cells (B cells, plasma cells), it seems unlikely that common factors directly could influence their *de novo* production. However, this does not rule out the possibility that they share factors that can enhance their production or release from their respective cell lineages. Another intriguing possibility is that they may be 'metabolically linked', at least within people with atopic asthma.

The regulation of IgE production in humans is controlled through several interconnected metabolic pathways [17]. Among these is regulation via the low affinity IgE receptor (Fc ϵ RII/CD23) present on lymphoid series cells and others. When CD23 is bound by IgE, IgE production is inhibited. However, if the IgE-receptor bond is disengaged, or if the receptor is cleaved from the cell surface by enzymatic activity, IgE production is enhanced.

A number of enzymes have shown this ability to participate in the regulation of IgE production [18, 19]. Although not connected to IgE production *per se*, a membrane-bound metalloproteinase produced by activated neutrophils has been shown to be responsible for enzymatic cleavage of CD23 [20]. However, though not specifically identified, the MMP in this report was an integral membrane type MMP, and not a GPI-anchored enzyme like leukolysin. This does not rule out the possibility of a leukolysin-CD23 connection as it has not been tested. Our results suggest that there are common factors that act upon cells of the granulocytic myeloid series (leukolysin release) and lymphoid series (IgE) among people with history of chronic airways inflammation. Further, serum leukolysin may be a novel metabolic marker associated with atopic asthma or COPD patients who are also atopically sensitized.

In summary, these results point to a novel MT-MMP, leukolysin, as a candidate metabolic marker found in serum associated with atopic asthma and COPD patients who are also atopically sensitized to allergens. Also, due to its limited cellular distribution, it is an excellent model for studying MMP production and its regulation *in vivo*.

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