Published in final edited form as:

J Allergy Clin Immunol. 2011 November ; 128(5): 1124–1127. doi:10.1016/j.jaci.2011.07.046.

Plasma metalloproteinase levels are dysregulated in signal transducer and activator of transcription 3 mutated hyper-IgE syndrome

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To the Editor:

Hyper-IgE syndrome (HIES) is caused by a heterozygous mutation in signal transducer and activator of transcription 3 (*STAT3*)^{1,2} and characterized by increased serum IgE levels along with dermatitis, boils, cyst-forming pneumonias, retained primary dentition, bone abnormalities, and coronary artery aneurysms.³ Because the HIES morphotype suggests abnormal tissue remodeling and *STAT3* is known to regulate matrix metalloproteinases (MMPs) for extracellular matrix turnover and degradation, especially in the lungs,^{4,5} we investigated MMP levels in plasma from patients with HIES.

Patients with heterozygous *STAT3* mutations were enrolled in National Institute of Allergy and Infectious Diseases protocols, and they or their parents provided informed consent. The control subjects were healthy blood bank donors on appropriate National Institutes of Health protocols. Plasma samples were analyzed with the Bio-plex (Bio-Rad Laboratories, Hercules, Calif) to determine MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12, and MMP-13 concentrations. We tested a single stored plasma sample from each patient with HIES, each of which was collected at a time when the patient did not have a known infection. For statistical analysis, we used the Mann-Whitney *U* test (GraphPad Prism; GraphPad Software, Inc, La Jolla, Calif). Because of multiple comparisons, we used the Bonferroni correction (dividing the standard significance level of .05 by the 6 comparisons performed), requiring a *P* value of less than .0083 to achieve statistical significance.

Of our 37 patients with HIES, 19 were male subjects; 23 had mutations in the DNA-binding region and 14 had mutations in the SH2-binding region of *STAT3*. The mean age was 32.6 years (95% CI 27.1–37.69 [SD 6.14.69 years]), and 5 patients were less than 16 years of age. MMP-1 levels (patients, 1,323 pg/mL [SE, 172.3 pg/mL]; control subjects, 803.7 pg/mL

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Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

[SE, 141.8 pg/mL]) were higher in patients ($P = .0130$, Fig 1), whereas MMP-2 levels (patients, 122,148 pg/mL [SE, 4,044 pg/mL]; control subjects, 139,426 pg/mL [SE, 7,870 pg/mL]; $P = .0164$) were lower, but neither achieved the required level for significance. MMP-3 levels were significantly lower in patients (6,196 pg/mL [SE, 1,240 pg/mL]) than in control subjects (15,624 pg/mL [SE, 1,596 pg/mL], $P < .0001$). MMP-7 levels did not differ. MMP-8 levels were significantly higher in patients (6,336 pg/mL [SE, 1,268 pg/mL]) than in control subjects (2,206 pg/mL [SE, 318.4 pg/mL], $P = .0025$). MMP-9 levels were also significantly higher in patients (152,295 pg/mL [SE, 24,717 pg/mL]) than in control subjects (54,975.7 pg/mL [SE, 6,023 pg/mL], $P = .0004$). MMP-12 and MMP-13 were undetectable in all samples.

Because there is evidence for age-related changes in plasma MMP levels during childhood,⁶ we repeated our analyses without the 6 patients younger than 16 years. Even without the youngest patients, the differences remained significant, suggesting that age did not skew the data. Our secondary analyses comparing SH2- and DNA-binding domains showed no differences in MMP levels. We also found no association between patients' MMP levels and total HIES scores⁷ using regression analysis for MMP-3, MMP-8, and MMP-9. We found no organ- or system-specific HIES manifestations correlated with MMP levels (eg, bone disease and MMP-3 levels, skin involvement and MMP-8 levels, pneumatocoles and MMP-3 and MMP-9 levels, or aneurysms and tortuosity with MMP-9 levels). We generated a heat map displaying the patient-MMP pattern relative to the mean level for each MMP (Fig 2). Patients with HIES and control subjects were clustered separately. Most patients with HIES with relatively high MMP-8 and MMP-9 levels had low MMP-3 levels. Control subjects had relatively low MMP-8 and MMP-9 levels but higher MMP-3 levels. To investigate the variability or fluctuation of MMP levels over time, we tested multiple samples from 8 patients with HIES collected and stored independent of infection or surgical status over a period of 20 years. Fluctuations were not wide, and there was reasonable stability over time (Fig 1).

Median MMP-8 and MMP-9 levels were approximately 3-fold higher in patients than in control subjects, and both of these MMPs are involved in tissue remodeling after acute lung injury.⁴ MMP-8 promotes acute inflammation in the lungs, whereas MMP-9 has an important role in vascular smooth muscle cell migration, macrophage infiltration, and elastolytic activity and has been associated with abdominal aortic aneurysms.⁸ Therefore increased MMP-8 and MMP-9 levels in patients with HIES might be etiologically linked to the disease-specific predilection to aneurysm development and postinfectious lung injury.

In contrast, MMP-3 is an important proteinase for vascular and cardiac matrix remodeling and was detected at only about one third the normal level in patients with HIES. MMP-3 regulation has a wide range of actions influencing fibrinolysis and angio-genesis. In a murine cell line model, mutated *STAT3* has been shown to cause a decrease in MMP-3 production.⁹ Therefore increased MMP-8 and MMP-9 levels and reduced MMP-3 levels in patients with HIES might be implicated in formation of aneurysms or pneumatocoles.

Mutations in *MMP2* disrupt skeletal and craniofacial development and decrease bone mineralization because of defects in osteoblasts and osteoclasts. The failure to identify

significant abnormalities in levels of MMP-2 and other MMPs might reflect that there are no significant differences between patients and control subjects or that resting plasma is not the correct compartment or situation. STAT3 plays a significant role in binding to the *MMP2* promoter and can exert transcriptional control over other MMPs as well. To better understand the potential regulatory roles of *STAT3* on MMP expression, we used positional weight matrix analysis and identified multiple STAT3-binding sites throughout the MMP promoters.¹⁰ However, we only found significant differences in plasma levels of MMP-3, MMP-8, and MMP-9. Although MMP-9 has an experimentally proved STAT3-binding site upstream of exon 1, *MMP3* and *MMP8* have positional weight matrix–predicted binding sites upstream and downstream of their first exons, respectively.

MMP-3, MMP-8, and MMP-9 levels were significantly different between patients with HIES and control subjects and might contribute to some of the characteristic features of HIES. Abnormal STAT3 signaling in HIES and the role for STAT3 in MMP regulation provides an attractive link between the manifestations of HIES and MMP regulation by STAT3. *In situ* tissue expression and regulation of MMPs in patients with HIES might be more relevant. Understanding MMP regulation in patients with HIES might guide efforts to prevent or mitigate some of the disease's most threatening end-organ effects.

Acknowledgments

Supported by the Division of Intramural Research, National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, Md. The views expressed in this article are those of the authors and do not reflect the official policy of the US Government.

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FIG 1.

Meanplasma concentrations of MMP-3, MMP-8, and MMP-9 are compared between control subjects and patients with HIES (*left panels*). MMP-3 levels are significantly lower in patients with HIES, whereas MMP-8 and MMP-9 levels are significantly higher. Changes over time for MMP-3, MMP-8, and MMP-9 levels are plotted against normal mean values (*dotted line*) for 8 patients from whom multiple stored frozen plasma samples were retrieved over a duration of 20 years (*right panels*). Each line represents a patient.

FIG 2.

Heat map analysis of MMP-3, MMP-8, and MMP-9 showing clustering of patients (*red*) and control subjects (*purple*). MMP-8 and MMP-9 cluster together, and patients' levels are significantly higher than those of control subjects. MMP-3 is independent of MMP-8 and MMP-9.