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Association of Macrophage Migration Inhibitory Factor (MIF) Polymorphisms with Risk of Meningitis from *Streptococcus pneumoniae*

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Abstract

Macrophage migration inhibitory factor (MIF) is an upstream proinflammatory cytokine encoded by a functionally polymorphic locus. This study of 119 patients explored the potential relationship between MIF genotype and invasive *Streptococcus pneumoniae* infections. We observed an association between a high-expression *MIF* allele and occurrence of pneumococcal meningitis.

Keywords

Macrophage migration inhibitory factor; cytokine; polymorphism; meningitis; inflammation

1.1 Introduction

Macrophage migration inhibitory factor (MIF) is an innate cytokine with diverse activating functions within the host immune response [1]. Clinical studies of sepsis demonstrating elevation of circulating MIF levels and an association between high MIF levels and fatal outcomes support an important role for MIF in human infections [2].

The promoter region of the human *MIF* gene contains two polymorphisms. A variable nucleotide tandem repeat at position –794 comprises five to eight CATT repeats (referred to henceforth by numbers from 5–8, rs5844572) [3]. Gene reporter assays show a proportional increase in transcription with CATT repeat number; the 5-repeat allele leads to low expression, and the 6-, 7-, and 8-repeat alleles lead to correspondingly higher expression of

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Conflicts of interest: US patent WO/2006/116688, MIF Agonists and Antagonists and Therapeutic Uses Thereof (Yale University).

MIF [4]. High-expression (non-5) *MIF* alleles are associated with the severity or incidence of a variety of autoimmune and inflammatory diseases [5]. There also appears to be an association between high-expression *MIF* alleles and infection, specifically *Pseudomonas* carriage and inflammatory lung damage in patients with cystic fibrosis [6]. A second *MIF* promoter polymorphism comprises a G-to-C single nucleotide polymorphism (SNP) at position -173 (rs755622), which is in strong linkage disequilibrium with -794 7-CATT and is associated with arthritis clinical severity and higher serum and synovial fluid MIF levels [7,8]. This allele also has been reported to confer improved survival in patients with outpatient pneumonia [9].

Streptococcus pneumoniae is a common cause of infection in the human host. Pneumococci reside in the nasopharynx of many individuals, especially during winter, without causing disease. What determines susceptibility to invasive disease versus nasopharyngeal carriage or localized respiratory infection is unclear, although both host and microbial factors likely play a role. In the present report, we performed an initial examination of whether *MIF* promoter genotype is associated with development of meningitis in patients hospitalized with invasive *Streptococcus pneumoniae* infections.

2.1 Methods

This study was comprised of two groups of patients, one from the USA (State of Connecticut) and the other from Germany (State of Schleswig-Holstein).

The USA study population consisted of 30 hospitalized adult patients (16 M, 14 F) over 18 years of age (range: 28–87 years) with positive cultures for *Streptococcus pneumoniae* isolated from sterile body fluids. Subjects were identified consecutively in a prospective manner by the microbiology laboratories at the Hospital of Saint Raphael, Saint Francis Hospital, and Yale-New Haven Hospital between September 2004 and September 2005. Written informed consent was obtained from all subjects or their surrogates. The respective hospital Investigational Review Boards and the Yale Human Investigation Committee approved the study protocol. Classification of a case as meningitis among the USA cohort was based either on positive pneumococcal cultures grown from the cerebrospinal fluid (CSF) or from treating physician diagnosis of meningitis based on clinical symptoms and signs and lumbar puncture results plus positive blood cultures.

The German study population included 89 adult Caucasian patients (52 M, 37 F) over 18 years of age (range: 24–96 years) with positive blood, CSF, bronchoalveolar lavage, sputum/tracheal secretion, and/or pleural fluid cultures growing *Streptococcus pneumoniae*. Subjects were identified at three German hospitals in a consecutive and prospective manner between December 1998 and May 2004. The study was approved by the local ethics committee, and written informed consent was obtained from subjects or their surrogates. Cases of meningitis were defined by clinical presentation and a marked neutrophilic pleocytosis in the CSF and/or detection of pneumococci in CSF gram stain or culture.

Genomic DNA from patient serum was isolated per manufacturer protocol using DNAzol Reagent (Invitrogen) or from dried, whole blood-spotted filter paper using the QIAmp DNA Blood Mini kit (Qiagen). Genomic DNA was amplified by multiple displacement amplification (MDA) overnight according to previously described methods [10]. The -794 CATT repeat and the -173 G/C SNP in the *MIF* gene were analyzed at the Yale University Keck Affymetrix Facility using an ABI Prism 7900 machine, and the allelic data were analyzed with SDS v2.2 software. Levels of MIF in the plasma of subjects were measured by sandwich ELISA [11].

The data were analyzed using GraphPad Prism software (GraphPad Software, La Jolla, CA), STATA 11 (StataCorp, College Station, TX), and Microsoft Excel (Microsoft, Redmond, WA). Differences between two means were calculated using independent two-tailed t-tests. Distributions were presumed to be normal unless noted in the text. The relationship between genotype and disease type was examined using the Fishers' exact test for contingency tables containing small numbers. Prevalence ratios are reported for the relationship between genotype and meningitis since this study was cross-sectional. A p-value of less than 0.05 was defined as statistically significant for all tests.

3.1 Results

A total of 30 samples from the USA population and 89 samples from the Germany population were obtained. Of those 119, allelic data was obtained on 106 samples for the -794 CATT repeat and on 108 samples for the -173 G/C SNP. As noted in Table 1, we observed an association between the 7-CATT allele and *Streptococcus pneumoniae* meningitis ($p=0.02$, PR = 3.344 [1.339–8.353]). There was no association between the -173 (G/C) SNP and rates of meningitis ($p = 0.76$, PR = 1.242 [0.462–3.340]).

MIF levels were measured in the USA cohort only. The mean MIF level for all subjects was 20.4 ± 13.13 ng/ml (range = 5–48 ng/ml). This mean level is considerably higher than that reported previously by our laboratory for non-infected, disease-free individuals (mean MIF levels over 24 hours 1.8–4.5 ng/ml, standard deviation 0.4–2.1, $n = 5$) [12]. MIF levels did not differ significantly with central nervous system (CNS) invasion ($p = 0.52$), 5-CATT allele carriage ($p = 0.30$), or 7-CATT allele carriage ($p = 0.27$).

4.1 Discussion

In this first examination of *MIF* alleles in the clinical expression of a gram-positive infection, we observed an association between the high-expression 7-CATT *MIF* allele and incidence of meningitis. The potential association between high-expression *MIF* genotypes and meningitis supports a role for the inflammatory pathogenesis of invasive *S. pneumoniae* disease. One manner in which MIF may increase risk of meningitis is via promotion of expression of adhesion molecules in the cerebral vascular endothelium. MIF up regulates the expression of intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) in different animal models of inflammatory disease, and MIF has been shown to disrupt the blood brain barrier leading to neuro-invasive flavivirus infection [13,14]. In experimental models of *S. pneumoniae* meningitis, the innate immune system plays an important role in both adherence to endothelium via the laminin receptor and penetration of the blood brain barrier via the platelet activating factor pathway [15,16]. Upregulation of these molecules by MIF or a downstream cytokine may result in a more leaky blood brain barrier and predispose to central nervous system invasion.

The finding of an elevated level of circulating MIF in all patients in this study is in accord with research showing higher median MIF levels among patients with septic shock (17.8 ng/ml) and sepsis (12.2 ng/ml) as compared with healthy controls (3 ng/ml) [17]. MIF levels in the present study did not differ by genotype or by CNS invasion, which may be attributed to an infection-related induction in MIF production that is greater than the influence of particular alleles or the presence of CNS disease. Sera were collected from study subjects only at single time points and at different times after the onset infection, which makes comparisons difficult. Finally, the plasma compartment is an imperfect measure of cytokine production at the site of tissue infection, where allele-specific effects may predominate. Future studies exploring the relationship between MIF levels, genotype, and meningitis are

warranted and should incorporate serial sampling over the full clinical course of the infection.

Our conclusion of a possible association between *MIF* genotype and *S. pneumoniae* meningitis must be tempered by the methodological limitations of this exploratory study. First, we did not evaluate a control population of racially/ethnically-matched individuals who were admitted to the hospital with fever but no positive blood cultures or contemporaneous racially/ethnically-matched healthy control individuals. Second, our patient identification and selection prevented the inclusion of those with fulminant disease (i.e., who died before enrollment) or with mild disease (i.e., who were discharged before culture documentation). As a result, our study population represented those with moderate-severe disease. Posthumous collection of patient samples as well as the enrollment of discharged patients would have helped to eliminate this bias. Third, our analyses included two separate populations: one from Germany and another from the USA. Although this may impact the results because of the possibility of different gene frequencies in the two populations, the prevalence of *MIF* alleles appear similar in Caucasian populations previously studied in the USA and Europe [18].

4.1.1 Conclusion

In diseases with a complex genetic basis, it is likely that multiple polymorphisms contribute to susceptibility for a particular individual. While association studies can be useful in elucidating which genes play a role, eventually the contribution of multiple different genes will need to be examined in individual patients. Nonetheless, these data point to an intriguing link between *MIF* promoter polymorphisms and incidence of *Streptococcus pneumoniae* meningitis. In the future, individuals with certain genetic profiles may be candidates for earlier preventative interventions and more specific targeted therapies.

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Table 1

Association between genotype and disease type.

Genotype (n)	Meningitis, # (%)	No meningitis, # (%)
CATT genotype (106)		
XX	7 (8.9)	72 (91.1)
77 or 7X	8 (29.6) [*]	19 (70.4)
SNP genotype (108)		
CC or GC	5 (16.1)	26 (83.9)
GG	10 (13.0) ^{**}	67 (87.0)

X = 5 or 6 allele. No subjects were found to have the 8 allele.

^{*}
p = 0.02 for comparison between CATT genotypes.^{**}
p=0.76 for comparison between SNP genotypes.