SUPPLEMENT ARTICLE

Macrophage Migration Inhibitory Factor: Gene Polymorphisms and Susceptibility to Inflammatory Diseases

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The cytokine macrophage migration inhibitory factor (MIF) is a constitutive element of the host antimicrobial defenses and stress response that promotes proinflammatory function of the innate and acquired immune systems. MIF plays an important role in the pathogenesis of acute and chronic inflammatory or autoimmune disorders, such as sepsis, acute respiratory distress syndrome, asthma, rheumatoid arthritis, and inflammatory bowel diseases. Polymorphisms of the human *MIF* gene (that is, guanine-to-cytosine transition at position −173 or CATT-tetranucleotide repeat at position −794) have been associated with increased susceptibility to or severity of juvenile idiopathic and adult rheumatoid arthritis, ulcerative colitis, atopy, or sarcoidosis. Whether these *MIF* polymorphisms affect the susceptibility to and outcome of sepsis has not yet been examined. Analyses of *MIF* genotypes in patients with sepsis may help to classify patients into risk categories and to identify those patients who may benefit from anti-MIF therapeutic strategies.

The innate immune system assumes an essential role in the natural host defenses against microbes [1, 2]. Sensing of microbial pathogens, either in tissue in contact with the host's environment or in the systemic circulation after invasion of the bloodstream, is carried out by macrophages, dendritic cells, natural killer cells, granulocytes, and monocytes acting as sentinels of the innate immune system. Binding of microbial products to pathogen recognition molecules activates signal-transduction pathways and the transcription of immune genes, resulting in the expression of costimulatory molecules at the cell surface and in the release of immunoregulatory effector molecules in the extracellular compartment [1, 3].

Failure to recognize pathogens at an early stage of invasion, for example because of genetic defects in the ability of macrophages to detect and kill microbial pathogens, facilitates unrestricted microbial growth and the development of overwhelming and potentially life-threatening infections [4, 5]. Among numerous effector molecules involved in the antimicrobial host defenses, cytokines have a crucial role because they kick off the host inflammatory response and coordinate the cellular and humoral responses aimed at the eradication or the containment of invasive pathogens [6]. The increased susceptibility to infection of transgenic animals with qualitative or quantitative defects of their cytokine response, due to mutations or deletions of cytokine or cytokine receptor genes, is an example of the critical role played by cytokines or cytokine receptors in antimicrobial host defenses [6]. However, exuberant production of proinflammatory mediators may also become life-threatening, as observed in patients with severe sepsis or septic shock [7–9], indicating that a tight control of cytokine production is essential for balanced innate immune responses.

MACROPHAGE MIGRATION INHIBITORY FACTOR

Investigations of the delayed-type hypersensitivity reaction conducted 40 years ago led to the identification of one of the first cytokine activities: macrophage migration inhibitory factor (MIF). It was described orig-

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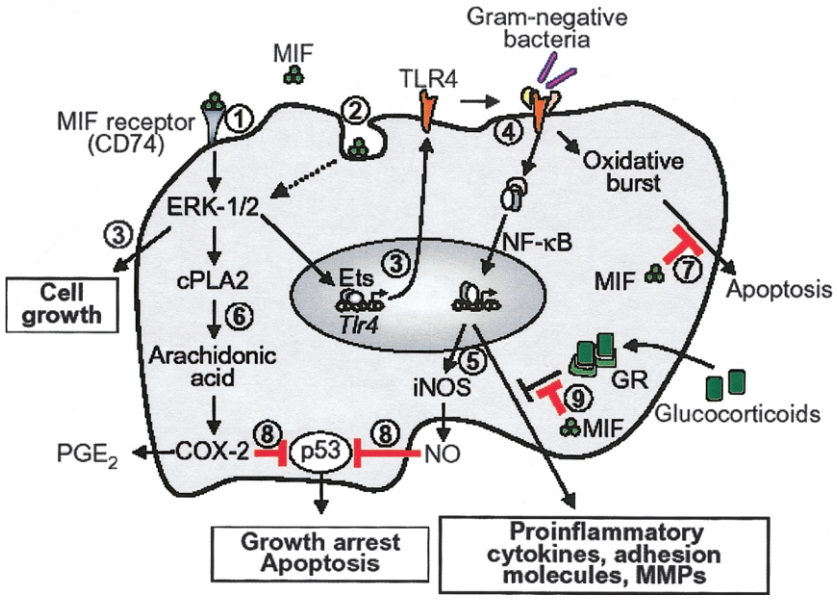

Figure 1. Mode of action of macrophage migration inhibitory factor (MIF). MIF may exert its biological effects either via the binding to a cognate receptor *(1)* or via a nonclassical endocytic pathway *(2)*. MIF is associated with activation of the extracellular signal–regulated kinase-1/2 (ERK-1/2), promoting cell growth and activating Ets transcription factors *(3)* shown to be critical for the expression of the Toll-like receptor 4 (TLR4) gene *(Tlr4)* encoding for the signal-transducing molecule of the endotoxin receptor complex. By up-regulating TLR4 expression, MIF facilitates the sensing of endotoxin-containing particles *(4)*, thereby promoting the production of proinflammatory mediators, including cytokines (such as MIF) and nitric oxide (NO) *(5)*. MIF activates a series of events initiated by the phosphorylation of ERK-1/2 and followed by the production of cytoplasmic phospholipase A2 (cPLA2), arachidonic acid, and prostaglandin E2 (PGE_2) *(6)*. Via the generation of oxidoreductase activity, NO and cyclooxygenase (COX)–2, MIF prevents activation-induced apoptosis mediated by the oxidative burst *(7)* and by p53 *(8)*. Finally, MIF counterbalances the immunosuppressive effects of glucocorticoids *(9)*. GR, glucocorticoid receptor; iNOS, inducible nitric oxide synthase; MMPs, matrix metalloproteinases; NF-κB, nuclear factor–κB. Adapted from Calandra et al. [14].

inally as a factor released by activated lymphocytes that inhibited the random migration of exudate cells, hence its name [10, 11]. Until the cloning of a human MIF complementary DNA in 1989 [12] and its rediscovery in 1991 as a pituitary-derived peptide released after exposure to endotoxin [13], MIF had remained a mysterious cytokine. The intriguing observation that MIF was a neuroendocrine mediator potentiating host responses to microbial products (endotoxin) suggested that MIF was at the crossroads of the endocrine and immune systems. It also helped to uncover an important feature of this molecule, namely its capacity to promote proinflammatory immune functions. Over the past decade, several studies have revealed that MIF is a regulator of inflammatory and innate immune responses (reviewed in [14]).

MIF is constitutively expressed by a broad variety of cells and tissues, including such innate immune cells as monocytes and macrophages [15], and is rapidly released after exposure to microbial products (cell wall components and toxins) and proinflammatory mediators and in response to stress [16–18]. Once released in the extracellular milieu, MIF promotes proinflammatory biological activities, acting in an autocrine, paracrine, or endocrine manner as a regulator of immune responses. MIF has been shown to counterregulate the immunosuppressive effects of glucocorticoids on immune cells [17], to activate the extracellular signal–regulated kinase–1/2 (ERK-1/2) mitogen-activated protein kinase pathway [19], to inhibit the activity of JAB-1/CSN5, a coactivator of the activator protein–1 (AP-1) [20], to up-regulate the expression of Toll-like receptor 4, to facilitate the sensing of endotoxin-bearing bacteria [21], and to sustain proinflammatory function of macrophages by inhibiting p53-dependent apoptosis [22] (figure 1). As a proinflammatory mediator, MIF has been shown to be implicated in the pathogenesis of severe sepsis and septic shock [13, 16, 17, 23, 24], acute respiratory distress syndrome [25], and several other inflammatory and autoimmune diseases [26], including rheumatoid arthritis [27, 28], glomerulonephritis [29, 30], and inflammatory bowel diseases [31].

A single *MIF* gene located on chromosome 22q11.2 has been identified in the human genome [32, 33]. This region is in syntenic conservation with a region of mouse chromosome 10 containing the mouse *Mif* gene [34, 35]. The human *MIF* gene is short, composed of 3 exons of 205, 173, and 183 bp and 2 introns of 189 and 95 bp (figure 2) [12, 32, 34–38]. Genes with a high degree of homology with human and mouse *Mif* have been identified in the genome of several other mammals (rats, gerbils, cattle, and pigs), where they are expressed as a single

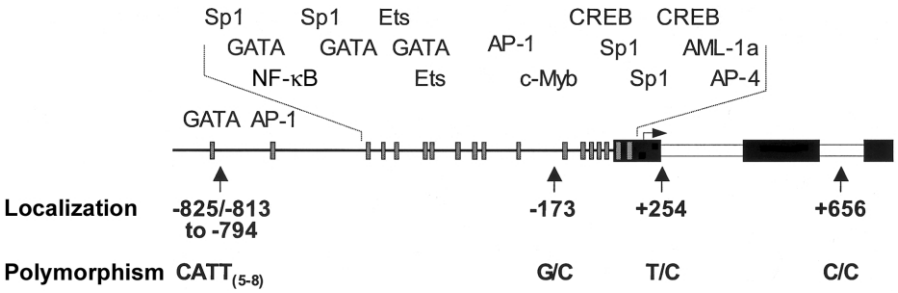

Figure 2. Structure of the human macrophage migration inhibitory factor *(MIF)* gene. The 3 exons, 2 introns, and putative transcription factor–binding sites are represented by black, white, and gray boxes, respectively. Arrows indicate the positions of the 3 single-nucleotide polymorphisms and of the $CATT_{(5-8)}$-tetranucleotide microsatellite. AP, activator protein; CREB, cyclic adenosine 3′,5′-monophosphate–responsive element–binding protein; Ets, E–twenty-six; NF-κB, nuclear factor–κB; Sp1, specificity protein 1.

copy per haploid genome. In contrast to other mammalian genomes, the mouse genome also contains several processed (intronless) pseudogenes [34, 35, 38]. Homologues of the human *MIF* gene, encoding for proteins sharing ~30% identity with MIF at the amino acid level, have been identified in chickens, jawless and jawed fish, ticks, parasites, plants, and cyanobacteria [36, 39–42]. The high degree of conservation of the MIF protein across different animal species suggests that it may exert important biological functions.

The promoter region of the *MIF* gene contains several putative DNA-binding sequences for transcription factors, including AP-1, nuclear factor (NF)–κB, Ets, GATA, specificity protein 1, and cyclic adenosine 3′,5′-monophosphate (cAMP)–responsive element-binding protein (figure 2). Whether these DNA-binding sequences are implicated in the control of the expression of the human *MIF* gene is not known. Of note, however, a cAMP-responsive element DNA-binding site located in the proximal promoter region of the mouse *Mif* gene, which is conserved in the human *MIF* gene promoter, has been implicated in *Mif* gene activation of AtT-20 mouse pituitary cells induced by forskolin, an activator of the cAMP-dependent protein kinase A pathway [43]. To improve our understanding of the factors implicated in the regulation of the expression of the *MIF* gene, it remains imperative to identify which regions of the *MIF* promoter and their cognate transcription factors are implicated in the control of the basal and stimulus-induced expression of the *MIF* gene. The *MIF* 5′ flanking region lacks a TATA box but is rich in GC nucleotides, 2 characteristics usually associated with the presence of multiple transcriptional start sites. Yet, on the basis of primer extension and 5′-rapid amplification of complementary ends PCR analyses of the human *MIF* gene, there is a single RNA initiation start site located 97 bp upstream of the methionine codon [32]. In agreement with this finding, a single transcriptional start site was also identified in the mouse *Mif* gene. A single MIF mRNA species of ~800 bp was observed in human, mouse, or rat cell lines or tissues. The 345-bp open-reading frame of MIF mRNA encodes for a 115–amino acid nonglycosylated protein of 12.5 kDa. Crystallographic studies of the human and rat proteins have revealed that MIF is a homotrimer [44, 45].

MIF GENE POLYMORPHISMS AND SUSCEPTIBILITY TO INFLAMMATORY DISEASES

Genetic studies of twins and adoptees have revealed that host factors are essential determinants of susceptibility to infectious and autoimmune diseases [46]. Immunogenetic analyses have linked genes of the major histocompatibility complex (MHC), as well as non-MHC genes, to increased susceptibility or resistance to several infectious diseases, such as malaria, tuberculosis, leprosy, AIDS, and viral hepatitis [47]. Of the non-MHC genes, polymorphisms within the promoter region of cytokines (e.g., TNF, IL-1, IL-4, and IL-10) and of cytokine or chemokine receptors (e.g., IL-7R, IFN-γR, IL-12R, and CCR5) have been associated with mostly enhanced, but sometimes also reduced (CCR5), predisposition to inflammatory and infectious diseases.

In recent years, the advent of modern sequencing tools and the development of high-throughput technologies has greatly facilitated the study of gene polymorphisms and their impact on the pathogenesis of human diseases. Susceptibility to infection and propensity to develop severe inflammatory and immune diseases are likely to be strongly influenced by genetic factors. By analogy with other cytokines and given the role of MIF in the control of inflammation and innate immune responses to microbial invasion, it was reasonable to postulate that mutations in the human *MIF* gene would predispose affected hosts to altered susceptibility to or severity of inflammatory or infectious diseases. Indeed, loss-of-function MIF mutations may affect the capacity of the host to mount inflammatory and innate immune responses. Alternatively, gain-of-function *MIF* mutations may predispose the host to more-severe inflammatory and immune reactions. Over the last 3 years, a rapidly growing body of literature has linked *MIF* gene

polymorphisms with susceptibility to or severity of inflammatory diseases in which increased MIF concentrations had been associated with severe clinical manifestations, high severity scores, and often poor outcome.

MIF *gene polymorphisms.* Four polymorphisms of the human *MIF* gene have been reported thus far (figure 2): a 5–8-CATT tetranucleotide repeat at position −794 (−794 $CATT_{(5–8)}$) and 3 single-nucleotide polymorphisms (SNPs) at positions −173 (−173*G/C), +254 (+254*T/C), and +656 (+656*C/G) [48–50]. The +254 and +656 SNPs are positioned in introns and, thus, do not affect the coding sequence of the *MIF* gene. *MIF* genotyping studies have focused on the −794 $CATT_{(5–8)}$ microsatellite and the −173*G/C polymorphisms but have not examined the impact of the other 2 known polymorphisms [48–56]. Table 1 shows a summary of the allele frequencies of these 2 polymorphisms in cohorts of healthy persons from different countries. In white subjects from the United Kingdom or United States and in Japanese, the frequency of the CATT alleles followed the same ranking order: $CATT_6$, followed by $CATT_5$, $CATT_7$, and $CATT_8$ [49, 50, 55]. The $CATT_8$ allele was rare (<1%) in all ethnic groups examined. The $CATT_6$ allele was predominant in white subjects from the United Kingdom and United States, whereas the $CATT_5$ and $CATT_7$ alleles were more frequent in Japanese subjects. In all populations studied, the −173*G allele (75%–90%) was far more common than the −173*C allele (15%–20%). Of note, in the 2 studies from Japan, the frequency of the −173*C allele (19.3% and 22.3%) was almost twice as high as that among the white population from the United Kingdom (12%). Surprisingly, the −173*C allele frequency among the German population studied was similar to that among the Japanese subjects [54–56]. Unfortunately, the ethnic background of the German population was not reported. Evidence for strong linkage disequilibrium between the 4 polymorphisms was reported in a cohort of 342 white subjects from the United Kingdom. The $CATT_5$/−173*C allele was extremely rare (1.3%), whereas the $CATT_7$/−173*G allele was not observed.

Arthritis. Previous studies have revealed a role for MIF in the pathogenesis of rheumatoid arthritis (reviewed in [27, 28]). Increased MIF levels have been detected in the serum and synovial fluids of children with juvenile idiopathic arthritis (also called juvenile rheumatoid arthritis) and of adults with rheumatoid arthritis [58, 59]. Immunoneutralization of MIF was observed to inhibit the development of adjuvant- or collagen-induced arthritis in experimental animal models [60–62]. It was therefore not surprising that *MIF* genotyping studies have been conducted in cohorts of patients with arthritis with the aim to look for possible associations between *MIF* gene polymorphisms and susceptibility to or severity of inflammatory arthritis (table 2).

The −173*G/C SNP was the first *MIF* gene polymorphism identified in 2001 by Donn et al. [48], who screened for mutations within 1 kb of the 5′ flanking region of the human *MIF* gene in 32 unrelated healthy white subjects in the United Kingdom. Compared with expression of the −173*C allele among 172 healthy subjects, the frequency was increased 2-fold (20.5% vs. 10.2%) in a cohort of 117 patients with systemic-onset juvenile idiopathic arthritis. Similar results were obtained when 526 patients with juvenile idiopathic arthritis were compared with 259 healthy white subjects in the United Kingdom [49], suggesting that the −173*C allele is likely to confer susceptibility to juvenile idiopathic arthritis. Interestingly, patients with the −173*C allele had increased levels of MIF in the circulation or in synovial fluids (patients with juvenile idiopathic arthritis) [49, 63]. Moreover, the presence of a −173*C allele in patients with juvenile idiopathic arthritis was predictive of a shorter duration of clinical response to corticosteroid therapy [63]. To begin to study the molecular mechanism by which the −173*C SNP may affect *MIF* gene expression, a −775 to +84 region of the *MIF* gene (excluding the CATT repeat region) was cloned in a luciferase reporter vector and tested for promoter activity in CEMC7A human T lymphoblasts and A549 human lung epithelial cells [49]. Although the −173*C promoter was more active than the −173*G promoter in CEMC7A cells, the opposite results were obtained in A549 cells, suggesting that the −173 SNP may affect promoter activity in a cell type–specific manner. On the basis of sequence analysis, it was proposed that the presence of a cytosine at position −173 creates a binding site for the transcription factor AP4. However, binding of AP4 to that potential site has not yet been demonstrated.

In 2002, Baugh et al. [50] reported the association between the −794 $CATT_{(5–8)}$ microsatellite and disease severity in patients with rheumatoid arthritis. The study included 159 healthy white subjects in the United States and 184 patients from Wichita, Kansas, with either mild (105 patients) or severe (79 pa-

Table 1. Allele frequencies of macrophage migration inhibitory factor *(MIF)* promoter polymorphisms, $CATT_{(5–8)}$ and −173*G/C, in healthy subjects from various countries.

Reference	Country of origin[a]	No. of subjects	Allele frequency, %[b] No. of CATT repeats 5	6	7	8	−173 SNP G	C
[57]	UK	342	25.3	65.6	8.8	0.3	88	12
[56]	Germany	390	ND	ND	ND	ND	79	21
[51]	Spain	122	ND	ND	ND	ND	85	15
[50]	US	159	27.7	60.7	11	0.6	ND	ND
[54]	Japan	750	ND	ND	ND	ND	80.7	19.3
[55]	Japan	155	39.4	42.6	17.4	0.6	77.7	22.3

NOTE. ND, not determined; SNP, single-nucleotide polymorphism.

[a] UK, United Kingdom; US, United States.

[b] The frequencies of the +254*T, +254*C, +656*C, and +656*G alleles have been determined only in white subjects in the United Kingdom and were 88.8%, 11.2%, 86.3%, and 13.7%, respectively [49].

Table 2. Association between macrophage migration inhibitory factor gene *(MIF)* polymorphisms and inflammatory diseases.

Reference	Type of disease	Country[a]	Relationship between *MIF* polymorphisms and disease
[48]	Systemic-onset juvenile idiopathic arthritis	UK	−173*C allele increased susceptibility
[49]	Juvenile idiopathic arthritis	UK	−173*C allele increased susceptibility
[50]	Rheumatoid arthritis	US	$CATT_5$ allele reduced disease severity
[56]	Rheumatoid arthritis and juvenile rheumatoid arthritis	Germany	$CATT_5$ allele increased susceptibility to juvenile rheumatoid arthritis
			−173*C allele reduced susceptibility to rheumatoid arthritis
[52]	Inflammatory polyarthritis	UK	$CATT_7$/−173*C haplotype increased susceptibility
[51]	Sarcoidosis in erythema nodosum	Spain	−173*C allele increased susceptibility
[55]	Atopy	Japan	$CATT_7$/−173*C haplotype increased susceptibility
			$CATT_5$/−173*G haplotype reduced susceptibility
[54]	Ulcerative colitis	Japan	−173*C/C genotype increased disease severity

[a] UK, United Kingdom; US, United States.

tients) rheumatoid arthritis. The $CATT_5$ allele was associated with a lower disease severity, because it was present in 50% of the control subjects and 39% and 32% of patients with mild or severe rheumatoid arthritis, respectively. Using luciferase reporter assays in COS-7 monkey kidney fibroblasts, the authors showed that the $CATT_5$ promoter construct exhibited reduced basal and serum or forskolin-stimulated transcriptional activity, compared with that of any of the $CATT_6$, $CATT_7$, or $CATT_8$ promoter constructs. The effect of the −173*G/C polymorphism was not studied. Recently, the combined effects of the −794 CATT and the −173 polymorphisms were assessed in a cohort of 343 white subjects in the United Kingdom and 438 patients with inflammatory polyarthritis [52]. The −173*C allele, the $CATT_7$ allele, and the $CATT_7$/−173*C haplotype were associated with a 1.5-, 1.7-, and 3.0-fold increased risk, respectively, of developing inflammatory polyarthritis ($P = .0001$, $P = .02$, and $P = .0001$, respectively), strongly suggesting the presence of a linkage disequilibrium between the −173*C and $CATT_7$ alleles. In contrast to what had been observed in the cohort of patients with rheumatoid arthritis in the United States [50], *MIF* polymorphisms were surprisingly not associated with disease severity in the UK population.

Arthritis is a complex and polygenic chronic systemic inflammatory disease. To investigate possible associations between candidate genes and adult or juvenile rheumatoid arthritis, Miterski et al. [56] analyzed the polymorphisms of 13 genes, including *MIF,* in ~400 German patients and 300–400 control subjects. None of the candidate genes investigated (among which were *HLA-DRBI, TNF, TNFRI,* and *TNFRII*) was found to be associated with adult or juvenile rheumatoid arthritis. Of note, the $CATT_5$ and −173*G alleles were unexpectedly significantly more frequent in patients with either adult or juvenile rheumatoid arthritis than in control subjects. Unfortunately, the ethnic background of the case-patients and control subjects was not reported. Given the critical importance of control subjects in such studies, it is impossible to draw firm conclusions from the apparently conflicting results of this study. One approach to avoid that problem is to perform family-based analyses. In an elegant study, Donn et al. [53] investigated the transmission of *MIF* promoter polymorphisms in 321 white families in the United Kingdom with one child with juvenile idiopathic arthritis. The $CATT_7$/−173*C haplotype was observed to be transmitted in excess in the patients with juvenile idiopathic arthritis. Moreover, both conditional and pairwise extended transmission disequilibrium tests predicted functional interaction between the 2 polymorphisms. Functional studies comparing the activities of all possible combinations of the various CATT and −173 alleles suggested the existence of an association between the length of the CATT repeat and either the −173*C allele (CEMC7A T cells) or the −173*G allele (A549 epithelial cells) in a cell type–dependent manner. However, in both cell lines, $CATT_7$/−173*C promoter activity was similar to that of $CATT_5$/−173*G and $CATT_6$/−173*G promoters, a finding in apparent contradiction with the hypothesis that $CATT_7$ and −173*C alleles enhance promoter activity.

Other inflammatory diseases. Three case-control studies conducted in other patient populations confirmed and extended the observations made in patients with arthritis. In the first study performed in Spain, the frequency of the −173*C allele was significantly higher in 28 patients with erythema nodosum secondary to sarcoidosis (34%) than in 70 patients with erythema nodosum due to other causes (12%) or in 122 matched control subjects (15%) [51]. Moreover, the presence of the −173*C allele was found to increase the risk of developing sarcoidosis in erythema nodosum patients. In the second study, *MIF* genotyping was performed in 221 patients with ulcerative colitis and in 438 healthy control subjects from Japan [54]. There was no difference in the distribution of the −173 genotypes between control subjects and patients with ulcerative colitis. Yet, there was an overrepresentation of the −173 C/C

genotypes in the subgroup of patients with severe pancolitis (i.e., extending to the cecum) (OR, 10.7; 95% CI, 1.3–86.6; $P = .007$), suggesting a possible relationship between the −173*G/C polymorphism and disease severity in Japanese patients with ulcerative colitis. The third case-control study conducted in Japanese patients with atopy and asthma (349 subjects with atopy, 197 of whom had asthma, and 235 subjects without atopy, of whom 80 had asthma) confirmed the existence of association between the CATT and −173 promoter polymorphisms in atopic but not in patients with asthma [55]. Indeed, the risk of atopy was reduced in carriers of the $CATT_5$/−173*G haplotype, whereas it was increased in carriers of the $CATT_7$/−173*C haplotype. However, analyses of *MIF* promoter activity in A549 lung epithelial cells yielded conflicting results, as the $CATT_7$/−173*C promoter exhibited lower activity than the $CATT_5$/−173*G or the $CATT_6$/−173*G promoters. These and other results reported above argue in favor of a complex regulation of the transcriptional activity and expression of the *MIF* gene. Given the lack of information on the identity of proteins potentially interacting with the CATT and −173 regions of the *MIF* promoter, the mechanisms by which these polymorphic sites may modulate *MIF* transcription remain unknown.

MIF polymorphisms and sepsis. The studies described above strongly suggest that *MIF* gene polymorphisms predispose to the development of inflammatory diseases. Given that MIF is an important mediator of innate immunity and sepsis (reviewed in [14]), we postulate that genetic variations within the *MIF* gene also may influence predisposition to or outcome of sepsis. To verify this hypothesis, we are currently investigating whether the −794 $CATT_{(5–8)}$ and −173*G/C *MIF* polymorphisms play a role in the pathogenesis of human sepsis.

CONCLUSIONS

In recent years, a rapidly growing amount of literature has revealed an important role for MIF in innate immunity, inflammation, and sepsis. Increased systemic or local MIF concentrations have been associated with severe clinical manifestations, high severity scores, and often poor outcome of sepsis and inflammatory and autoimmune diseases. Similarly, polymorphisms of the *MIF* gene have been linked to susceptibility to or severity of chronic systemic inflammatory diseases. Given these observations, one would predict that polymorphisms of the human *MIF* gene would also predispose affected hosts to altered susceptibility to or outcome of sepsis. Work is in progress to verify these assumptions in a cohort of patients with severe sepsis and septic shock.

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