The D-dopachrome tautomerase (DDT) gene product is a cytokine and functional homolog of macrophage migration inhibitory factor (MIF)

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AUTHOR SUMMARY

The therapeutic targeting of immunologic hormones or cytokines has shown clinical success in the treatment of chronic inflammatory diseases. A proinflammatory cytokine known as macrophage migration inhibitory factor (MIF) functions upstream in the inflammatory cascade and is considered a promising therapeutic target for rheumatoid arthritis (1). Inflammatory or invasive stimuli trigger the rapid release of MIF from immune cells; MIF then acts to promote immune cell activation and survival, and it enables the maximum production of downstream inflammatory mediators (2). The binding of MIF to its cell surface receptor, a protein called CD74, induces the recruitment of a second protein, CD44, into a signaling complex. Formation of this complex triggers a signaling cascade that promotes cell survival and proliferation (3, 4). In addition, MIF binds the chemokine receptors CXCR2 and CXCR4 and thus modulates the migration of cells of the immune system to sites of inflammation (5).

Whereas mice lacking the MIF receptor exhibit a similar phenotype to mice that fail to produce MIF, recent studies have shown that the phenotype is more pronounced in the former group of mice. Furthermore, although therapeutic antibodies against MIF have proved highly effective in experimental studies, these antibodies do not completely abolish the activation of the MIF receptor. We hypothesized that this discrepancy suggests the existence of a second MIF-like molecule, possibly a protein encoded by the homologous D-dopachrome tautomerase, D-DT (DDT) gene. The genes for MIF and D-DT are located in close proximity to each other and genetic analysis suggests that the genes arose by an ancestral duplication event. Indeed, MIF and D-DT share 34% amino acid sequence identity and crystallography has confirmed the almost identical 3D structures of the two proteins.

We cloned and produced the D-DT protein and observed its high-affinity binding to the MIF receptor. Investigation of D-DT’s activation properties revealed that it induces a MIF-like signaling cascade, inhibits macrophage migration, and counterregulates the inhibitory effect of glucocorticoids on inflammation. D-DT thus is a cytokine, and it triggers the same immunologic pathways as MIF, supporting an overlapping functional spectrum of the two proteins.

Stimulation of macrophages with lipopolysaccharide (LPS) (or endotoxin), a component of the bacterial cell wall that causes inflammation, leads to the release of D-DT with kinetics comparable to those of MIF. Exposure of mice to LPS also results in a time-dependent increase of D-DT concentration in the serum that mimics the increase observed for MIF. Sepsis, which occurs when a bacterial infection enters the bloodstream, is characterized by a severe inflammatory response in the entire body. Endotoxin in the bloodstream causes the systemic production of potent cytokines such as TNF-α and IL-1β. High levels of these cytokines lead to vasodilation and hypotension, diminished myocardial contractility, vascular injury, and activation of the coagulation system, culminating in lethal disseminated intravascular coagulation.

Administration of therapeutic antibodies specific to MIF, a process known as immunoneutralization, rescues mice from sepsis and previous studies have demonstrated that this protection is reflected in the reduction of numerous inflammatory mediators such as TNF-α and IL-1β. Treatment of endotoxemic mice with an anti-DDT antibody boosted the survival of mice from 20% to 79%, comparable to the level of protection conveyed by anti-MIF antibody. An analysis of circulating cytokine concentrations showed that D-DT neutralization was associated with a significant reduction in the plasma concentration of proinflammatory cytokines (TNF-α, IL-1β, IFN-γ, and IL-12) previously implicated in shock pathogenesis (Fig. 1).

Finally, an analysis of clinical samples showed that circulating levels of D-DT are highly elevated in patients with sepsis or with
malignancy, and a positive correlation between disease severity and D-DT concentration was observed, suggesting that this protein could serve as a useful therapeutic target for patients with bacterial infections or cancer. Furthermore, a positive correlation between MIF and D-DT levels was detected, both in healthy and in patient samples with a stronger correlation observed in patient samples.

In conclusion, our study implicates D-DT as a previously undescribed cytokine and a close functional homolog of MIF. On the basis of our findings, we propose that D-DT be designated “MIF-2.” Finally, we note that the current emphasis on the development MIF-directed therapies, which have shown strong efficacy in different animal models of disease, may be enhanced by the simultaneous targeting of D-DT.