

### **A cytokine designated GIF or MIF directly regulates CD4 cell differentiation toward Th effectors.**

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A 13-kDa cytokine, macrophage migration inhibitory factor(MIF), is implicated in inflammation and the responsiveness to bacterial products. Although the function of this cytokine was quite elusive, recent experiments using MIF<sup>-/-</sup> mice suggested that this protein is involved in Th1-type immune responses. MIF<sup>-/-</sup> mice are more susceptible to *Leishmania major* infection than wildtype animals. The mutant mice also fail to develop experimental colitis. However, the mechanism by which this protein regulates the CD4-dependent immune response remains unclear.

We have called this cytokine glycosylation-inhibiting factor(GIF). Although GIF and MIF were independently studied, they are encoded by the same and single gene. GIF secreted from T cells is posttranslationally modified, whereas the same protein contained in the cytosol is unmodified. The modification, i.e., the binding of a free cysteine to C60 of GIF, is required for GIF to bind to the receptor. The receptors are detected on activated T and B cells but not on macrophages or dendritic cells. Injection of GIF secreted from T cells inhibits the IgG1 and IgE antibody response to T-dependent antigens. rGIF cysteinylated at C60, but not unmodified rGIF, inhibits the antibody formation *in vivo*. C60-modified rGIF inhibits B cell secretion of IgG1 and IgE induced by LPS and IL-4 and reduces antigen uptake and presentation mediated through BCR. Since GIF receptors are also expressed on activated T cells, we hypothesized that cysteinylated GIF is secreted from CD4 cells and directly acts on the same cells to regulate their effector function.

To test this, we crossed GIF(MIF)<sup>-/-</sup> mice to BALB/c and generated DO11.10 TCR Tg mice on GIF<sup>+/+</sup> and <sup>-/-</sup> backgrounds. Naïve CD4 cells from GIF<sup>+/+</sup> and <sup>-/-</sup> DO11.10 Tg mice were stimulated with antigen and T-depleted spleen cells purified from either GIF<sup>+/+</sup> or <sup>-/-</sup> mice. GIF<sup>-/-</sup> naïve CD4 cells secreted more IL-4 than GIF<sup>+/+</sup> cells, whereas they secreted similar amounts of IL-2 and IFN- $\gamma$  to <sup>+/+</sup> cells. Upon restimulation with antigen, GIF<sup>-/-</sup> CD4 cells secreted more IL-4 and less IFN- $\gamma$  than GIF<sup>+/+</sup> cells. GIF <sup>-/-</sup> CD4 cells were polarized toward a Th2 phenotype no matter whether T-depleted spleen cells as APCs were derived from GIF<sup>+/+</sup> or <sup>-/-</sup> mice. Therefore, GIF derived from T cells but not APCs inhibits Th2 differentiation and/or induces Th1 differentiation. rGIF cysteinylated at C60 inhibited secretion of IL-4 from GIF<sup>-/-</sup> naïve CD4 cells, without affecting that of IL-2 and IFN- $\gamma$ . Unmodified rGIF had no effect, which recapitulates the requirement of C60-modification for the bioactivity of this cytokine. GIF<sup>-/-</sup> mice immunized with Ova in CFA generated reduced Th1 effector cells *in vivo*. These experiments demonstrate that GIF is a T cell cytokine that acts on CD4 cells to inhibit Th2 and/or induce Th1 differentiation.