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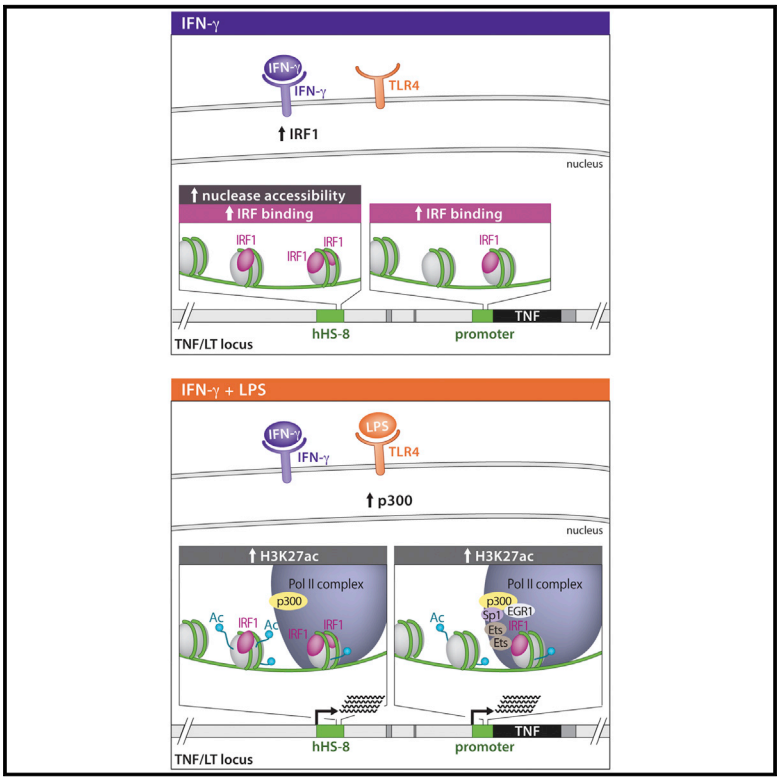
A Distal Locus Element Mediates IFN-γ Priming of Lipopolysaccharide-Stimulated *TNF* Gene Expression

Graphical Abstract



Highlights

IFN-γ priming requires the IRF1-dependent distal *TNF/LT* locus element hHS-8

IFN-γ priming promotes chromatin accessibility and recruitment of IRF1 at hHS-8

IFN-γ priming enriches LPS-stimulated H3K27ac and induces eRNA synthesis at hHS-8

Targeting the hHS-8 IRF1 binding site in vivo with Cas9 abolishes IFN-γ priming

Authors

Nancy A. Chow, Luke D. Jasenosky, Anne E. Goldfeld

Correspondence

anne.goldfeld@childrens.harvard.edu

In Brief

Interferon γ (IFN-γ) priming is a critical immune event that enhances the monocyte and macrophage response, particularly expression of the *TNF* gene, to toll-like receptor (TLR) signaling. Chow et al. demonstrate that IFN-γ priming requires a distal enhancer element within the *TNF/LT* locus, thereby expanding the role of distal regulatory elements in the innate immune response.

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A Distal Locus Element Mediates IFN-γ Priming of Lipopolysaccharide-Stimulated *TNF* Gene Expression

Nancy A. Chow,[1] Luke D. Jasenosky,[1] and Anne E. Goldfeld[1,*]
[1]Program in Cellular and Molecular Medicine, Children's Hospital Boston, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, USA
*Correspondence: anne.goldfeld@childrens.harvard.edu
http://dx.doi.org/10.1016/j.celrep.2014.11.011

SUMMARY

Interferon γ (IFN-γ) priming sensitizes monocytes and macrophages to lipopolysaccharide (LPS) stimulation, resulting in augmented expression of a set of genes including *TNF*. Here, we demonstrate that IFN-γ priming of LPS-stimulated *TNF* transcription requires a distal *TNF/LT* locus element 8 kb upstream of the *TNF* transcription start site (hHS-8). IFN-γ stimulation leads to increased DNase I accessibility of hHS-8 and its recruitment of interferon regulatory factor 1 (IRF1), and subsequent LPS stimulation enhances H3K27 acetylation and induces enhancer RNA synthesis at hHS-8. Ablation of IRF1 or targeting the hHS-8 IRF1 binding site in vivo with Cas9 linked to the KRAB repressive domain abolishes IFN-γ priming, but does not affect LPS induction of the gene. Thus, IFN-γ poises a distal enhancer in the *TNF/LT* locus by chromatin remodeling and IRF1 recruitment, which then drives enhanced *TNF* gene expression in response to a secondary toll-like receptor (TLR) stimulus.

INTRODUCTION

Produced by natural killer cells and activated Th1 lymphocytes, interferon γ (IFN-γ) sensitizes circulating monocytes and tissue-resident macrophages, leading to augmentation of macrophage activation after microbial recognition and toll-like receptor (TLR) signaling (Murray, 1988). This phenomenon, known as IFN-γ priming, results in enhanced gene expression of inflammatory cytokines such as tumor necrosis factor (TNF), interleukin 12 (IL-12), and IL-6 (Lorsbach et al., 1993; Ma et al., 1996; Pace et al., 1983; Sanceau et al., 1991). In the case of TNF, de novo transcription of *TNF* is enhanced in human monocytes primed by IFN-γ and then stimulated by lipopolysaccharide (LPS) (Hayes and Zoon, 1993). However, the molecular mechanisms that control IFN-γ priming, and whether these mechanisms are gene specific, are poorly understood.

The *TNF* gene and the genes encoding lymphotoxin-α and lymphotoxin-β (*LTA* and *LTB*) comprise the ~20 kb *TNF/LT* locus region, which lies within the histocompatibility locus on human chromosome 6 and mouse chromosome 17. *TNF* is highly and rapidly expressed in both lymphocytes and monocytes (Goldfeld and Maniatis, 1989; Goldfeld et al., 1990, 1993), and its transcriptional regulation occurs in a cell-type- and inducer-specific manner. Distinct sets of transcription factors and coactivators, including chromatin-modifying enzymes, are recruited to DNA elements in the *TNF* promoter depending on the type of cell and the type of stimulus that is received (Falvo et al., 2000a, 2000b, 2010; Tsai et al., 2000; Tsytsykova and Goldfeld, 2000). Furthermore, the formation of higher-ordered structures, or enhanceosomes, is required for *TNF* gene expression in specific cell types (Tsytsykova and Goldfeld, 2002; Barthel and Goldfeld, 2003). Distal hypersensitive (DH) elements upstream and downstream of the *TNF* transcription start site (TSS) have been identified in the *TNF/LT* locus. A subset of these DH sites also varies by cell type (Barthel and Goldfeld, 2003; Tsytsykova et al., 2007; Taylor et al., 2008; Biglione et al., 2011). For example, DH sites ~9 kb upstream and ~3 kb downstream of the murine gene act as NFATp-dependent enhancers in T cells and participate in activation-induced intrachromosomal interactions with the promoter (Tsytsykova et al., 2007), whereas a myeloid-specific DH site ~7 kb upstream of the TSS functions as a matrix attachment region (Biglione et al., 2011).

In this study, we show that a DH site ~8 kb upstream of the human *TNF* TSS (human hypersensitive site −8 kb [hHS-8]) is required for and mediates IFN-γ-stimulated augmentation of LPS-induced *TNF* gene expression in human monocytes/macrophages. The highly conserved hHS-8 noncoding element exhibits increased nuclease accessibility in response to IFN-γ stimulation, and interferon regulatory factor 1 (IRF1) is recruited. Upon subsequent LPS stimulation of IFN-γ-primed cells, increased acetylation of H3K27 and synthesis of enhancer RNA (eRNA) at hHS-8 occur. IFN-γ priming of *TNF* is abrogated with the ablation of IRF1, disrupting the IRF1 site in reporter assays, or by targeting the IRF1 binding element in hHS-8 with the catalytically inactive form of Cas9 linked to the Krüppel-associated box (KRAB) domain of Kox1 (Margolin et al., 1994; Gilbert et al., 2013) in human monocytic cells. Thus, IRF1 expression and an intact hHS-8 IRF1 binding element are required for IFN-γ priming of *TNF* in vivo.

Our results expand the functional role of distal regulatory elements in the innate immune response to IFN-γ priming and