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IRF3 polymorphisms induce different innate anti-Theiler's virus immune responses in RAW264.7 macrophages

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ABSTRACT

Persistent viral infections can lead to disease such as myocarditis. Theiler's murine encephalomyelitis virus (TMEV) infects macrophages of SJL/J (H-2s) mice establishing persistent infections leading to demyelinating disease. In contrast macrophages from B10.S (H-2s) mice clear TMEV. Activation of the transcription factor IRF3 induces IFNβ, ISG56, and apoptosis for viral clearance, but also inflammatory cytokines, such as IL-23 and IL6, which contribute to disease. Here we identify polymorphisms in the IRF3 of SJL/J versus B10.S mice that are located in DNA binding, nuclear localization, and autoinhibitory domains. SJL-IRF3 expression in RAW264.7 macrophage cells with or without TMEV infection decreased IL-23p19 promoter activity compared with B10S-IRF3. In contrast SJL-IRF3 increased IL-6, ISG56 and IFNβ in response to TMEV. B10S-IRF3 expression augmented apoptotic caspase activation and decreased viral RNA in TMEV-infected macrophages while SJL-IRF3 increased viral replication with less caspase activation. Therefore IRF3 polymorphisms contribute to viral persistence and altered cytokine expression.

Introduction

Humans become infected by viruses throughout life. After the acute stage of each infection, viruses are controlled by the innate and adaptive immune systems either by eliminating the viruses and virus infected cells entirely or by tolerating a persistent lifelong infection (Virgin et al., 2009). The bases for persistent viral infections are unclear, but virus interference with host cytokine production and apoptosis is thought to play a role. Macrophages are essential to anti-viral immunity because they destroy phagocytized viruses and produce cytokines that influence innate and adaptive immune responses. Cytokine production is induced when viral nucleic acids in the late endosome bind to pattern recognition receptors (PRRs), activate cell signaling pathways and transcription factors for cytokine responses. For instance, Toll-like receptor (TLR)-3 recognition of viral dsRNA (Diebold et al., 2004; Heil et al., 2003; Latz et al., 2004) leads to activation of the IRF3 transcription factor (Horng et al., 2002; Takeuchi et al., 2004). However, macrophages are sometimes infected by viruses. Cytokine production can occur if macrophages are infected with viruses when cytoplasmic helicases recognize viral RNA. Retinoic Acid Inducible gene-I (RIG-I) recognizes uncapped 5′-triphosphorylated-RNA that is unique to viruses (Hornung et al., 2006; Pichlmair et al., 2006) and melanoma differentiation-associated gene (MDA)-5 recognizes RNA from picornaviruses that are triphosphorylated and capped with viral protein (VPg) (Kato et al., 2006; Yoneyama et al., 2005). Activation of either RIG-I and MDA5 pathways via mitochondrial (Kawai et al., 2005;·Seth et al., 2005) and peroxisomal (Dixit et al., 2010) MAVS (also known as IPS-1, CARDIF, VISA) also leads to activation of IRF3. As a result of IRF3 activation, IFNβ, IL-6, and interferon stimulated genes (ISG) such as ISG56 are expressed. We also found that IRF3 represses IL-12 p35 (Dahlberg et al., 2006) and induces IL-23 p19 expression from macrophages challenged with Theiler's Murine Encephalomyelitis Virus (TMEV). Therefore, activation of IRF3 is a common feature of macrophage anti-viral responses following both phagocytosis of and infection by viruses in order to achieve the correct cytokine and anti-viral factors for innate and adaptive immunity.

In addition to its role in gene expression, IRF3 is also involved in virus induced apoptosis in cells infected by viruses (Heylbroeck et al., 2000). IRF3 activation induces proapoptotic caspase-3, 8, 9 activities. Induction of apoptosis is dependent on IRF3 interaction with Bax at aa366-427, the apoptosis activation domain (AAD) of IRF3 (Chattopadhyay et al., 2010). Following interaction of IRF3-AAD with Bax, the Bax/IRF3 dimer translocates to mitochondria causing release of cytochrome C, activation of the apoptosome and caspase 9, followed by activation of caspase 3. It is thought that induction of apoptosis in virus infected cells limits the

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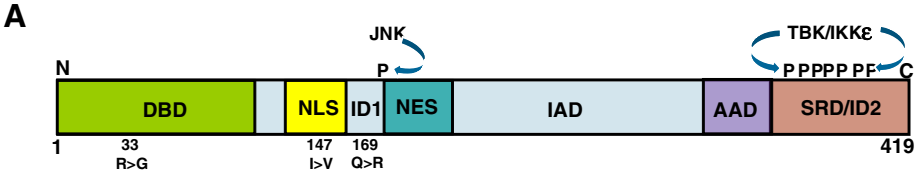
viral infection while inhibition of apoptosis plays a role in viral persistence (Chattopadhyay et al., 2011).

IRF3 is constitutively expressed in macrophages, is autoinhibited by two unphosphorylated inhibitory domains (ID), and is activated via hyperphosphorylation of the IDs. Activated TBK1/IKKε phosphorylates carboxy terminus serine/threonines of IRF3 positioned between aa385 and 405 at one of the inhibitory domains (ID1) (Fig. 1A). In addition, activated JNK-MAPK phosphorylates serine-173 of IRF3, which is near the other ID (ID2) and nuclear export a signal (NES) domain (Zhang et al., 2009). These phosphorylations rearrange the IDs and permit dimerization with IRFs at the IRF association domain (IAD), nuclear entry, interaction with the CBP/p300 transcription factor (Weaver et al., 1998), and binding to promoter elements via the N-terminal DNA binding domain (DBD). Promoters responsive to IRF3 after viral infection of cells include the IFNβ (Panne, 2008), IL-6 (Sweeney et al., 2010) and ISG56 (Grandvaux et al., 2002) genes. Therefore, activation of IRF3 is critical to the immediate/early response of macrophages to virus infection through expression of cytokines and ISGs, as well as induction of apoptosis. Because the human population has several polymorphisms at the IRF3 locus (rs79173361, rs2230667, rs968457, rs7251) that change single amino acids near the DBD and ID1 it is imperative to understand how polymorphisms at these IRF3 domains change the responses of macrophages to viruses.

Theiler's murine encephalomyelitis virus (TMEV) persistently infects and causes Multiple Sclerosis-like neuroinflammatory disease in SJL/J mice (H-2s) but not B10.S mice (H-2s) (Aubagnac et al., 2002; Monteyne et al., 1999; Vigneau et al., 2003). Thus comparison of TMEV induced responses in SJL mice with those of B10.S mice identifies non-MHC genes that are involved in persistent viral infections that lead to disease. One of the differences between these two strains is TMEV's chronic infection of macrophages that infiltrate the white matter of the CNS in SJL/J but not B10.S mice. We have found that the cellular and electrophoretic activities of IRF3 from SJL/J macrophages are distinctly different than that of IRF3 in B10.S macrophages (Dahlberg et al., 2006). This is significant because viral replication in infected macrophages is higher, survival of infected macrophages is greater, macrophage expression of IL-12 is lower, and IFNβ expression is higher during innate anti-TMEV immune responses of SJL/J macrophages compared with B10.S macrophages (Petro, 2005a). We have previously seen that the IL-23 p19 promoter in RAW264.7 cells is activated following challenge with TMEV (Al-Salleeh and Petro, 2007). These data suggest that IRF3 polymorphisms could be the bases for the different phenotypes of SJL/J and B10.S macrophage responses to TMEV. The present study extends the analysis of these differences in IRF3 by comparing the amino acid sequences, the TMEV-induced transcriptional activities, and the TMEV-induced apoptotic activities of IRF3 from SJL/J and B10.S mice. IRF3 from SJL/J mice is different than that from B10.S mice in that there are three single nucleotide polymorphisms that predict three amino acid differences in the protein. RAW264.7 macrophage cells overexpressing IRF3 from SJL/J mice expressed more



B

SJL	METPKPRILPWLVSQLDLGQLEGVAWLDESRTGFRIPWKHGLRQDAQMAD	50
B10S	METPKPRILPWLVSQLDLGQLEGVAWLDESRTRFRIPWKHGLRQDAQMAD	50
SJL	FGIFQAWAEASGAYTPGKDKPDVSTWKRNFRSALNRKEVLRLAADNSKDP	100
B10S	FGIFQAWAEASGAYTPGKDKPDVSTWKRNFRSALNRKEVLRLAADNSKDP	100
SJL	YDPHKVYEFVTPGARDFVHLGASPDTNGKSSLPHSQENLPKLFDGLVLGP	150
B10S	YDPHKVYEFVTPGARDFVHLGASPDTNGKSSLPHSQENLPKLFDGLILGP	150
SJL	LKDEGSSDLAIVSDPSQRLPSPNVNNFLNPAPQENPLKQLLAEEQWEFEV	200
B10S	LKDEGSSDLAIVSDPSQQLPSPNVNNFLNPAPQENPLKQLLAEEQWEFEV	200
SJL	TAFYRGRQVFQQTLFCPGGLRLVGSTADMTLPWQPVTLPDPEGFLTDKLV	250
B10S	TAFYRGRQVFQQTLFCPGGLRLVGSTADMTLPWQPVTLPDPEGFLTDKLV	250
SJL	KEYVGQVLKGLGNGLALWQAGQCLWAQRLGHSHAFWALGEELLPDSGRGP	300
B10S	KEYVGQVLKGLGNGLALWQAGQCLWAQRLGHSHAFWALGEELLPDSGRGP	300
SJL	DGEVHKDKDGAVFDLRPFVADLIAFMEGSGHSPRYTLWFCMGEMWPQDQP	350
B10S	DGEVHKDKDGAVFDLRPFVADLIAFMEGSGHSPRYTLWFCMGEMWPQDQP	350
SJL	WVKRLVMVKVVPTCLKELLEMAREGGASSLKTVDLHISNSQPISLTSDQY	400
B10S	WVKRLVMVKVVPTCLKELLEMAREGGASSLKTVDLHISNSQPISLTSDQY	400
SJL	KAYLQDLVEDMDFQATGNI	419
B10S	KAYLQDLVEDMDFQATGNI	419

Fig. 1. Comparison of the predicted amino acid sequences of IRF3 from B10.S and IRF3 from SJL. A. Domains of the 419 aa IRF3 include the DNA binding domain (DBD), Nuclear Localization Signal (NLS), Autoinhibitory Domain (ID) 1 and 2, Nuclear Export Signal (NES), IRF association domain (IAD), Apoptosis Activation Domain (AAD), Signal Response Domain (SRD) and the sites phosphorylated by JNK and TBK/IKKε. The 33R(B10.S)>G(SJL/J), 147I>V, and the 169Q>R polymorphisms are noted. B. Alignment of SJL/J IRF3 amino acid sequence with B10.S IRF3 amino acid sequence. Following extraction from agarose and cloning into pTARGET expression vector, the inserted cDNA was sequenced and the predicted amino acid sequence was generated. The DNA binding domain (__), the autoinhibitory domain 1 (~~~), and the signal response/autoinhibitory domain 2 (▪▪▪▪) are noted.