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Targeting interferon regulatory factors to inhibit activation of the type I IFN response: Implications for treatment of autoimmune disorders ☆

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ABSTRACT

The type I interferon (IFN) response plays a critical role in autoimmunity and is induced by innate receptor ligation and activation of IFN-regulatory factors (IRF). The present study investigated the roles and functional hierarchy of IRF3, IRF5, and IRF7 in expression of cytokines, chemokines, and matrix metalloproteinases in human THP1 monocytic cells. Targeted IRF knockdown was followed by evaluation of gene expression, promoter activation, and mRNA stability to determine the role of IRF as potential targets for modulating IFN responses in patients with autoimmune diseases. IRF played a distinct role in regulation of type I IFN gene expression in human monocytic cells and specifically regulated gene expression through the IFN-stimulated response element, with no contribution to transcription of traditionally AP-1 or NF-kB regulated genes. IRF7 regulated IL-6 gene expression by increasing IL-6 mRNA stability. IRF regulation of inflammation and induction of the IFN signature might contribute to the pathogenesis of autoimmune diseases and therefore represent novel therapeutic targets.

1. Introduction

Activation of the type I interferon (IFN) system contributes to the pathogenesis of many rheumatic diseases, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). In SLE patients, autoantibody production and immune complex formation result in tissue injury and secretion of type I IFN and activation of innate immunity [1–4]. Peripheral blood mononuclear cells (PBMC) from SLE patients demonstrate increased expression of type I IFN and other IFN-regulated genes known as the IFN signature [5,6]. Innate immune responses also play a critical role in synoviocyte activation and recruitment into the synovial tissue of RA patients [7–12]. In fact, the gene expression profile in RA synovial tissue reflects exposure to toll-like receptor (TLR) ligands and displays characteristic features of the type I IFN signature [13–16]. In addition to the synovium, an interferon profile has also been reported in peripheral blood cells of a subset of RA patients [17].

Innate signaling is triggered by TLR-dependent and independent recognition of bacterial and viral products as well as endogenous self-nucleic acids containing RNA, DNA, and nuclear protein in immune complexes [18–20]. These immune complexes can activate membrane TLR and cytoplasmic innate pattern recognition receptors that induce the type I IFN response [21,22]. Binding to innate receptors results in activation of several transcription factors, including interferon regulatory factors (IRF), c-Jun/ATF2, and NF-κB, which are involved in production of the type I IFN response through formation of the IFN enhanceosome [23]. The IRF family is composed of nine transcription factors that regulate the type I IFN system and host defense [24]. Distinct functions and differential regulation of IRF have been elucidated in a variety of cell types and in response to viral infection and other triggers of innate receptors [9,10,25–27].

Because immune complexes contribute to the pathogenesis of RA and SLE and are often cleared from the circulation by the mononuclear phagocytic system, we evaluated the relative roles and functional hierarchy of IRF in the human monocytic cell line THP1. The present study investigated the role of IRF3, IRF5, and IRF7 in promoter activation and gene expression of cytokines, chemokines, and MMP in THP1 monocytic cells. We evaluated the hu-

Abbreviations: AP-1, activator protein 1; FLS, fibroblast-like synoviocyte; IFN, interferon; IKK, IκB kinase; IP-10, IFN inducible protein 10; ISRE, IFN-stimulated response element; IRF, IFN regulatory factor; MIP, macrophage inflammatory protein; MMP, matrix metalloproteinase; MCP1, monocyte chemotactic protein-1; PBMC, peripheral blood mononuclear cell; poly (I-C), polyinosinic and polycytidylic acid; RANTES, regulated upon activation, normal T-cell expressed, and secreted; RA, rheumatoid arthritis; siRNA, small interfering RNA; SLE, systemic lupus erythematosus; TNF, tumor necrosis factor; TLR, toll-like receptor.

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man cell line THP1 because monocytes from patients with autoimmune diseases are functionally and phenotypically distinct from healthy controls and participate in clearance of immune complexes from the circulation, possibly contributing to disease pathogenesis. Initial studies of the pathogenesis of SLE were somewhat more focused on the adaptive immune system and lymphocyte abnormalities; however, this has shifted more recently toward innate immunity. Monocytes and macrophages are an essential part of the innate immune response and contribute to phagocytosis and cytokine and IFN production. Aberrations of monocyte or macrophage phenotype and function are increasingly recognized in SLE and other autoimmune diseases. Targeted IRF knockdown was followed by evaluation of protein and gene expression, promoter activation, and mRNA stability to determine the specific roles of IRF and potential as therapeutic targets in SLE or RA patients.

2. Materials and methods

2.1. Reagents

Monoclonal anti-IRF7 (sc-74472) and GAPDH (sc-32233) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal anti-IRF5 (3255) was purchased from Cell Signaling Technology (Danvers, MA). Polyinosinic and polycytidylic acid [poly (I-C)] and LPS were purchased from Sigma Aldrich (St. Louis, MO). CpG ODN2006 (tlrl-hodnb-1) was obtained from Invivogen (San Diego, CA). TNF and IL-1 were purchased from R&D systems (Minneapolis, MN).

2.2. Cell culture

Human monocytoid THP-1 cells were purchased from ATCC (Manassas, VA) and grown in serum-free RPMI 1640 medium (Life Technologies, Grand Island, NY) supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 50 μg/mL gentamicin, and 10% heat inactivated fetal bovine serum (Gemini Bio-Products, Calabasas, CA).

2.3. Western blot analysis

Cells were cultured in 6-well plates at 5×10^5 cells/mL. Cells were incubated with poly (I-C) (10 μg/mL), LPS (1 μg/mL), CpG (10 μg/mL), IL-1 (1 ng/mL), and TNFα (10 ng/mL) for 6 h. Dose response studies were performed with poly (I-C) for various times points up to 24 h. Cells were washed with cold PBS, and protein was extracted using kinase lysis buffer (50 mM HEPES, 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1 mM $MgCl_2$, 1.5 mM EDTA pH 8.0, 20 mM β-glycerophosphate, 50 mM NaF, 1 mM Na_3VO_4, 10 μg/mL aprotinin, 1 μM pepstatin A, and 1 mM PMSF). The protein concentrations were determined using Micro BCA protein assay kit (Thermo Scientific, Rockford, IL). Samples containing 50 μg protein were resolved via 4–12% SDS–PAGE and transferred to a polyvinylidene diflouride membrane. The membranes were blocked and incubated with primary antibody at 4 °C overnight, followed by HRP-conjugated secondary antibody for 1 h. Proteins were visualized with chemiluminescence using the VersaDoc 4000 MP imaging system (BioRad, Hercules, CA).

2.4. Small interfering RNA transfection

Dose–response and kinetic studies were performed using 1, 3, and 5 μg siRNA at days 3, 5, and 7 to standardize and confirm knockdown. A total of 1×10^6 cells were transfected with 5 μg IRF3, IRF5, IRF7, or scramble control Smartpool small interfering RNA (Dharmacon, Lafayette, CO) using the Cell Line Nucelofector Kit V according to the manufacturer's instruction (Amaxa, Gaithersburg, MD). Approximately 75–85% decrease in protein expression is achieved using this method. Transfected cells were allowed to recover overnight and stimulated 6 h with poly (I-C) prior to lysis on day 3 post-transfection.

2.5. Quantitative real-time PCR

After siRNA transfection, cells were cultured in RPMI 1640 with 10% fetal bovine serum at 37 °C overnight. Cells were treated with either medium or poly (I-C) (10 μg/mL) for 6 h. Total RNA isolation with RNA STAT and cDNA synthesis by RT-PCR were performed as previously described [28]. Gene expression was evaluated using TaqMan PCR analysis and the GeneAmp 7300 Sequence Detection System as previously described [29]. Forward and reverse primers as well as fluorogenic TaqMan FAM/TAMRA-labeled hybridization probes were used (Assay on Demand; Applied Biosystems, Foster City, CA). To control for sample cellularity, human GAPDH forward and reverse primers and labeled probe were included in separate PCR. The threshold cycle C(t) was determined for each sample using GeneAmp software. Standard curves are generated by linear regression using log (C[t]) versus log (cell number). The cell equivalent (CE) number for samples was calculated using the standard curve. Data are expressed as the ratio between gene of interest CE and GAPDH CE, yielding the relative gene expression (RE).

2.6. Reporter gene assay

After siRNA transfection, THP-1 monocytic cells were cultured for 3 days, and subsequently, 1×10^6 cells were transfected with 1 μg reporter plasmid DNA and 0.1 μg *Renilla reniformis* luciferase construct as internal control for transfection efficiency (a generous gift from Dr. M. David UCSD). Reporter constructs containing IFN-stimulated response element (ISRE)-luciferase, which has five repeats of the ISRE sequence from IFN-stimulated gene 15 kD gene promoter, NF-kB-luciferase, or activator 1 (AP-1)-luciferase, and the full length IL-6 promoter-luciferase were individually transfected with control. After overnight incubation, transfected cells were stimulated with 10 μg/mL poly (I-C) for 6 h. Luciferase activity was measured using a dual luciferase assay kit (Promega, Madison, WI).

2.7. Analysis of mRNA stability

After siRNA transfection and poly (I-C) treatment, THP-1 cells were treated with 5 μg/mL actinomycin D to inhibit transcription (A1410) purchased from Sigma (St. Louis, MO), for 0.5, 1, 2, 6, or 18 h. Cells were harvested using RNA STAT-60 and cDNA was isolated for Q-PCR according to the methods described.

2.8. Statistical analysis

Statistics were performed using the paired Student's *t* test. A comparison was considered significant if $p < 0.05$.

3. Results

3.1. Activation of IRF5 and IRF7 in human THP1 monocytes

To characterize the protein expression of IRF5 and IRF7 in human THP1 responses, cells were stimulated with cytokines or TLR ligands followed by Western blot analysis to detect IRF5 and IRF7 induction (Fig. 1, top panel). Because IRF5 and IRF7 were inducible, we measured the increase in IRF5 and IRF7 protein expression. Quantification by densitometry of IRF5 and IRF7 protein expression in THP1 stimulated with each ligand is also shown in Fig. 1 (bottom