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Type I interferons activate apoptosis in a Jurkat cell variant by caspase-dependent and independent mechanisms

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Abstract

Although the antiviral actions of interferons (IFNs) are observed in most types of cells, the antiproliferative effects of IFNα/β are variable as are the mechanisms of growth inhibition that may or may not be due to the induction of apoptosis. To understand more about the mechanisms that are responsible for IFNα/β-stimulated apoptosis, we have characterized a new human Jurkat T cell variant named H123 where IFNα activates programmed cell death (PCD). No differences in IFNα-stimulated, Stat-dependent gene expression were detected between H123 cells and the parental Jurkat cells, which are growth inhibited, but do not undergo apoptosis with IFNα. Although IFNα stimulates the activity of both caspase 3 and 9 in H123 cells, the general caspase inhibitor Z-VAD only partially reverses the apoptotic actions of IFNα. Induction of apoptosis by IFNα occurs through a mitochondrial-dependent pathway in H123 cells, as demonstrated by the release of cytochrome C from the mitochondria. Furthermore, IFNα treatment of H123 cells stimulates the release of the serine protease HtrA2/Omi from the mitochondria, suggesting that it plays a role in the apoptotic actions of this cytokine. These results provide evidence for a novel type 1 IFN-mediated pathway that regulates apoptosis of T cells through a mitochondrial-dependent and caspase-dependent and independent pathway.

Keywords: Type; One; Interferone; Signaling; Jak/Skat; Apoptosis

1. Introduction

The antiviral, antigrowth, and immunomodulatory activities of interferons (IFNs) are controlled, in part, by a set of cellular genes which are rapidly induced upon type 1 (IFNα/β) or type 2 (IFNγ) binding to specific cell surface receptors. Type 1 IFNs consist of several subtypes of IFNα, a single IFNβ as well as IFNτ and IFNω. In most cellular systems, the actions of IFNα and IFNβ are generally the same although IFNβ has been shown to be more potent and elicit IFNβ-dependent gene activation at lower concentrations than IFNα in several cell types. Interferon stimulated gene factors (ISGFs) mediate gene induction by binding to enhancers within the promoters of both IFNα/β- and IFNγ-induced genes [1,2]. Isolation of the promoters of several IFNα/β-stimulated genes permitted the mapping of an enhancer termed the interferon-stimulated response element (ISRE), which was demonstrated to be necessary and sufficient for activation of these genes [3,4]. Several genes have also been characterized whose expression is rapidly increased after incubation of cells with IFNγ. The promoter regions of these genes contain an element referred to as the gamma activation sequence (GAS) or gamma response elements (GRR) [5,6]. Latent cytoplasmic transcription factors called Stats are required for the expression of early response genes by IFNs. Stats are covalently modified by tyrosine

Abbreviations: Stat, signal transducer and activator of transcription; ISG, interferon-stimulated gene; ISGF3, interferon-stimulated gene factor 3; ISRE, interferon stimulated response element; GRR, gamma response element; EMSA, electrophoretic mobility shift assay; PCD, programmed cell death; IFN, interferon.

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phosphorylation performed by the Jak family of tyrosine kinases [7], and subsequently translocate to the nucleus where they interact with enhancers needed for interferon-stimulated gene expression. The information gained concerning IFN signaling has now been extended to include a broad network of cytokine-regulated signaling systems that use tyrosine phosphorylation of a family of structurally related Stat proteins to activate transcription of early response genes.

Type 1 IFNs are inhibitors of cell growth, and are presently used for the treatment of chronic myelogenous leukemia and other malignancies such as melanomas and renal cell carcinomas. The variety of mechanisms by which IFNα/β inhibits cell growth has not, for the most part, been correlated with activation of the Jak/Stat pathway. Although it is clear that IFNαβ activation of the Jak/Stat pathway is required for its antiproliferative effects [8], the mechanisms by which these cytokines inhibit cell proliferation vary. For instance, treatment of the Daudi Burkitt lymphoma B cell line with IFNα/β results in cell cycle arrest at the G0/G1 phase, while in the Jurkat T cell line, IFNα induces a slowing of the cell cycle without inducing cell cycle arrest or apoptosis [9]. Daudi cells also do not undergo apoptosis after treatment with IFNα/β; however, they show a suppression of the DNA-binding activity of the E2F transcription factor as well as decreased levels of phosphorylation of the retinoblastoma protein [10,11].

A variety of hematopoietic derived T and B cell lines, certain melanomas, and other cell lines derived from solid tumors have been reported to undergo apoptosis when incubated with type 1 IFNs [12–18]. The best described system to examine IFNα/β-stimulated apoptosis of primary cells is with murine, IL-7-dependent bone marrow derived pro-B cells. These cells exhibit inhibition of cell growth as well as apoptosis when incubated with IFNα/β. Similar apoptotic effects have been seen in several IL-7-dependent murine pro-B cell lines treated with IFNα/β [18]. Two reports using animal models confirm in vitro studies concerning the ability of IFNα/β to stimulate apoptosis in pro-B cells. IFNα/β treatment of newborn mice inhibits the development of both T and B cell populations, and infection of mice with lymphocytic choriomeningitis virus (LCMV) causes a transient bone marrow aplasia due to the production of type 1 IFNs [19,20]. In both of these reports, the actions of either IFNα/β or infection of mice with LCMV did not occur in mice lacking the IFNAR1 subunit of the type 1 IFN receptor. It remains to be determined whether under these conditions type 1 IFNs inhibit cell proliferation by inducing apoptosis. The inhibition of cell growth in primary murine B cells does not require the expression of Stat1 but appears to require IFNα/β-stimulated expression and translocation of Daxx [21].

In most cells where type 1 IFNs induce apoptosis, it has not been elucidated whether the actions of IFNα/β are mediated through the intrinsic or extrinsic apoptotic pathways. Some reports suggest that both pathways contribute to IFNα/β-induced programmed cell death [12,13]. It remains unclear as to the role caspase activation plays in the apoptotic actions of these cytokines. To gain a better understanding of the molecular events that govern the apoptotic effects of IFNα/β, we have taken advantage of a Jurkat cell variant called H123. These cells undergo massive apoptosis when incubated with IFNα/β while the parental Jurkat cells exhibit an antigrowth response, but do not undergo apoptosis under the same conditions. Comparison of the H123 and parental Jurkat cells has allowed us to define a previously uncharacterized set of events that play an important role in the apoptotic actions of these cytokines.

2. Materials and methods

2.1. Cell culture, transfection and reagents

The human leukemic T-cell line Jurkat, (subclone E6) and the H123 Jurkat variant line were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), containing 2 mM L-glutamine, penicillin and streptomycin (Invitrogen Corp.) at 37 °C and 5% CO_2. H123 cells were obtained from parental Jurkat mutagenized with ICR-191 and stable cell lines deficient in Ca^{2+} signaling were isolated as described [22]. Stable lines of H123 cells expressing CrmA and dominant negative FADD or overexpressing Bcl-2 were generated by transfection with Superfect reagent (Qiagen). Cells were maintained in the presence of 500 μg/ml of G418. Transient transfections with H123 cells were performed by electroporation according to the manufacturer's recommendations (BIORAD). A mixture of 5 μg of pEGFP-C1 plasmid (Clontech) with either 20 μg of pCEFL empty or HA-tagged cIAP1 plasmid cDNA, (a gift from Charles Tannenbaum, Cleveland Clinic Foundation, OH) was added to a total volume of 150 μl of cells followed by electroporation at 140 V, 1000 μF. Murine IL-7-dependent bone marrow derived B cells were isolated from 129 Sv mice as described [23]. Recombinant human IFNα-2a was a gift from Hoffman-LaRoche, N.J. Murine IFNβ was obtained from BiogenIdec [24].

2.2. Antibodies and chemical reagents

Anti-cytochrome C and anti-Fas monoclonal antibody were purchased from Pharmingen and used at 1:500 dilution and 100 ng/ml, respectively. Rabbit antiserum to tubulin was obtained from Santa Cruz Biotechnology and monoclonal actin antibody was purchased from Abcam. These antibodies were used at 1:1000 and 1:5000 dilution, respectively. Anti-SpI-2/CrmA monoclonal ascites was kindly provided by Richard Moyer and used at 1:500 dilution. Anti-Flag monoclonal antibody and staurosporine were obtained from Sigma. Anti-PARP and anti-HA monoclonal antibodies were purchased from Biomol and Covance, respectively. The HtrA2 inhibitor, ucf101, was generously provided by Dr. Antonis Zervos (University of Central Florida).

2.3. Preparation of whole cell and cytoplasmic extracts

Following treatment of cells with IFNα, cytosolic fractions were generated using a digitonin-based subcellular fractionation technique as described previously [25]. Briefly, 1×107 cells were harvested by centrifugation at 1000 rpm and washed in PBS pH 7.2, and re-pelleted. Cells were digitonin-permeabilized for 5 min on ice at a density of 1×10^7/ml in cytosolic extraction buffer (250 mM sucrose, 70 mM KCl, 137 mM NaCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2P0_4, pH 7.2, 100 μM PMSF, 200 μg/ml digitonin, 10 μg/ml leupeptin, and 2 μg/ml aprotinin). Plasma membrane permeabilization of cells was verified by staining the sample with 0.2% trypan blue. Cells were centrifuged at 1000 rpm for min at 4 °C. The supernatant fractions were collected and saved as cytosolic extracts. For whole-cell extracts, cells were lysed in whole-cell extraction buffer (20 mM Hepes, pH 7.5, 300 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$, 20% glycerol, 1% NP-40, 10 mM β-glycerophosphate, 1 mM sodium orthovanadate, 25 mM NaF, 200 μM PMSF, 0.5 mM dithothreitol). After centrifugation at 4 °C for 10 min, supernatants were collected. Protein concentration was measured by the Bio-Rad/Bradford protein assay.