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The DNA Damage Response Induces IFN

Sabrina Brzostek-Racine, Chris Gordon, Sarah Van Scyoc, and Nancy C. Reich

This study reveals a new complexity in the cellular response to DNA damage: activation of IFN signaling. The DNA damage response involves the rapid recruitment of repair enzymes and the activation of signal transducers that regulate cell-cycle checkpoints and cell survival. To understand the link between DNA damage and the innate cellular defense that occurs in response to many viral infections, we evaluated the effects of agents such as etoposide that promote dsDNA breaks. Treatment of human cells with etoposide led to the induction of IFN-stimulated genes and the IFN-α and IFN-λ genes. NF-κB, known to be activated in response to DNA damage, was shown to be a key regulator of this IFN gene induction. Expression of an NF-κB subunit, p65/RelA, was sufficient for induction of the human IFN-λ1 gene. In addition, NF-κB was required for the induction of IFN regulatory factor-1 and -7 that are able to stimulate expression of the IFN-α and IFN-λ genes. Cells that lack the NF-κB essential modulator lack the ability to induce the IFN genes following DNA damage. Breaks in DNA are generated during normal physiological processes of replication, transcription, and recombination, as well as by external genotoxic agents or infectious agents. The significant finding of IFN production as a stress response to DNA damage provides a new perspective on the role of IFN signaling. The Journal of Immunology, 2011, 187: 5336–5345.

An effective DNA damage response is critical for maintaining genomic integrity and preventing mutations that can lead to cancer. Double-strand breaks are the most severe lesions, and they can occur during DNA replication, lymphocyte V(D)J gene rearrangement, meiosis, viral infection, and in response to naturally occurring ionizing radiation (1–5). These DNA breaks are sensed rapidly, and accurate repair is essential to prevent permanent genomic damage. However, the cellular response to DNA damage engages more than just DNA repair machinery; it engages complex signaling pathways that can promote cell survival or cell death (6, 7). In this report, the activation of an additional network is revealed: the IFN signal pathway.

A primary transducer of the response to double-strand breaks is the nuclear kinase ataxia-telangiectasia mutated (ATM) (8). ATM belongs to a family of PI3K-related kinases, several of which are involved in the DNA damage response, including Rad53 and Rad53 related (ATR) and DNA-dependent protein kinase (DNA-PK) (9). ATM transduces the DNA damage response signal by phosphorylating downstream effectors such as the checkpoint kinases Chk1 and Chk2 and the p53 tumor suppressor. These effectors turn establish cell-cycle arrest to allow repair of damaged DNA or promote damage-induced apoptosis. Major alterations in gene expression occur during this time, and this reflects the action of not only p53, but also other transcription factors (10). One transcription factor that is activated in response to DNA damage is NF-κB (11). NF-κB regulates the expression of diverse genes involved in cellular responses that include survival, proliferation, tissue remodeling, inflammation, immunity, and stress. We have found that NF-κB activation in response to DNA damage directs the induction of the IFN system, a stress pathway best known for its ability to confer viral resistance.

IFNs play vital roles in both innate and adaptive immunity and consist of three families of cytokines that bind to distinct cell-surface receptors and are designated types I, II, and III (12). The genes encoding type I IFN (primarily α and β) and type III (λ) IFN are induced in response to viral or bacterial infection (13). The single type II (γ) IFN gene is induced primarily following receptor activation of T cells and NK cells. The regulated expression of the IFN-β gene in response to viral infection is a paradigm for cooperativity of DNA binding factors (14). NF-κB and IFN regulatory factors (IRFs) function along with activating transcription factor-2/c-Jun in the IFN-β enhancer. The IRFs were first characterized as regulators of type I IFN genes and IFN-stimulated genes (ISGs) and are now known to have diverse roles in immunity (15). Activation of ubiquitous IRF-3 during viral infection supports induction of a subset of ISGs and the IFN-β and IFN-α genes (16, 17). The IRF-1 and -7 genes are induced in response to secreted IFN and can play a role in the secondary response to IFN (18). IFNs bind to cell-surface receptors that activate Janus kinases and the tyrosine phosphorylation of STAT1 and STAT2 (19, 20). We report in this study that signaling via the DNA damage response in human cells primarily induces the IFN-λ and IFN-α genes. The promoters of the IFN-λ genes have been found to possess both IRF and NF-κB binding sites (21, 22). We demonstrate that NF-κB activation in response to DNA damage is sufficient and necessary to induce human IFN-λ.

This study identifies IFN signaling as part of the DNA damage response. IFNs are essential components of innate immunity and are well recognized for their ability to inhibit viral infection and activate immune effector cells (23). In addition, they are known for their antitumor effects by inhibiting proliferation of cancerous cells and promoting apoptosis (24–26). The IFN arm of the DNA...
damage response may have evolved as an antiviral mechanism in reaction to DNA damage induced by viruses, as a mechanism that reduces cellular proliferation to allow DNA repair, or as a mechanism to promote the death of cells with irreparable damage.

Materials and Methods

Cell culture, transfections, and infections

HeLa S3, HT1080, and THP-1 cells were obtained from American Type Culture Collection. THP-1 cells were maintained in RPMI 1640 with 10% FBS; other cells were maintained in DMEM with 8% FBS. Primary human monocytes were isolated from blood of healthy donors (Long Island Blood Services) using the Monocyte Isolation Kit II (Miltenyi Biotec, Auburn, CA) and maintained in RPMI 1640 with 10% FBS. Stable HT1080 transfectants with tetracycline-inducible expression of IFN-γ were generated according to the manufacturer’s instructions (T-Rex system; Invitrogen, Carlsbad, CA), and the gene was induced with 2 μg/ml doxycycline. Murine embryonic fibroblasts (MEFs) from NF-κB essential modifier (NEMO)/IκB kinase (IKK) γ knockout mice were a gift of Dr. Kenneth Marcu (Stony Brook University) (27). MEFs from IRF3 knockout mice were a gift of Dr. Tak Mak (University of Washington) (28) and MEFs from IRF7 knockout mice were a gift of Dr. Michael Gale (University of Washington) (29). DNA transfections were performed using FuGene 6 (Roche Diagnostics, Indianapolis, IN) or TransIt (Mirus, Madison, WI). Newcastle disease virus (NDV) (NJ-LaSota-1946) was a gift from Dr. Paula Pitha-Rowe (Johns Hopkins University, Baltimore, MD) and was propagated as described previously (30). Infections were performed at 200 hemagglutination units/ml.

Plasmids and luciferase assays

IFR-3, STAT1, and STAT2 constructs have been described (31–33). The dominant-negative IκBα plasmid (S32A/S36A) was a gift of Dr. Dean Ballard (Vanderbilt University, Nashville, TN) (34). The hemagglutinin (HA)-tagged ubiquitin–K63-only (HA-Ub0R63K) plasmid was a gift of Dr. Dafna BarSagi (New York University) (35). The reporter plasmid encoding the IFN-α promoter regulatng expression of the firefly luciferase gene (pα1−554/+14Luc) was a gift of Dr. Takashi Fujita (Kyoto University, Kyoto, Japan) (21). The human IFRFα gene was obtained from Dr. Joseph S. Pagano (University of North Carolina, Chapel Hill, NC) (36) and subcloned into the pcDNA/pMyc-polyhistidine (His) vector (T-Rex system; Invitrogen). The Dual-Luciferase reporter assay system was used for luciferase assays with the Renilla luciferase construct pRL-null as an internal control (Promega, Madison, WI).

Reagents

Etoposide was used at 40 μg/ml, and camptothecin, adriamycin, and mitomycin were used at the concentrations indicated (Sigma-Aldrich, St. Louis, MO). NF-κB inhibitor BAY 11-7082 was obtained from Alexis Biochemicals (San Diego, CA) and used at 5 μM, and the IKKβ inhibitor ML120B was a gift from Millennium Pharmaceuticals (Cambridge, MA) and used at 20 μM. The ATM inhibitor AZ12622702/KU55933 was a gift from Millennium Pharmaceuticals (Cambridge, MA). Doxycycline was added 24 h posttransfection with or without tetracycline-inducible expression of Myc-His–IRF-7 gene as described above. Etoposide activation of IFN signaling was analyzed using the LightCycler software (Roche Molecular Biochemicals), and values were normalized to actin mRNA levels. Human primers used in the studies corresponded to: IFN-α1 (37), IFN-α2 (38), IFN-α3/4 (38), actin (39), and pan-IFNs 5′-CACAGCAGGTTCCAA-GACCTC–3′ and 5′-TCTTCAGCACAAGGAAGCTC–3′; ISG54 (+1608) 5′-ATTCTATCCAAAGGGCCGG–3′ and (+1370) 5′-TGGAGTGCTGGAAGCTTACCTC–3′; IFN-α1 (+194) 5′-TACCCTCGGGAATTGCTTCG–3′ and (+316) 5′-AGGCTGTTGATATCTCCTCG–3′; IFN-γ (+194) 5′-TACCCTCGGGAATTGCTTCG–3′ and (+316) 5′-AGGCTGTTGATATCTCCTCG–3′; IFN-β (+275) 5′-TGCTCTCCTGTTGCTTCCAC–3′ and (+233) 5′-ATAATGATTGAACTAGCCGG–3′; IFN-β (+180) 5′-ATAATGATTGAACTAGCCGG–3′ and (+1343) 5′-GTGCTCTCGTGAAGCCTGA–3′; IFN-γ (+397) 5′-TCTTACGATCTGCTGACCTCTC–3′ and (+190) 5′-CTGATACAAACGGG–3′; and GAPDH (+1007) 5′-TACTCTTGAGGAGCCTA-TGTG–3′ and (+528) 5′-CAGACTTCCGATCCATCCTG–3’. Murine primers corresponded to: pan-murine IFN-α 5′-CCTGAGAGAGGAACACACACGG–3′ and 5′-TCTGCTCTGACACCTTCCAC–3′ (40); murine IFN-α2 (41); and murine Actin (42).

Immunoprecipitation and Western blot

For immunoprecipitation, cells were lysed in 50 mM HEPES (pH 7.2), 250 mM NaCl, 0.5% Nonidet P-40, 5 mM EDTA, 1 mM EDTA, 0.1 mM NaF, 0.1 mM Na3VO4, and protease inhibitor mixture (Sigma-Aldrich, St. Louis, MO). Lysates were cleared by centrifugation for 5 min at 15,000 × g and reacted with Abs for 3 h at 4°C. Immunocomplexes were collected with Protein G-conjugated agarose (Invitrogen). For direct Western blot, cells were lysed in 50 mM Tris (pH 7.5), 400 mM NaCl, 0.5% Nonidet P-40, 5 mM EDTA, 10% glycerol, 50 mM NaF, 0.1 mM Na3VO4, and protease inhibitors. The lysates were cleared by centrifugation and directly added to SDS sample buffer. Proteins were separated on 5% SDS-PAGE and transferred to nitrocellulose membrane (Pierce Biotechnology, Rockford, IL). Membranes were reacted with indicated Abs, and images were detected using the Odyssey infrared imaging system (Li-COR Biosciences, Lincoln, NE). Alternatively, secondary anti-rabbit or anti-mouse Abs linked to HRP (Amerham/GE Healthcare, Piscataway, NJ) were used, and the membrane was incubated in ECL reagents and exposed to film.

Fluorescence imaging

Cells were seeded on coverslips, fixed in 4% paraformaldehyde, and either visualized directly for GFP fluorescence or permeabilized in 0.1% Triton X-100 before reaction with anti-Myc Ab. Secondary Abs were conjugated to rhodamine (Jackson ImmunoResearch Laboratories, West Grove, PA). Coverslips were mounted in anti-fade solution (Vectashield; Vector Laboratories, Burlingame, CA). Images were captured with a Zeiss Axiovert 200 M digital deconvolution microscope or Zeiss LSM 510 META NLO two-photon laser scanning confocal microscope (Carl Zeiss).

Ubiquitination assay

The HA-Ub0R63K expression plasmid was transfected into a stable cell line expressing tetracycline repressor and responsive Myc-His–IFR-7 gene as described above. Doxycycline was added 24 h posttransfection with or without etoposide as indicated. Cells were lysed by sonication in 6 M guanidine HCl, 0.1 M Na2HPO4/NaH2PO4 (pH 8), and 10 mM imidazole. Lysates were incubated with NI-NTA agarose beads (Qiagen, Chatsworth, CA), and proteins were eluted with SDS loading buffer and analyzed by Western blot (43).

Results

Etoposide activation of IFN signaling

Cells respond to viral infection with the induction of ISGs, either directly by activation of the IFR-3 transcription factor or as an indirect response to autocrine IFN and STAT activation (15, 19, 30). Because the genes induced by IRF-3 or STATs can promote cell death, the DNA damage response to identify shared proapoptotic-induced genes (24, 41). Etoposide, an anticancer drug, was used to initiate the DNA damage response because it inhibits the ability of topoisomerase II to religate cleaved DNA (47, 48). The inhibition results in an accumulation of double-stranded breaks in DNA, particularly during DNA replication and S/G2 phases of the cell cycle. The increase of dsDNA breaks with time leads to the DNA damage response and can lead to the death of cells with irreparable damage.
damage response and cell-cycle arrest or apoptosis. Following cell treatment with etoposide, we noted induction of several of the ISGs. The response of primary human monocytes was examined, and induction of a representative gene, ISG54/Ifit2, is shown in Fig. 1A. The effect of etoposide gradually increases the number of dsDNA breaks, and accordingly, the levels of ISG54 mRNA increased with time. To ensure this response was not specific to cell type, we evaluated the response of HeLa cells to etoposide. These cells also responded with induction of the ISG54 gene.

To access whether IFN production was responsible for ISG54 induction, we evaluated the activation of the STAT1 and STAT2 transcription factors in etoposide-treated cells. Binding of type I IFNs to specific receptors leads to tyrosine phosphorylation and nuclear accumulation of STAT1 and STAT2 (49). Cells expressing GFP-tagged STAT1 or STAT2 were examined for their response, and nuclear accumulation was evident by 24 h of etoposide treatment (Fig. 1B). In addition, the tyrosine phosphorylation of endogenous STAT1 and STAT2 was clearly evident (Fig. 1C).

These results suggested that etoposide treatment could induce the production and action of IFN, and accordingly, we evaluated the induction of IFN-α, IFN-β, and IFN-λ genes in primary human monocytes. There was little response of the IFN-β gene to DNA damage, although the gene was robustly induced in response to infection by NDV (Fig. 2A). In contrast, IFN-α mRNA expression was evaluated with pan-specific primers and clearly displayed an induction in response to etoposide (Fig. 2B). To determine the induction of IFN-α gene family subtypes, we tested the expression of individual genes. Various IFN-α genes tested were induced (Fig. 2C). We next tested expression of the newest family of IFN, IFN-λ. Specific induction of the IFN-λ1 gene clearly increased with etoposide treatment (Fig. 2D).

**Induction of ISG54, IFN-α, and IFN-λ in response to various DNA damaging agents**

Etoposide elicits dsDNA breaks by forming an inactive ternary complex with topoisomerase II and inhibits the ability of the enzyme to religate cleaved DNA. Effectiveness can vary with proliferation of cell cultures, but 40 μg/ml etoposide was usually optimal for the IFN response (Supplemental Fig. 1). To determine whether IFN signaling is a general response to DNA damage or specific to etoposide, three diverse agents were tested: camptothecin, mitomycin C, and adriamycin. Camptothecin elicits ssDNA breaks by forming a ternary complex with topoisomerase I, mitomycin C is a DNA alkylating agent, and adriamycin causes DNA strand breaks by intercalation (50). Cells were treated with these agents and evaluated for ISG54 protein expression and mRNA levels of IFN-α and IFN-λ1 (Fig. 3). Despite the different mechanisms that elicit DNA damage by these agents, they all induced ISG54 and the IFN genes. The differences in fold induction may be a consequence of various peak response times due to different mechanisms of DNA damage. The results indicate that the production of IFN in etoposide-treated cells is a general DNA damage response and not one specific to a single drug or DNA insult.

**DNA damage activates IRF-1 and IRF-7 but not IRF-3**

IRF-3 is expressed constitutively in cells, and in response to viral infection, it is a critical transcription factor for the induction of the IFN-β gene, a subset of IFN-α genes, and the direct induction of a subset of ISGs (15, 16, 51, 52). It exists in a latent state primarily in the cytoplasm and is modified by specific serine phosphorylation following viral infection. Phosphorylation promotes IRF-3 nuclear accumulation, DNA binding, and association with CBP. We evaluated these parameters for the activation of IRF-3 in response to DNA damage. Cells expressing IRF-3 tagged with GFP were treated with etoposide and visualized microscopically to access the cellular localization of IRF-3. IRF-3 remained primarily cytoplasmic and did not show evidence of nuclear accumulation (Fig. 4A). The phosphorylated forms of IRF-3 can be detected by their reduced migration during SDS-PAGE. This can be easily observed following viral infection, but it was not evident.

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**FIGURE 1.** Induction of ISG54 mRNA and activation of STAT signaling in response to etoposide. A, Primary (1˚) human monocytes were untreated or treated with etoposide for 0, 18, or 24 h. RNA was extracted, and real-time RT-PCR was performed with primers specific for ISG54 and quantified (left panel). HeLa cells were untreated or treated with etoposide for 24 h. RNA was extracted, and real-time RT-PCR was performed with primers specific for ISG54 and quantified (right panel). B, HeLa cells were transfected with STAT1-GFP or STAT2-GFP and untreated (−) or treated with etoposide for 24 h. Fluorescent images are shown. C, HeLa cells were treated with etoposide for the hours indicated, and cell lysates were evaluated for endogenous STAT1 (top panels) or STAT2 (bottom panels) tyrosine phosphorylation by Western blot. Results represent duplicate determinations in three independent experiments.
in response to DNA damage elicited with camptothecin or etoposide (Fig. 4B). The ability of activated IRF-3 to form complexes with CBP was evaluated by coimmunoprecipitation and Western blot. Although IRF-3 can be readily detected in immunocomplexes with CBP during viral infection, there was no evidence of association with CBP following etoposide treatment.

The IRF-7 transcription factor is a key regulator of the IFN-α genes in response to viral or bacterial infection (18, 29, 51, 53). It is expressed at low levels in lymphoid cells, but it is induced in all cell types by IFN and can function in a secondary wave of IFN production. We evaluated induction of the IRF-7 gene in response to DNA damage and found mRNA levels were clearly induced following etoposide (Fig. 5A). mRNA levels increased by \(10^3\)-fold estimated by real-time PCR in 24 h (S. Brzostek-Racine and N. C. Reich, unpublished observations). IRF-7 can be activated in response to viral infection by serine phosphorylation. Although this modification may occur, it was not evident by a reduced migration in SDS-PAGE (S. Brzostek-Racine and N.C. Reich, unpublished observations) (54). Regulation of IRF-7 activity has also been demonstrated to occur by lysine 63-linked ubiquitination, and this modification was evaluated (55). A stable cell line was generated expressing a tetracycline/doxycycline-inducible IRF-7 gene tagged with the His and Myc epitopes. These cells were untreated or treated with etoposide, and IRF-7 protein was captured on nickel-charged resins and evaluated by Western blot (Fig. 5B). Following etoposide treatment, IRF-7 displayed slow migrating species indicative of its polyubiquitination and activation. Lysine 63-linked ubiquitin chains have been shown to regulate cellular processes including protein–protein interactions and to play a critical role in the DNA damage response (56).

In a latent state, IRF-7 resides primarily in the cytoplasm and accumulates in the nucleus following activation. The tetracycline-inducible cell line was used to evaluate IRF-7 localization by immunofluorescence during DNA damage (Fig. 5C). Following induction of IRF-7 expression, the protein was clearly cytoplasmic in untreated cells. However, IRF-7 accumulated in the nucleus following etoposide treatment, suggesting its activation. To evaluate the effects of inducible IRF-7 on IFN-α and IFN-λ1, their mRNA levels were measured (Fig. 5D). Cells were treated with etoposide, treated with tetracycline to induce IRF-7, or treated with both etoposide and tetracycline. Pan-specific primers were used to quantify expression of the IFN-α family. IFN-α was found to be induced in response to etoposide, but there was minimal
effect of tetracycline-induced IRF-7 alone. However, etoposide and tetracycline potently increased etoposide-induced IFN-α expression. The results indicate modification of IRF-7 protein in response to DNA damage contributes to induction of the IFN-α genes. Expression of IFN-α mRNAs was similarly evaluated, and IRF-7 activation by etoposide also was found to contribute to induction of the IFN-α gene.

Another member of the IRF family, IRF-1, has diverse roles in response to pathogens, development of the immune system, growth arrest, and apoptosis (15, 57, 58). The protein is constitutively nuclear but only expressed following induction by cytokines such as IFN. We evaluated IRF-1 mRNA expression and found mRNA levels increased by >10-fold estimated by real-time PCR following 24 h of etoposide treatment (Fig. 6A) (S. Brzostek-Racine and N.C. Reich, unpublished observations). To evaluate the possible role of IRF-1 in induction of the IFN genes during DNA damage, we tested its effect on expression of the IFN-α promoter driving a luciferase reporter gene. The promoter of IFN-α possesses IRF binding sites and a bona fide NF-κB site (21, 22). The IFN-α promoter activity was induced following etoposide treatment or by cotransfection with a plasmid encoding IRF-1 (Fig. 6B). Combined IRF-1 expression with etoposide induced significant expression of the IFN-α promoter. IRF-1 could also be demonstrated to bind to a site in the IFN-α promoter, suggesting direct action on the IFN-α gene (Supplemental Fig. 2).

dsDNA breaks that occur in response to etoposide lead to the recruitment and activation of the ATM kinase. To evaluate the potential role of ATM in the induction of IFN gene expression, we tested the effects of a pharmacological inhibitor, AZ12622702. Treatment of cells with the ATM inhibitor decreased the ability of etoposide to induce the IFN-α gene and the IFN-α genes (Fig. 6C). The results indicate ATM is a significant signaling kinase upstream of IFN gene induction, and the residual response may be due to other PI3K-related kinases that are activated during DNA damage.
Values are means of duplicate determinations in two independent experiments.

His–inducible cells by real-time PCR. Cells were untreated (−) or treated with etoposide (+) for 24 h. IRF-7 mRNA induction was evaluated by RT-PCR and displayed on agarose gels. Faint band in untreated sample is nonspecific. mRNA levels of GAPDH are shown as controls. B, HT1080 stable cell line expressing tetracycline-inducible IRF-7–Myc-His was transfected with the HA-Ub0R63K ubiquitin (K63Ub), and doxycycline (Dox) was used to induce IRF-7 expression in the absence or presence of etoposide for 24 h. IRF-7 was collected on nickel-charged resins, and samples were analyzed by Western blot with Ab to IRF-7.

IRF-7–Myc-His was induced with doxycycline in the stable cell line in the absence or presence of etoposide for 15 h before immunostaining with Abs to Myc. Imaging analysis of three independent experiments indicate nuclear accumulation of IRF-7 at this time in >50% of the cells. D, Pan-specific primers were used to quantify IFN-α mRNA levels (top panel), and specific primers were used to assess IFN-λ1 mRNA levels (bottom panel) in the IRF-7–Myc-His–inducible cells by real-time PCR. Cells were untreated (−) or treated with etoposide (E), doxycycline (D), or doxycycline and etoposide (D/E) for 24 h. Values are means of duplicate determinations in two independent experiments.

indicating NF-κB is sufficient to induce the human IFN-λ1 gene independent of viral infection or DNA damage (Fig. 7D). This result has obvious implications for the involvement of IFN action in the many signaling pathways that activate NF-κB.

Response of murine embryo fibroblasts

Our studies with human primary cells or established human cell lines provide clear evidence that the IFN-λ and IFN-α genes are induced in response to DNA damage by etoposide and that NF-κB is requisite for the induction. Although murine cells may not accurately reflect the human response, the murine system affords the ability to test cells from animals with specific gene knockouts. For this reason, we obtained MEFs from wild-type (wt) or gene knockout animals and tested their response to DNA damage. Wild-type MEFs or MEFs that lack NEMO were treated with etoposide, and IFN gene induction was evaluated by RT-PCR (Fig. 8). Because murine IFN-λ1 is a pseudogene, we assayed expression of murine IFN-λ2. Results showed etoposide treatment induced the genes encoding IFN-λ2 and IFN-α in wt cells, but not in NEMO knockout cells. The results are in accordance with our studies in human cells that demonstrated a requirement of NF-κB activation. In addition, MEFs from IRF3 knockout animals treated with etoposide induced the IFN-λ2 and IFN-α genes, supporting our studies with human cells that showed IRF3 did not play a major role in the DNA damage response. Analyses of MEFs that lack the IRF-1 gene indicated it was critical for IFN-λ2 gene expression, but not for IFN-α expression, whereas MEFs that lack the IRF-7 gene had a profound defect in the induction of both IFN-λ2 and IFN-α genes in response to etoposide. The promoters of the murine IFN-λ genes are not well characterized, and therefore, they may respond differently from the human IFN-λ genes during the DNA damage response. The levels of IFN induction in these spontaneously immortalized MEFs were modest but reproducible following etoposide treatment.

Expression of human IRF and IFN genes with time during the DNA damage response

Our studies with human cells indicate that NF-κB activated by DNA damage stimulates induction of the IFN-λ1, IRF-1, and IRF-7 genes. To determine the time course of expression of these genes, human THP-1 cells were treated with etoposide, and real-time PCR was used to quantify IFR and IFN mRNA levels. IRF-1 and IRF-7 mRNA levels displayed an initial peak of expression at 4 h of etoposide treatment and reached steady-state levels by ∼12 h (Fig. 9A). Expression of the IFN-λ1 gene showed a small increase at 4 h of etoposide treatment and peaked at 15 h with a kinetic profile similar to that of the IRFs. Expression of IFN-α mRNA trailed that of IFN-λ1 by 3 to 4 h, possibly indicating a greater dependency on IRF-7 induction and activation (Fig. 9B).

Discussion

The DNA damage response rapidly engages multimeric protein complexes to repair DNA and activate transcriptional programs that regulate cell-cycle checkpoints and cell survival (6, 8, 65, 66). The recruitment of ATM, ATR, and DNA-PK to DNA breaks initiates phosphorylation and ubiquitination events that lead to specific transcription factor activation and gene expression. ATM is activated primarily in response to dsDNA breaks, followed by ATR and DNA-PK in response to DNA single strands and ends generated.
during break resolution. One of the known substrates of ATM is NEMO, and phosphorylation promotes its ubiquitination, nuclear export, and activation of IKK complexes (62, 67, 68) (Fig. 9C).

IKK phosphorylation of IkB leads to release of NF-κB dimers and their ability to translocate to the nucleus and bind DNA targets. Our studies demonstrate that NF-κB is sufficient to induce the human IFN-λ1 gene during the response to DNA damage. The promoter of the human IFN-λ1 gene has a bona fide NF-κB binding site as well as an IRF binding site (21). NF-κB also induces the IFR-1 and IRF-7 genes that can influence expression of the IFN-α and IFN-λ genes. The induced IFNs can additionally amplify expression of the IRFs. These findings add a new dimension to the complexity of the DNA damage response. ATM appears to be primarily accountable for initial signal pathways that lead to human IFN gene expression by etoposide. Inhibition of ATM significantly reduces the induction of IFN-α and IFN-λ1 genes (Fig. 6). The ATM deficiency responsible for the development of ataxia-telangiectasia results in an array of clinical manifestations including cerebellar ataxia, oculocutaneous telangiectasia, immunodeficiency, and susceptibility to cancer (69, 70). The lack of IFN production in response to DNA damage that occurs through physiological processes in ataxia-telangiectasia speculatively may contribute to the immunodeficiency and tumor formation in the disease. Results of a few studies have suggested a potential role of IFN signaling during DNA damage (71–74). One study observed ISG expression correlated with resistance to radiation therapy (74), and another
Etoposide and IFN both have been used clinically for years for their antitumorigenic effects. Viruses have evolved mechanisms to inhibit activation or down-regulation of the DNA damage response (77–79). Viruses like HIV have RNA genomes but integrate viral DNA into the host genome, creating DNA strand breaks (4). Viruses with DNA genomes can generate ss- and dsDNA breaks during lytic replication. EBV, HSV1, and adenovirus are a few examples of DNA viruses that have been documented to activate a DNA damage response (4, 75, 76). More significantly, some of these DNA viruses that have been documented to activate a DNA damage response. EBV, HSV1, and adenovirus are a few examples of DNA viruses that have been documented to activate a DNA damage response. EBV, HSV1, and adenovirus are a few examples of DNA viruses that have been documented to activate a DNA damage response (4, 75, 76). More significantly, some of these DNA viruses that have been documented to activate a DNA damage response (77–79). Viruses may inhibit this pathway not only to block cell cycle arrest and apoptosis, but also to block the antiviral functions of IFNs that are produced by DNA damage.

Etoposide and IFN both have been used clinically for years for their antitumorigenic effects. IFNs produced in response to DNA damage may contribute to the antitumorigenic effects of etoposide. IFNs are recognized for their ability to cause growth arrest and/or apoptosis in neoplastic cells, although they can stimulate proliferation of healthy cells (24, 25, 46, 80–83). They also have vital immunoregulatory functions that include direct and indirect effects on activation of NK cells, macrophages, dendritic cells, T cells, and B cells (84, 85). The antiproliferative effects of IFN and the potential enhanced clearance of tumor cells may play a role in the in vivo DNA damage response pathway.

The novel observation of the convergence of the DNA damage response with IFN signaling stimulates speculation as to the possible function of IFN in reaction to genotoxic stress. In our experimental system, the addition of IFN did not block the apoptotic effects of etoposide or significantly contribute to cell death (S. Brzostek-Racine and N.C. Reich, unpublished observations). But IFNs are well characterized for their ability to inhibit viral infection, and this may reflect the evolutionary link. Many viral infections are known to stimulate DNA damage response pathways. Viruses like HIV have RNA genomes but integrate viral DNA into the host genome, creating DNA strand breaks (4). Viruses with DNA genomes can generate ss- and dsDNA breaks during lytic replication. EBV, HSV1, and adenovirus are a few examples of DNA viruses that have been documented to activate a DNA damage response (4, 75, 76). More significantly, some of these viruses have evolved mechanisms to inhibit activation or downstream function of the DNA damage response (77–79). Viruses may inhibit this pathway not only to block cell cycle arrest and apoptosis, but also to block the antiviral functions of IFNs that are produced by DNA damage.

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Disclosures

The authors have no financial conflicts of interest.

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