Molecular Pathways

The Two Faces of Interferon-γ in Cancer

M. Raza Zaidi and Glenn Merlino

Abstract

Interferon-γ is a cytokine whose biological activity is conventionally associated with cytostatic/cytotoxic and antitumor mechanisms during cell-mediated adaptive immune response. It has been used clinically to treat a variety of malignancies, albeit with mixed results and side effects that can be severe. Despite ample evidence implicating a role for IFN-γ in tumor immune surveillance, a steady flow of reports has suggested that it may also have protumorigenic effects under certain circumstances. We propose that, in fact, IFN-γ treatment is a double-edged sword whose anti- and protumorigenic activities are dependent on the cellular, microenvironmental, and/or molecular context. As such, inhibition of the IFN-γ/IFN-γ receptor pathway may prove to be a viable new therapeutic target for a subset of malignancies.

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Background

The canonical IFN-γ signaling pathway

Interferons are a group of pleiotropic cytokines that play important roles in intercellular communication during innate and acquired immune responses and host defense against viral and bacterial infections, as well as tumor surveillance (1). IFNs are divided into 2 main categories in mammals, type I and type II, both of which differ substantially in the relative potencies of their immunomodulatory and cell-surface molecular modification properties (2). The 2 major members of type I IFNs (IFN-α and IFN-β) are ubiquitously expressed and signal through the type I receptor. IFN-γ is the lone member of the type II IFN and is more restrictively expressed. It is structurally and functionally different from the type I IFNs and has its own receptor, consisting of IFN-γR1 and IFN-γR2 subunits (3, 4). The biologically active form of IFN-γ is an antiparallel dimer that interacts with the extracellular domain of the receptor subunit IFN-γR1 (3). Binding of the ligand engages the IFN-γR2 subunit, which is responsible for the intracellular transmission of the signal. The intracellular carboxy termini of IFN-γR1 and IFN-γR2 carry the nonreceptor tyrosine kinases Janus-activated kinase (JAK)1 and JAK2, respectively, which phosphorylate the receptor upon ligand binding (5–7). This phosphorylation creates binding sites for the STAT proteins, primarily STAT1 (4, 8, 9). Phosphorylation leads to translocation of STAT1 homodimers into the nucleus, where they bind to gamma-activated sequence (GAS) sites on the promoters of downstream target genes. One of the major primary response genes transactivated by IFN-γ–activated JAK/STAT signaling is the transcription factor IFN response factor 1 (IRF1). IRF1, in turn, activates a large number of secondary response genes (10). Figure 1 depicts a simplified canonical IFN-γ/JAK/STAT pathway. Details of IFN signaling pathways have been reviewed elsewhere (11).

Regulatory features

IFN-γ/JAK/STAT signaling is regulated at several levels by positive and negative processes (Fig. 1). The STAT1 homodimers exist in the cytoplasm in the inactive antiparallel configuration (12). Phosphorylation leads to a change into a parallel configuration, which exposes a nuclear localization signal leading to nuclear translocation and binding to the target GAS sequences (12–15). Intracellular dephosphorylation by phosphatases such as TCP45 inactivates the STAT1 homodimer and causes its exodus from the nucleus into the cytoplasm (16). The activation of STAT1 is negatively regulated by lysine acetylation through histone acetyltransferases (e.g., CBP), but deacetylation by histone deacetylases (e.g., HDAC3) enhances activation (16, 17). A major avenue of IFN-γ/JAK/STAT pathway regulation is the negative feedback inhibition by the suppressor of cytokine signaling (SOCS) molecules, which block the activity of JAKs (18, 19). It has also been proposed that the transcriptional activity of STAT1 is enhanced by kinases such as mitogen-activated protein kinase (MAPK), protein kinase C (PKC), and phosphoinositide 3-kinase (PI3K)/AKT, which phosphorylate STAT1 in the transactivation domain (20). At the same time, these kinases themselves are activated through IFN-γ–induced STAT1-independent pathways. Although STAT1 is the primary transactivator immediately downstream of IFN-γ, there is evidence that, under certain circumstances, some STAT1-independent transactivators

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are also activated directly by IFN-\(\gamma\)-mediated signaling, including STAT3, STAT5, AP1, and NF-kB (21, 22).

**Clinical–Translational Advances**

**Diverse biologic functions**

Through the activation of a panoply of downstream effector molecules, IFN-\(\gamma\) signaling does diverse biologic functions primarily related to host defense and immune regulation, including antiviral and antibacterial defense, cell cycle, apoptosis, inflammation, and innate and acquired immunity (11). The most well-characterized function of IFN-\(\gamma\) is the upregulation of the MHC class I molecules to aid in the priming and presentation of antigens in the professional antigen-presenting cells (23). IFN-\(\gamma\) regulates the differentiation and function of many types of immune cells. It is intimately involved in all aspects of Th1-mediated immune responses by regulating the differentiation, activation, and homeostasis of T cells; it inhibits Th2 cell development but promotes the development of regulatory T (T\(_{reg}\)) cells (24). It also activates macrophages and induces production of chemokines, which recruit specific effector cells to the site of inflammation (25).

The profound immunomodulatory functions associated with IFN-\(\gamma\) quickly inspired clinical applications in a variety of disease conditions, including chronic granulomatous disease; fungal infections; autoimmune diseases, such as rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and lupus nephritis; and cancer (26). IFN-\(\gamma\) has long been associated with cytostatic/cytotoxic and antitumor functions (27). Fibrosarcoma cell lines refractory to IFN-\(\gamma\) signaling, because of ectopic expression of dominant negative IFN-\(\gamma\)R1, were shown to grow better and resist rejection in syngeneic mice, suggesting that IFN-\(\gamma\) plays an important role in the detection and elimination of tumor cells (28). It was also suggested that IFN-\(\gamma\) takes part in tumor surveillance functions by enhancing tumor cell immunogenicity, as mice that were insensitive to IFN-\(\gamma\) (i.e., IFN-\(\gamma\)R\(^{-/-}\) and Stat1\(^{-/-}\) mice) exhibited enhanced methylcolanthrene-induced tumor growth (29). This observation was supported by the finding that approximately one third of melanoma and lung adenocarcinoma cell lines had inactivating mutations in the IFN-\(\gamma\)
pathway components (29), which raised the possibility that tumor insensitivity to IFN-γ may be a mechanism used by cancers to evade tumor surveillance. The effects of IFN-γ were shown to involve upregulation of MHC class I genes, which increase tumor immunogenicity (30). This avenue of tumor surveillance was determined to involve recognition and elimination of tumor cells by cytotoxic T lymphocytes (CTL), recruited to the tumor mass via IFN-γ-induced chemokine signaling (31, 32).

Recombinant IFN-γ was shown to be involved in anti-proliferative (33–35), antiangiogenic, (36–38), and pro-apoptotic (39) effects against cancer cells. It was first clinically used to treat chronic myelogenous leukemia, alone and in combination with recombinant IFN-α, but it failed to show any significant positive outcome (40, 41). Since then, IFN-γ has been used in the clinical management of a variety of malignancies, including bladder carcinoma, colorectal cancer, ovarian cancer, and adult T-cell leukemia; however, the results have been mixed (reviewed in ref. 26).

The first demonstration of the antiproliferative effects of IFN-γ in melanoma cells was reported by Fisher and colleagues (42). Subsequently, Brown and colleagues identified IFN-γ as one of the growth inhibitory factors present in conditioned media of activated T cells (27). Kortylewski and colleagues reported that IFN-γ had significant growth inhibitory activity on 4 different human melanoma cell lines, although the extent of growth inhibition was inconsistent (43). The growth inhibition was dependent on STAT1 activation. Curiously, however, although STAT1 was activated by a low concentration of IFN-γ, the growth inhibition was only evident at a much higher concentration, indicating the presence of complex and even divergent signals emanating from the IFN-γ/STAT1 axis. Further support of this notion came from a study showing that IFN-γ upregulated c-jun and c-myc in a Stat1-independent manner (44).

The dark side of IFN-γ

At about the same time that IFN-γ was being touted as a promising antitumor agent, the opposite was being reported as well. Taniguchi and colleagues showed that IFN-γ was a much more potent enhancer of lung colonization of i.v. inoculated B16 melanoma cells than either IFN-α or IFN-β (45). Low-dose IFN-γ treatment of B16 cells enhanced resistance to natural killer (NK) cells and was accompanied by upregulated expression of MHC class I molecules H-2Kb and H-2Dβ. Human lymphocytes expressing low levels of IFNGR2 showed antipapoptotic and proliferative responses to IFN-γ, whereas those expressing high IFNGR2 levels showed a proapoptotic phenotype (46). Intratumoral expression of IFN-γ was shown to be associated with expression of MHC class II molecules and a more aggressive phenotype in human melanomas (47). Garbe and colleagues reported that treatment of human melanoma cells in culture induced characteristics of a biologically aggressive phenotype (48). Gorbacheva and colleagues showed that IFN-γ accelerated the proliferation of NIH-3T3 cells by upregulating guanylate-binding protein 2 (GBP2; ref. 49). Autocrine IFN-γ signaling was shown to enhance experimental metastatic ability of IFN-γ gene-transfected TS/A mammary adenocarcinoma cells and was attributed to increased resistance to NK cells (50).

Despite these early indications, IFN-γ was taken into clinical trials for melanoma. Early small-scale clinical trials were largely inconclusive (51–54). However, because of moderate success of recombinant IFN-α in melanoma clinical trials, IFN-γ was further tested in relatively larger studies. Schiller and colleagues reported a phase II to III clinical trial of IFN-γ for melanoma patients with a good prognosis (55). This study failed to detect any efficacious effects of IFN-γ, as the response rate was only 5%, with significant side effects. Importantly, suppression of helper T cells was observed (55). Yet another melanoma trial for adjuvant application of IFN-γ had to be prematurely terminated because the IFN-γ-treated patients fared worse than the untreated population (56, 57).

These failed attempts to treat melanoma with recombinant IFN-γ, combined with the occasional but conspicuous reports of its progrowth activities, raise the possibility that IFN-γ has, in fact, two faces; it can have cytostatic/cytotoxic as well as cytoproliferative effects depending on the context (Fig. 2). Such a scenario is not a new concept. TGF-β and TNF are well-known examples of secreted factors that display this kind of dual contrasting behavior (58, 59).

Possible mechanisms underlying protumorigenic IFN-γ

For the past 3 decades, IFN-γ has established a reputation for being an immunologic guardian against neoplastic disease. Schreiber and colleagues have implicated IFN-γ as a central player in their “immunoediting” model of the war between the immune defense systems of the host (tumor surveillance) and the oncogenic machinery of the tumor bent on escape (60, 61). Their model suggests that, whereas most oncogenic cells are recognized and eliminated by the immune system, some evolve strategies to survive and live in a dormant state in which equilibrium with the immune system is achieved. Further accumulation of capabilities (mutations) may push the tumor to the stage of complete evasion of the immune system, leading to overt disease.

Several lines of evidence place IFN-γ at the elimination stage of the immunoediting paradigm (62). However, evidence is now emerging that IFN-γ may also be involved at the equilibrium and/or evasion stages, roles that may be more protumorigenic. If so, under what conditions and by which mechanisms might IFN-γ behave as a “bad guy”? One key may lie in the homeostatic functions of IFN-γ. Although the well-known primary function of IFN-γ is to enhance the inflammatory response, it also plays a crucial role in limiting the destruction of tissues in the aftermath of inflammation. The IFN-γ–induced inflammatory cascade summons a variety of immune-related cell types, such as macrophages, NK cells, and CTLs, which play a central role
As Resanc for malignant progression and resistance to leading to upregulation of MHC class II antigens, which activates constitutive expression of CIITA in melanoma cell lines treated with IFN-γ lost Melan-A and gp100 tumor antigen processing, enabling the tumor cells to evade CTL recognition.

Suppression of CTL- and NK-cell–mediated immune responses is central to tumor immune escape, and a number of studies have indicated that IFN-γ may be intimately involved in these immunosuppressive mechanisms. It has been shown that IFN-γ upregulates the development of Treg and suppresses CTLs by inducing the expression of indoleamine 2,3-dioxygenase (IDO) in melanoma cells (64–66). IFN-γ attenuates infiltration of neutrophils and myeloid cells into the tissue microenvironments (67, 68). It activates constitutive expression of CIITA in melanoma, leading to upregulation of MHC class II antigens, which are associated with malignant progression and resistance to Fas-L+ T-cell–mediated apoptosis (69–71). Two separate studies have shown that incubation of IGR39D, FO-1, and MELA melanoma cell lines with IFN-γ decreases NK-cell–mediated cytolysis, with or without activation of MHC class I antigens (72, 73). Using the CT26 colon carcinoma tumor model, Beatty and colleagues showed that IFN-γ enhanced the expression of MHC class I molecules, which led to reduced tumor recognition and CTL-mediated lysis (74). Morel and colleagues reported that melanoma cell lines treated with IFN-γ lost Melan-A and gp100 tumor antigen processing, enabling the tumor cells to evade CTL recognition.

The presence of monocytic and granulocytic myeloid-derived suppressor cells (MDSC), both of which are dependent on IFN-γ, in the tumor microenvironment causes suppression of T-cell response (76). Nonclassical MHC class I molecules (e.g., HLA-G and HLA-E) are IFN-γ–regulated genes that are implicated in resistance to CTL and NK cell responses and in immune escape in a variety of cancers (77, 78). Recently, Cho and colleagues attributed the clinical failure of melanoma peptide vaccines to IFN-γ–driven expression of nonclassical MHC class I molecules, which enable melanoma cells to evade CTL-mediated cytolysis (79).

We have provided evidence that a substantial proportion of human melanomas harbor IFN-γ–producing macrophages, consistent with the proposed role for infiltrating macrophages in the IFN-γ–driven protumorigenic microenvironment created in UVB-irradiated skin (80, 81). Although strong data implicate lymphocytic infiltration in primary melanoma as a favorable prognostic marker (82, 83), little is known about the prognostic significance of macrophage infiltration. The prospect of validating IFN-γ–producing macrophages as a new and simple cellular marker of poor prognosis deserves further study.
investigation. It is also noteworthy that the presence of IFN-γ in serum has already been implicated as an independent prognostic indicator for disease recurrence in melanoma patients (84).

In conclusion, recent advances have provided evidence for the existence of a dark side of IFN-γ. IFN-γ seems capable of driving novel cellular and molecular inflammatory mechanisms that may underlie tumor initiation, immunoevasion, survival, and/or outgrowth. Which side wins the tussle between the anti- and protumorigenic functions of IFN-γ seems to be dependent on the contexts of tumor specificity, microenvironmental factors, and signaling intensity (Fig. 2). Despite the frequent, albeit typically ineffective, use of high-dose type I IFNs as conventional chemotherapy (85), we believe a case can now be made for exploring a paradigm-shifting strategy in which IFN-γ, IFN-γR, and/or downstream pathway members become viable therapeutic targets for at least a subset of melanomas, and perhaps other cancers as well.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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