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Interferon-Gamma at the Crossroads of Tumor Immune Surveillance or Evasion

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Interferon-gamma (IFN-γ) is a pleiotropic molecule with associated antiproliferative, pro-apoptotic and antitumor mechanisms. This effector cytokine, often considered as a major effector of immunity, has been used in the treatment of several diseases, despite its adverse effects. Although broad evidence implicating IFN-γ in tumor immune surveillance, IFN-γ-based therapies undergoing clinical trials have been of limited success. In fact, recent reports suggested that it may also play a protumorigenic role, namely, through IFN-γ signaling insensitivity, downregulation of major histocompatibility complexes, and upregulation of indoleamine 2,3-dioxygenase and of checkpoint inhibitors, as programmed cell-death ligand 1. However, the IFN-γ-mediated responses are still positively associated with patient's survival in several cancers. Consequently, major research efforts are required to understand the immune contexture in which IFN-γ induces its intricate and highly regulated effects in the tumor microenvironment. This review discusses the current knowledge on the pro- and antitumorigenic effects of IFN-γ as part of the complex immune response to cancer, highlighting the relevance to identify IFN-γ responsive patients for the improvement of therapies that exploit associated signaling pathways.

Keywords: type II interferon, immunoregulation, cancer microenvironment, immunotherapy, immune contexture

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INTRODUCTION

Interferons (IFNs) are pleiotropic cytokines with antiviral, antitumor and immunomodulatory properties, being central coordinators of the immune response (1). The term "interferons" comes from the description of molecules protecting cells by "interfering" with viral infection (2, 3). Three major types of IFNs are distinguished by their sequence identity, genetic loci, cell of origin, nature, and distribution of their receptors and resulting stimuli (**Table 1**).

The human type I IFN family comprises 17 distinct proteins, mainly represented by IFN-α and IFN-β, which are ubiquitously expressed and signal through their cognate receptor, composed by IFNαR1 and IFNαR2 subunits [reviewed in Ref. (4)]. IFN-γ is the lone member of type II IFN family. It is more restrictively expressed and is structurally and functionally different from the other types of IFNs. Most recently, a type III IFN family was described to be composed of four homologous proteins (IFNλ1–4), which bind the IFNλR1 and interleukin (IL)-10Rβ heterodimeric receptor [reviewed in Ref. (8)]. To date, type I and type III IFNs have been mainly involved in host–pathogen

TABLE 1 | Comparison of human type I, type II, and type III IFN production and signaling.

Properties	Type I IFN (IFN-α, IFN-β)	Type II IFN (IFN-γ)	Type III IFN (IFN-λ)
Members	17 proteins: 13 IFN-α, IFN-β, IFN-ε, IFN-κ, IFN-ω	1 protein: IFN-γ	4 proteins: IFN-λ1, IFN-λ2, IFN-λ3, IFN-λ4
IFN-producing cells	All nucleated cells	T cells, B cells, NK cells, NKT cells, and APCs	All nucleated cells, mainly mDCs, pDCs, and epithelial cells
IFN-responding cells	All nucleated cells	All nucleated cells	Lung, intestine, and liver epithelial cells
Stimuli	DAMPs and PAMPs	IL-12, IL-15, IL-18, type I IFN, and PAMPs	DAMPs and PAMPs
IFN receptor	IFN type I receptor (IFNαR): IFNαR1 and IFNαR2 subunits	IFN type II receptor (IFNγR): IFNγR1 and IFNγR2 subunits	IFN type III receptor (IFNλR): IFNλR1 and IL10Rβ
Signaling molecules	TYK2, JAK1, all STATs, CRKL, and IRS	JAK1, JAK2, STAT1, and STAT3	TYK2, JAK1, STAT1, STAT2, and IRF9
Transcription factor binding sites	ISRE (canonical) GAS (non-canonical)	GAS (canonical) ISRE (non-canonical)	ISRE
Functions	Antiviral, antiproliferative response, regulation of cell survival/apoptosis, and immunoregulation	Antiviral, antiproliferative, immunomodulatory, and antitumor response	Antiviral response, mucosal immunity
Reference	(4, 5)	(6, 7)	(8)

APCs, antigen-presenting cells; CRKL, CT10 regulator of kinase-like; DAMPs, damage-associated molecular patterns; GAS, gamma-activated site; IFN, interferon; IFN-γ, interferon-gamma; IFNαR1–2, type I receptor; IFNγR, type II receptor; IFNλR, type III receptor; IL, interleukin; IRF, interferon-regulatory factor; IRS, insulin receptor substrate; ISRE, interferon-sensitive response element; JAK, Janus kinase; mDCs, myeloid dendritic cells; NK, natural killer; NKT, natural killer T cells; PAMPs, pathogen-associated molecular patterns; pDCs, plasmacytoid dendritic cells; STAT, signal transducer and activator of transcription; TYK, tyrosine kinase.

interactions, and their expression is activated through immune system sentinel receptors, such as pattern recognition receptors. Despite the similar function of type I and III on antiviral infections, it is the viral tropism that dictates the relative contribution of each IFN (9). Moreover, whereas almost all nucleated cells respond to type I IFN, type III IFNs response is restricted to tissues with a high risk of viral exposure and infection, as the mucosal surfaces. The role of type II IFN in promoting host immune response to microorganisms is similarly well documented. Notably, it is also known to play a pivotal function on cancer immune surveillance, stimulating antitumor immunity and promoting tumor recognition and elimination (10–16).

This review focuses on type II IFN signaling, cellular functions, and directed therapies and was encouraged by novel findings revealing regulatory mechanisms of IFN-γ and its prognostic as well as therapeutic potential. In fact, since Wheelock who reported that IFN-γ inhibited viral replication in 1965 (17), it took around 30 years to envisage this cytokine as a target of antitumor immunity (18).

Interferon-gamma is a homodimer formed by the non-covalent association of two 17 kDa polypeptide subunits. During synthesis, after multiple N-glycosylation, both subunits bind in an antiparallel manner, constituting a mature 50 kDa molecule (19, 20). Notably, the IFN-γ symmetry suggests that a single molecule can bind simultaneously to two receptors, amplifying the underlying responses. Cellular responses induced by IFN-γ may also involve cross-communication with IFN-α/β receptors, amplifying IFN-γ signaling and its effects (21, 22).

Interferon-gamma is secreted predominantly by activated lymphocytes such as CD4 T helper type 1 (Th1) cells and CD8 cytotoxic T cells (23–26), γδ T cells (27–33), and natural killer (NK) cells (34, 35) and, to a less extent, by natural killer T cells (NKT), B cells (36–39), and professional antigen-presenting cells (APCs) (40–42). Its expression is induced by mitogens and cytokines, such as IL-12 (43, 44), IL-15 (45), IL-18 (46, 47), and type I IFN (48, 49). IFN-γ pleiotropic functions are mediated by cell-specific expression of hundreds of IFN-γ-regulated genes that encompass inflammatory signaling molecules, apoptosis and cell cycle regulators, and transcriptional activators (50). Autocrine IFN-γ produced by APCs can act locally and contribute to sustain self and neighbor cell activation (51–53), crucial for early control of pathogen spreading, while T lymphocytes are the major paracrine source of IFN-γ in adaptive immunity. Under physiological conditions, the constitutive expression of type I and II IFNs is tightly controlled, remaining localized to tissues, without systemic effects (54–56). For instance, constitutive expression of endogenous IFN-γ contributes to the homeostasis of immune cell functions (57), maintenance of the hematopoietic stem cell niche (58), and bone formation (59). Combination approaches to boost innate immune activation have been explored to converge onto IFN pathways. However, IFN-γ-related signaling can also have suppressive immunoregulatory effects on antiviral (60, 61), autoimmune (62, 63), as well as on antitumor responses (64, 65). Unveiling cellular targets of IFN-γ is critically important for its therapeutic application, to predict patient responses, particularly in cancers where this cytokine can exert protumorigenic effects. Therefore, the cellular and molecular effects of IFN-γ, with particular emphasis on its dual role on tumor immunity and how to overcome its limitations, will be the major focus of this review.

CANONICAL SIGNALING AND REGULATORY MECHANISMS

The IFN-γ Receptor

The IFN-γ receptor is composed of two ligand-binding IFNγR1 chains associated with two signal-transducing IFNγR2 chains, which are responsible for connecting to the cytoplasmic transduction machinery (see **Figure 1**). The *IFNGR1* and *IFNGR2* are localized in chromosome 6 and 21, respectively, and their

expression differs significantly. While IFNγR1 is constitutively expressed at moderate levels on the surface of almost all cells, IFNγR2 is constitutively expressed at low levels, and its expression is tightly regulated, according to the state of cellular differentiation or activation (66). For example, CD4 T helper cell subsets differ in their ability to respond to IFN-γ (67, 68). Remarkably, IFN-γ activates the signal transducer and activator of transcription (STAT) 1 that maintains the expression of T-bet, the master transcription factor that controls IFN-γ expression in T cells (69). This signaling constitutes a positive feedback loop that maximizes Th1 immunity (70–72). Notably, Th1 cells are more resistant to the antiproliferative effects of IFN-γ than Th2 cells. This is likely due to lower levels of expression of the IFNγR2 subunit that allows Th1 cells to continue to proliferate during IFN-γ signaling. By contrast, Th2 cells that do not produce IFN-γ express higher levels of the IFNγR2 subunit, rendering them particularly susceptible to the presence of IFN-γ that inhibits their proliferation (67, 68, 73). Nevertheless, IFNγR2 downregulation may be also induced in Th2 cells when they are exposed to IFN-γ (68). Thus, IFN-γ appears to regulate the expression of its own receptor on specific cell types, representing a regulatory mechanism of cellular desensitization in response to cytokines present at the local microenvironment. As a result, IFNγR2 expression can be a limiting factor in IFN-γ responsiveness and functional outcome that can dictate the Th1–Th2 phenotype switch and modulate the subsequent immune response.

JAK/STAT Signaling Pathway

The biological effects of IFN-γ are elicited through activation of intracellular molecular signaling networks, mainly *via* the JAK/STAT pathway, which modulates the transcription of hundreds of genes and mediates diverse biological responses (50, 74–76). Upon IFN-γ binding, the intracellular domains of IFNγR2 oligomerize and transphosphorylate with IFNγR1, activating

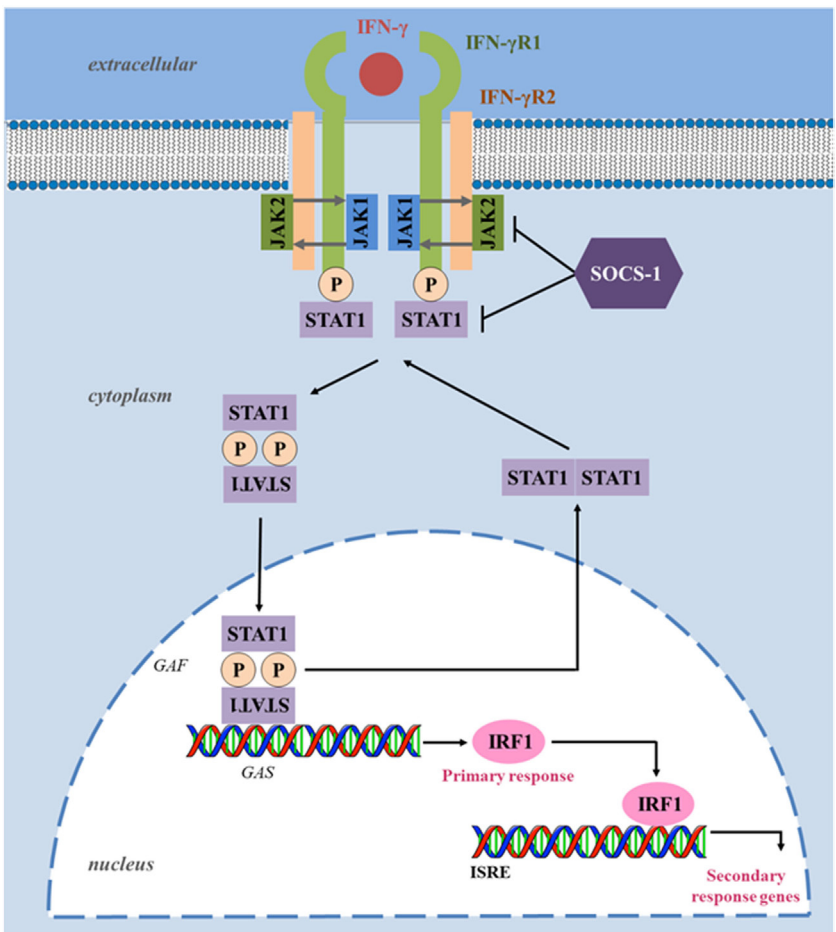

FIGURE 1 | Interferon-gamma (IFN-γ) canonical signaling pathway. Upon ligand binding, IFNγR1 and IFNγR2 oligomerize and transphosphorylate, activating Janus activated kinase (JAK) 1 and JAK2. These, in turn, phosphorylate IFNγR1, creating a docking site for the signal transducer and activator of transcription (STAT) 1. Phosphorylated STAT1 homodimerizes in an antiparallel configuration, forming a complex gamma-activated factor (GAF), which translocates to the nucleus and binds to gamma-activated site (GAS), located at the promoters of primary response genes, increasing their transcription. Upon induction, transcription factor interferon-regulatory factor 1 (IRF1) binds to interferon-stimulated response element (ISRE) and enhances the transcription of several secondary response genes responsible for several immunomodulatory functions. Suppressor of cytokine signaling (SOCS) proteins negatively regulate the IFN-γ pathway by inhibiting JAKs and STAT1 phosphorylation. Through dephosphorylation and deacetylation, the configuration of STAT1 homodimers reverts to parallel, triggering their exit from the nucleus.

the downstream signaling components, JAK1 and JAK2. The activated JAKs phosphorylate the intracellular domain of the receptor (tyrosine 440 on human IFNγR1), creating binding sites for STAT1 (77). STAT1 is then phosphorylated in the C-terminus on tyrosine Y701 residues by JAK, resulting in the formation of STAT1 homodimers complexes, known as gamma-activated factors (GAFs), which translocate to the nucleus and regulate gene expression through binding to gamma-activated site (GAS) elements in the promoters of interferon-stimulated genes (ISGs) (78). One of the major primary response genes induced by STAT1 signaling is the transcription factor interferon-regulatory factor 1 (IRF1), a member of the IFN regulatory transcription factor family (79). IRF1 functions as a transcription activator of interferon-stimulated response elements (ISRE), leading to the transcription of a large number of secondary response genes (**Figure 1**). For instance in breast cancer cells, a genome-wide identification of IFN-γ-induced IRF1 activation reveals over 17,000 binding sites, with "apoptosis" or "cell death" as the most enriched target processes underlying the direct tumoricidal property of the cytokine (80). However, tumor cells also develop resistance to IFN-γ through differential IRF1 responsiveness, pointing out that the JAK/STAT signaling pathway needs to be tightly regulated to avoid detrimental consequences of excessive stimulation and highlighting its role on immune responses and tumorigenesis (81). STAT1 targets of the IFN-γ-mediated signaling also include the SMAD family member 7 (SMAD7), and proteins involved in cell cycle regulation, such as c-Myc and the cyclin-dependent kinase inhibitor 1A (82–84).

The JAK/STAT signaling pathway is regulated at several levels by positive and negative mechanisms. In particular, deregulation or inhibition of the JAK/STAT pathway leads to lowered immunity and is often associated with increased tumorigenesis (85, 86) or metastatic dissemination (87). STATs are also involved in the development and function of the immune system and play a role in maintaining tumor surveillance [reviewed in Ref. (88)]. STAT1, as a tumor suppressor, is deducted for its expression in tumor cells, modulates their immunological status and consequently their response to antitumor immune responses. Indeed, STAT1-deficient tumor cells were more susceptible to NK cells while STAT1-proficient tumor cells were more sensitive to $CD8^+$ T cells (89). In the same way, STAT1-deficient mice that are impaired in Th1 cell polarization, exhibited reduced IFN-γ expression and compromised cytolytic and NK lytic activity, failing to control tumor growth in contrast with wild-type mice (90). In addition, cell-autonomous tumor-suppressor functions of STAT1 have also been reported in breast cancer (91). However, there is growing evidence that STAT1 also acts as a tumor promoter (92–94) since it can enhance resistance to chemotherapeutic agents and radiation in carcinoma (95). Importantly, STAT1 also participates in the signaling from different cytokines, including IL-21, IL-27, and IL-35. These cytokines have been proposed to limit antitumor immunity in specific cellular, molecular, and microenvironmental contexts (96–101). Thus, STAT1 phosphorylation reflects not only the threshold and magnitude of IFN-γ response but also of other immune mediators, highlighting the importance of the regulation of STAT1 phosphorylation. One of the most important negative regulators of the JAK/STAT signaling pathway is the suppressor of cytokine signaling (SOCS) proteins, which expression is increased in response to IFN-γ signaling through IRF1 (102, 103). SOCS blocks the activity of JAKs by a negative feedback loop, but also regulates other cytokines downstream signaling. SH2 domains in SOCS proteins directly bind to phosphorylated tyrosine residues of activated JAKs, blocking the recruitment of signal transducer adaptors, such as STATs, and JAK activity (102). Furthermore, SOCS promote interactions that lead to ubiquitination and proteasome degradation of components of the JAK/STAT signaling (104, 105). SOCS1 even prevents regulatory T (Treg) cells from producing IFN-γ by suppression of STAT1, avoiding the conversion of Treg cells into effector cells (106). In addition, SOCS2-deficient mice showed a reduction in lung metastases and an increase in survival following melanoma challenge (107).

Alternatively, the transcriptional activity of STAT1 can be positively regulated by other signaling cascades triggered by IFN-γ binding, such as the mitogen-activated protein kinase pathway, protein kinase C, and PI3K/AKT, which phosphorylate STAT1 in its transactivation domain (108). Adding to the complexity, under certain circumstances, IFN-γ also can activate STAT1-independent pathways through other transcription factors, namely STAT3 (109), STAT5 (110), nuclear factor-kappa B (NF-κB) (111), and activator protein 1 (112). In conclusion, the primary response of IFN-γ is mediated by GAF that acts on genes with GAS binding sequence in their promoter, while the primary response of type I IFNs is mediated by ISGF3 (STAT1/STAT2/IRF9 complex) that induces genes that have ISRE in their promoter. Thus, some of the ISGs are regulated by both types of IFNs, whereas others are selectively regulated by each type of IFN, consequently potentiating the diversity of biological responses.

BIOLOGICAL FUNCTIONS

IFN-γ Actions on Immune Cells

Interferon-gamma signaling pathway coordinates several biological responses, primarily involved in host defense and immune surveillance but also in the establishment of adaptive immunity (**Figure 2**) and in the regulation of inflammation, apoptosis and cell cycle. One of the first described biological effects of IFNs was the upregulation of the major histocompatibility complex (MHC) molecules (113, 114) as well as the upregulation of the whole MHC I and II antigen processing and presentation machinery including transporter associated with antigen processing (TAP) 1/2, invariant chain, and the expression and activity of the proteasome (115–122). Furthermore, in some tumor types, such as multiple myeloma and melanoma cells, IFN-γ can also upregulate the MHC class II transactivator (CIITA) that leads to MHC class II expression (123, 124). Thus, IFN-γ initiates an immune-antigenic exposure program in the target cells, and this ensures the rapid recognition of stressed tissues. IFN-γ is a major product of Th1-mediated immune response and orchestrates Th1 effector mechanisms, as further activation of innate immunity (macrophages and NK cells) in a positive feedback loop. Upregulation of cell surface MHC class I by IFN-γ is crucial for host response to intracellular pathogens and tumor cells, due to cytotoxic T cell activation, promoting cell-mediated immunity.

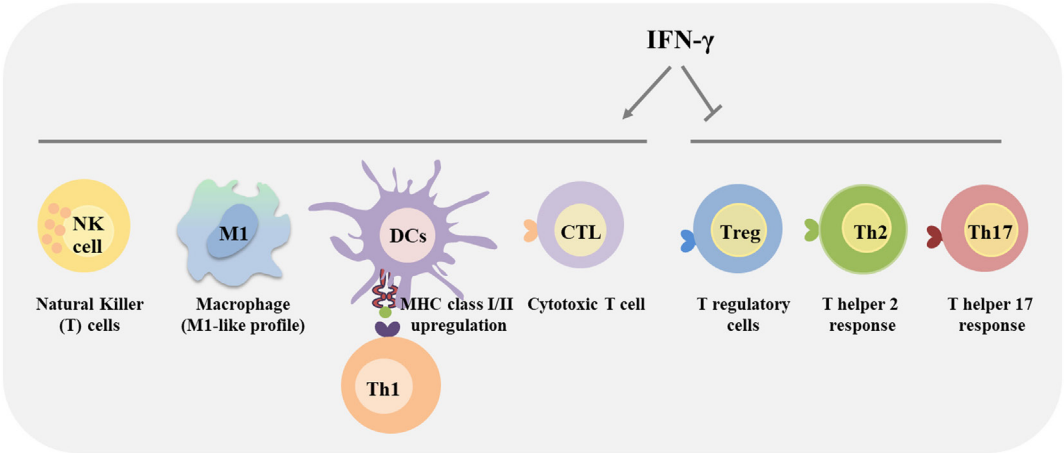

FIGURE 2 | Immunomodulatory effects of interferon-gamma (IFN-γ). IFN-γ produced by immune cells affects the behavior of distinct immune cells within the tumor microenvironment. Specifically, IFN-γ plays a major role in activating anticancer immunity, by promoting the activity of CD4 T helper type 1 cells, CD8 cytotoxic T lymphocyte (CTL), natural killer (NK) cells, dendritic cells (DCs), and macrophages, promoting the antigen presentation. Additionally, IFN-γ activates macrophages towards a more pro-inflammatory and tumoricidal phenotype (M1-like). Alternatively, IFN-γ inhibits regulatory T (Treg) cells, Th2 and Th17 differentiation and functions.

IFN-γ directly acts as a cytotoxic CD8 T cell differentiation signal, and it is essential for the induction of cytotoxic T cell precursor proliferation (125, 126). IFN-γ also upregulates cell surface MHC class II on APCs, thus promoting peptide-specific activation of CD4 T cells (25, 127–129). In addition, IFN-γ activates macrophages toward a pro-inflammatory profile, exhibiting an increased phagocytic ability as well as enhanced microbial killing activity (130). In fact, IFN-γ was initially shown to induce "classical" activation of macrophages and polarization toward a tumoricidal phenotype (131). Interestingly, the original name of IFN-γ was macrophage activation factor (132, 133). IFN-γ controls specific gene expression programs involving more than 290 genes related to cytokine and chemokine receptors, cell activation markers, cellular adhesion proteins, MHC proteins, proteasome formation, protein turnover, and signaling mediators and regulators (134). The ability of IFN-γ to induce tumor cell killing includes the activation of the NADPH-dependent phagocyte oxidase system, nitric oxide production, tryptophan depletion and upregulation of lysosomal enzymes (121, 135, 136). These events result in recruitment of effector cells to help in the inflammation resolution process (137, 138). In addition, as a major cytokine of Th1 cells, IFN-γ maintains Th1 lineage commitment through a positive feedback loop that stabilizes the Th cell phenotype (72, 139–141) and cross-inhibits the differentiation to other Th cell subsets (**Figure 2**). Indeed, IFN-γ inhibits Th2 cell differentiation (142, 143) and consequently IL-4 production. This regulation involves the inhibition of the IL-4/STAT6 pathway, required for Th2 cell differentiation, and it is mediated at least by IFN-γ-induced SOCS1 that inhibits IL-4R signaling (144, 145). Furthermore, IFN-γ-induced T-bet inhibits Th2 cell differentiation by directly interfering with the activity of Th2 cell-specific transcription factor, GATA-3 (146). Höfer and colleagues, using mathematical models, proposed that IL-4 also acts to propagate Th2 cell differentiation (147). A high IL-4 level promotes increased GATA-3 expression that further enhances GATA-3 transcriptional imprinting for Th2 differentiation (147, 148). This model proposed that high expression state of GATA-3 can be suppressed by strong inhibition of autoactivation, as observed in the presence of Th1-polarizing conditions (147, 149). IFN-γ was also described to downregulate the IL-4-inducible gene expression (150). The cross-regulation of Th1 and Th2 cells was also demonstrated in STAT6-deficient mice, which lack Th2 phenotype and associated immune responses. These animals displayed augmented tumor-specific IFN-γ production and cytotoxic T cell activity and, consequently rejected the tumor cell line that grew progressively in the wild-type control (151).

Interferon-gamma produced by Th1 cells also counteracts Th17 cell development and their effector functions (152–154). Several mechanisms can be considered as the inhibition of molecules involved in the Th17 differentiation (155, 156), the inhibition of STAT3 by STAT1 (157) and recently, T-bet was demonstrated to prevent differentiation of Th precursors into Th17 cells by blocking the expression of the Th17 cell lineage-specific transcription factor, RORγt (158). Furthermore, IFN-γ also exerts regulatory functions to limit tissue damage associated with inflammation (63, 159–162) (**Figure 2**). IFN-γ has been classically considered as a pro-inflammatory cytokine, involved in the regulation of anti-inflammatory responses, by antagonizing the IL-10 (157, 163) and TGF-beta (164) signaling pathways. Consequently, IFN-γ inhibits Treg cell differentiation and functions (165, 166). However, in some chronic inflammation conditions, IFN-γ plays a crucial role in attenuating tissue destruction. In this case, IFN-γ might be protective (62, 167) by promoting the number and function of Treg cells (168–170). In addition, IFN-γ production by Treg cells themselves was shown to be a key feature of the Treg cells that are capable of dampening Th1 cell responses (171–174). Thus, IFN-γ dictates the differentiation of specialized $Foxp3^{+}T\text{-}bet^{+}$ Treg cells that selectively suppress Th1 cells, and constitute a negative feedback loop to minimize the detrimental effect of IFN-γ. IFN-γ also promotes the differentiation of

myeloid-derived suppressor cells (MDSCs) that restrain overactivation of effector T cells, maintaining tissue homeostasis (175, 176). Other regulatory mechanisms involving IFN-γ signaling that dampen the magnitude of the immune response have been reported, as the induction of indoleamine 2,3-dioxygenase (IDO) by Treg cells, monocytes and stromal cells (177–180), and of the programmed cell death 1 (PD-1) ligand (PD-L1) on immune and transformed cells, inhibiting T cell responses (181–183).

IFN-γ Actions on Transformed Cells and on the Tumor Microenvironment

Interferon-gamma is involved in antiproliferative (18), antiangiogenic (184) and pro-apoptotic effects established against neoplastic cells. How IFN-γ induces the signaling pathways initiating and propagating the apoptotic cascade remains to be elucidated. The level of complexity is demonstrated by the fact that the mechanism might depend on the tumor cells themselves. For example, while in a glioblastoma cell line the induction of apoptosis was due to suppression of the PI3K/AKT pathway, in another glioblastoma cell line apoptosis occurred independently of the PI3K/AKT pathway but required NF-κB (185). It was also shown that IFN-γ induces apoptosis of human pancreatic carcinoma cells in a caspase-1-dependent manner (186). A review covered in detail the mechanism of induction of programmed cell death (187). So far, the known biological functions of IFN-γ indicate that, although it can act as a potent inducer of antitumor immunity, it actually has a dual role and may also favor tumor immune evasion.

IFN-γ IN CANCER

The first reports pointing to the relevance of IFN-γ in antitumor immunity came from studies with the fibrosarcoma (Meth A) cell line, refractory to IFN-γ signaling, since it lacks the expression of the IFNγR1 subunit. IFN-γ-insensitive Meth A cells displayed enhanced tumorigenicity compared with control cells and were not rejected in syngeneic tumor mice models, suggesting that IFN-γ plays an important role in tumor cell elimination (18). This finding was further supported by experiments using 129/SV IFN-γ insensitive mice, lacking the IFNγR1 subunit or STAT1, which developed 3-methylcholanthrene (MCA)-induced sarcomas more rapidly and more frequently than their wild-type counterparts (12). Similarly, these IFN-γ-insensitive mice lacking the tumor-suppressor protein p53 formed spontaneous tumors more rapidly than IFN-γ-sensitive p53-deficient mouse (12). In addition, C57BL/6 mice that lack the gene encoding IFN-γ also displayed higher susceptibility to experimental (B6, RM-1 prostate carcinoma) and spontaneous (BALB/c, DA3 mammary carcinoma) models of primary and metastatic tumors (13, 14). Notably, further studies described that IFN-γ may cooperate with other molecules to prevent tumor formation. Mice deficient in both granulocyte/macrophage colony-stimulating factor (GM-CSF) and IFN-γ developed lymphoma and non-lymphoid solid tumors at a higher rate than did mice deficient in GM-CSF or IFN-γ alone (15). Additional studies revealed that mice insensitive to IFN-γ, or that lack the recombination activating gene (RAG) protein (failing to produce mature B and T lymphocytes), or that lack both, showed similar incidence of MCA-induced sarcomas, suggesting that the T cell–IFN-γ axis is involved in immune surveillance (10).

The role of IFN-γ on cancer immunoediting emerged from studies assessing the immunogenicity of tumors from immunocompetent versus immunodeficient mice. Kaplan et al. showed that MCA-induced sarcoma cells from IFNγR1-deficient mice (unresponsive to IFN-γ signaling) grow as aggressively in immunocompetent as in IFNγR1-deficient mice. However, when IFN-γ responsiveness was conferred on the tumor cells by introducing the IFNγR1 subunit, they became more immunogenic and were rejected through a T cell-dependent manner (12). This constitutes the first demonstration that IFN-γ sensitivity of the tumor is fundamental for an efficient antitumor response. Other studies revealed that wild-type hosts rejected 40% of MCA-induced sarcomas derived from RAG2-deficient mice, showing that these tumors were more immunogenic than those from wild-type mice (10). In addition, human tumors were evaluated for their ability to upregulate MHC I expression in response to IFN-γ stimulation. These studies revealed that 33% of 33 melanoma tumor cell lines showed a reduction in IFN-γ sensitivity while 4 of 17 lung adenocarcinoma cell lines were totally unresponsive to IFN-γ (12). This lack of response resulted from cellular defects on IFNγR1 and of JAK proteins and may explain the ability of many tumor cells to evade the immune response. Recently, JAK1/2 deficiency was demonstrated to protect melanoma cells from antitumor IFN-γ activity and results in T-cell-resistant melanoma lesions (188). Others reported the lack of STAT1 in melanoma cell lines and in some chronic myeloid leukemia cells (189). Furthermore, DNA methylation that selectively represses CIITA, in colorectal and gastric cancer cell lines, was associated with the absence of IFN-γ-induced HLA-DR, suggesting that this epigenetic alteration of CIITA enables some gastrointestinal cancer cells to evade the immune system (190). Concomitantly, epigenetic alterations repressing *MHC2TA* were described in T cell leukemias, B cell lymphomas, and in several cancer cells, such as small cell lung cancer and neuroblastoma cells that were unable to express MHC II upon IFN-γ stimulation (191–194). Consistently, IFN-γ upregulates CIITA expression on multiple myeloma and melanoma cells increasing their MHC II expression (123, 124). These findings indicate that IFN-γ acts on tumor cells, enhancing their recognition by CD8 T cells as well as by CD4 T cells, and unveiling a key role in the promotion of tumor immunogenicity. Altogether, these works pave the way for the elaboration of the stepping-stone concept of immunoediting promoted by IFN-γ (195, 196).

IFN-γ-Mediated Mechanisms Underlying Antitumorigenic Effects

As described earlier, the mechanisms by which IFN-γ exerts its antitumor effects depend on multiple processes. IFN-γ is described as an antiproliferative agent that regulates the expression of cyclin-dependent kinase inhibitor 1 (p21) through STAT1 activation in tumor cells (84, 197). Moreover, IFN-γ is able to promote tumor cells apoptosis by upregulating the expression of caspase-1, -3, -8 (198, 199) and by enhancing the secretion of FAS and FAS ligand (200) and TNF-related apoptosis-inducing

ligand (201, 202). Recent studies showed that IFN-γ also induces its tumoricidal effects through a form of regulated necrotic death (also named as necroptosis) that relies on the activity of the serine–threonine kinase RIP1 (203). Importantly, IFN-γ is also involved in the inhibition of angiogenesis, impairing the proliferation and survival of endothelial cells, inducing ischemia in the tumor stroma (184, 204, 205). In particular, IFNγR is expressed on blood endothelial cells and engagement of the receptor results in blood vessel destruction and necrosis, an important mechanism that leads to tumor rejection (206).

Considering the effect of IFN-γ on the host immune cells present at the tumor microenvironment, major efforts have been made for the development and establishment of combined clinical therapeutic applications (90, 151, 207). IFN-γ is critical for T cell, NK and NKT cell trafficking into the tumors through CXCL9, CXCL10, and CXCL11 chemokine induction (208, 209). Accordingly, T cells fail to migrate to tumor site in IFNγ-deficient mice (65). In commitment, dipeptidylpeptidase 4 inhibition, a protease that inactivates these chemokines, enhanced tumor rejection by increasing lymphocytes trafficking into the tumor (210). Lately, galectin-3 secreted by several tumors was demonstrated to bind glycosylated IFN-γ at the tumor extracellular matrix, avoiding IFN-γ diffusion and the formation of an IFN-γ-induced chemokine gradient required for T cell recruitment and infiltration (211). In addition, CXCL10 also prevents tumor angiogenesis by blocking endothelial cell proliferation (212) and consequently a decrease in microvessel density as observed in melanoma tumor xenografts (213). Apoptosis of endothelial cells by IFNs causes restriction of blood flow within the tumor vasculature, leading to tumor shrinkage (214). This is an effect of IFN-γ, not directly targeted to the tumor cell, but to the tumor vasculature, with drastic and desirable effects on tumor growth. A recent report also showed that IFN-γ was essential for the initial priming and differentiation of cytotoxic T cells residing in the periphery of the eye, contributing to the regression of intraocular tumors (215). Supporting data from therapy models showed that IFN-γ induces *survivin* and *ifi202*, two genes involved in T cell maturation, survival, and proliferation, in tumor-specific T cells (216). Overall, these studies demonstrated the relevance of IFN-γ on T cell-mediated antitumor immunity.

Interferon-gamma is also involved in macrophages tumoricidal activity (217). This cytokine supports a CD4 T cell/macrophage effector axis which acts as immune surveillance mechanism for MHC II-negative cancer cells (25). Indeed, upon recognition of tumor antigens present in the context of MHC II by macrophages, CD4 T cells secrete IFN-γ that further activates macrophages in the tumor, leading to tumor growth inhibition (25). This collaboration between CD4 T cells and macrophages was also essential for successful cancer immune surveillance in non-solid cancers, as myeloma and B-cell lymphoma. Indeed, Th1-secreted IFN-γ was shown to trigger a cytotoxic activity of tumor-associated macrophages (TAMs) and also induces CXCL9/MIG and CXCL10/IP-10 secretion by macrophages, which may affect the tumor progression by angiogenesis inhibition (129). IFN-γ-activated macrophages also acquire a tumoricidal phenotype with the upregulation of cytotoxicity-associated markers including granzyme A/B, and NKG2D (129). In addition, in STAT6-deficient mice, that display increased levels of IFN-γ, rejection of metastatic disease after removal of the primary tumor involved the generation of pro-inflammatory macrophages, also termed M1-like macrophages, and a decrease in MSDCs that accumulated during primary tumor formation (218). Studies from $APC^{Min/+}$ mice (that are highly susceptible to spontaneous intestinal adenoma formation) lacking IFN-γ signaling showed an accumulation of TAMs, more prone towards protumoral (M2-like) polarization, and upregulation of matrix metalloproteases. These results suggest that IFN-γ unresponsiveness contributes to the creation of an anti-inflammatory microenvironment, favorable to intestinal tumorigenesis (219). The properties of IFN-γ to reverse the myeloid immunosuppressive functions were also demonstrated in protumor role of human ovarian TAMs (220) and human M2-like macrophages (221).

Importantly, IFN-γ has also a key role on IL-12 production, supporting the activity of this later cytokine in cancer immune surveillance (222–225). Indeed, exogenous IL-12 administration into fibrosarcoma-bearing mice resulted in a complete tumor regression (222). This observation was extended to primary tumorigenesis models treated with exogenous IL-12 (226, 227). Consistent with this, chimeric antigen receptor-redirected T cells engineered to produce IL-12 where found to secrete increased IFN-γ levels and to display enhanced antitumor cell activity (228–230).

Regarding the importance of IFN-γ in cancer diagnostics, IFN-γ-associated signatures have a predictive value in cancer immune phenotypes (81, 231, 232). In addition, IFN-related gene signature is a predictive marker for chemotherapy and radiotherapy efficiency for breast cancer (94) as well as to PD-1 or cytotoxic T lymphocyte antigen-4 (CTLA-4) blockade in various types of malignancies (233–235). Consistently, immunotherapy using immune checkpoint blockers (anti-CTLA-4 and/or anti-PD-1) combined with anticancer vaccines, clearly associate inhibition of tumor growth with increased proportion of IFN-γ-producing effector T cells (236, 237). This is also verified in clinical trials, through which the anti-CTLA-4 therapy was associated with an increase of IFN-γ-producing $ICOS^+$ (inducible costimulatory) CD4 T cells and of T effector/Treg cell ratio in bladder cancer samples (238). In addition, PD-1 blockade was demonstrated to enhance T cell infiltration by promoting IFN-γ-inducible chemokines (239). In other way, it was recently shown that IFN-γ-induced Treg cell fragility (loss of suppressive function) is required for response to anti-PD-1 therapy (240).

Altogether, the versatility of IFN-γ and its fine-tuned biological effects highlight its relevance for therapeutic applications, and some clinical trials have already encouraging results. In fact, 75% of metastatic melanoma patients were non-responders to anti-CTLA-4 therapy, and this was associated with genomic defects of IFN-γ signaling genes on tumors (241). Recently, apelin receptor (*APLNR*) was described to regulate JAK/STAT signaling, modulating IFN-γ responses. Multiple loss-of-function mutations in *APLNR* were identified in patient tumors refractory to immunotherapy (242). The inclusion of IFN-γ in the first-line treatment of ovarian cancer resulted in benefit regarding progression-free survival, with acceptable toxicity (243). IFN-γ treatment also appears to be effective against bladder tumors by recruitment and activation of intratumoral

leukocytes (244). In a phase I clinical trial, which combined adoptive T cell therapy with intralesional administration of adenovirus expressing IFN-γ in metastatic melanoma, 38.5% of the patients had an overall objective response and 46% were able to control the disease (245).

IFN-γ-Mediated Mechanisms Underlying Protumorigenic Effects

It is becoming increasingly clear that IFN-γ can exert certain effects supporting tumorigenesis. Immune evasion can operate through tumor cells losing responsive to IFN-γ signaling to avoid its antiproliferative, pro-apoptotic, and immunoregulatory actions. This has been demonstrated with the tumor cells losing the receptor for IFN-γ or a component of JAK/STAT signaling (12, 18). In addition, constitutive activation of inhibitory molecules of this pathway, as SOCS1 and SOCS3, limits the actions of IFNs on human melanoma cells (246) and favors the activation of alternative signaling pathways, as STAT3, which is associated with tumor progression (247). These evidences suggest that tumor cells develop IFN-γ-dependent strategies to evade the immune system, leading to the emergence of very aggressive tumors, which are on the basis of immunoediting. In 2011, Zaidi and Merlino proposed that IFN-γ actions might play a physiological role in protecting cells from damage in a setting of tissue remodeling and repair, while on cells harboring oncogenic mutations, the same mechanisms may prevent cell destruction and allow complete transformation (248). Consistent with this, NF-κB in tumor cells was shown to act as a protective mechanism against IFN-γ-induced necroptosis (203).

Indeed, there are significant evidences that tumor cells can take the advantage of IFN-γ as an inducer of anti-inflammatory responses and protumor effects. The first report of the negative potential effects was in 1987 by Taniguchi and colleagues who proposed that IFN-γ changes the metastatic ability of the B16 melanoma cells in a cell-autonomous manner (249). Data from experiments using the CT26 colon carcinoma model showed that IFN-γ promotes tumor escape through the downregulation of the endogenous tumor antigen gp70 (250). IFN-γ expression by human melanoma samples was associated with enhanced expression of MHC class II molecules and the acquisition of a more aggressive phenotype (251, 252).

One of the principal mechanisms of tumor immune escape is the suppression of cytotoxic T cells and of NK cell-mediated immune responses. Brody and colleagues showed that IFN-γ upregulates IDO in melanoma cells and recruits Treg cells to avoid immune recognition (253). Curiously, IFN-γ induced IDO competence on human monocyte-derived DCs but had no effect on pro-inflammatory cytokine release, suggesting that IFN-γ triggers IDO activity and pro-inflammatory cytokine release as distinct cellular programs. In addition, IDO-competent DCs induced regulatory activity on allogeneic T cells (179). IFN-γ was also described to be involved in the accumulation of MDSCs in inflamed liver, which leads to T cell suppression (254). MDSCs producing nitric oxide decreased IFN-γ responsiveness of immune cells, such as T and NK cells (255).

One important aspect is the ability of IFN-γ to induce PD-L1 expression in cancer, stromal and myeloid cells to impair effector tumor immunity (181). Abiko and colleagues demonstrated that the contact between tumor cells and CD8 T cells is necessary for the induction of PD-L1, underlying the importance of paracrine exposure to IFN-γ (256). Recent reports suggest that loss of IFN-γ pathway genes, such as JAK1 and JAK2, is associated with resistance to anti-PD-1 therapy (257, 258). Prolonged IFN-γ signaling in tumors was also shown to coordinate PD-L1-dependent and PD-L1-independent resistance to immune checkpoint blockade and to other therapeutic combinations, such as radiation and anti-CTLA-4, through a multigenic resistance program (259). In addition, other inhibitory pathways are reinforced by IFN-γ, including CTLA-4 and CD86/CD80 interaction (260).

Interferon-gamma was used in clinical trials for melanoma but no significant improvement for patients was observed (261–264). In fact, IFN-γ treatment had no contribution to the outcomes of patients with metastatic renal cell carcinomas (265), leukemia (266), pancreatic carcinoma (267), breast cancer (268), or into the postoperative surgical therapy for colon cancer (269). Furthermore, a phase 3 trial of IFN-γ plus standard treatment with carboplatin/paclitaxel versus carboplatin or paclitaxel alone, for treated advanced ovarian tumors, was early terminated due to a higher incidence of serious hematological toxicities in patients receiving combined therapy compared with chemotherapy alone (270). The failed attempts to treat cancer patients with exogenous IFN-γ raised several concerns: the absence of tumor immunogenicity, the lack of IFN-γ-signaling components, the upregulation of IFN-γ signaling inhibitors, the immunosuppressive tumor microenvironment, the lack of effector T cells, or presence of anergic T cells and, in some cases toxicity. These accumulating evidences reinforce the importance to determine the grade of patients' IFN-γ-responsiveness. For example, in cases with low IFN-γ actions, active immunization either *via* IFN-γ treatment or *via* adjuvants of the immune system, as toll-like receptor ligands, should be considered, as demonstrated recently by using bacterial outer membrane vesicles that eradicate established tumors in an IFN-γ-dependent mechanism (271). The combination with radio- and chemotherapy is expected to be useful through immunogenic cell death that also elicits the innate immune system. Promising results were obtained with combination of low-dose 5-fluorouracil with recombinant interferon-gamma (IFN-γ) in patients with advanced hepatocellular carcinoma (272). In cases with high levels of IFN-γ signaling, the therapy with anti-PD-1/anti-PD-L1 is expected to be important.

Overall, these findings indicate that the local immune microenvironment of tumors is complex and variable and that for an effective therapy it is essential to evaluate, individually, the immune profile of patients or immune contexture [reviewed in Ref. (232, 273)], taking into account that it may evolve and modify throughout the anticancer therapy (**Figure 3**).

IFN-γ IN THERAPY—WHERE ARE WE AND WHERE ARE WE GOING?

Interferon-gamma therapy has ensued in clinical applications approved by the Food and Drug Administration in the treatment of chronic granulomatous disease, in 1999 and severe

malignant osteopetrosis, in 2000. Despite the promising therapeutic applications of IFN-γ in several settings, its limited success in cancer-immunotherapy trials might be due to cancer cell unresponsiveness to this cytokine, the failure to deliver it locally or with the adequate periodicity to achieve a therapeutic effect. Moreover, IFN-γ clinical use has also been restricted due to several limitations inherent to its molecular properties. Essentially, these include stability problems, such as acid degradation, and also the tendency to aggregate irreversibly under mild denaturing conditions, with subsequent loss of biological activity [the pharmacological aspect of IFN-γ is reviewed in Ref. (274, 275)]. Furthermore, IFN-γ is rapidly cleared from the blood when administered intravenously (276), requiring frequent re-administrations of high cytokine concentrations, to elicit an effective response at the target site, leading to systemic toxicity and side effects, such as fever, fatigue, nausea, vomiting, diarrhea, neurotoxicity, and leukopenia (277). These adverse effects are caused mainly by high serum concentration of the protein, due to an unequal distribution between body fluids and tissues (276) and, additionally, to the ubiquity of receptors which are expressed at the membrane of the majority of human cells (278, 279) and also to the existence of a circulating soluble form (which function remains elusive) (280).

These constraints in the clinical use of IFN-γ have encouraged the development of alternative delivery methods with the purpose of achieving higher therapeutic outcomes and, simultaneously, weaken its toxicity. Numerous reports have focused mainly on efficient routes of delivery rather than on systemic applications (281–287). In fact, IFN-γ is naturally produced in a paracrine manner, with local secretion and diffusion to the surrounding cells and microenvironment throughout the extracellular fluids (288). Therefore, a localized delivery of this cytokine has been

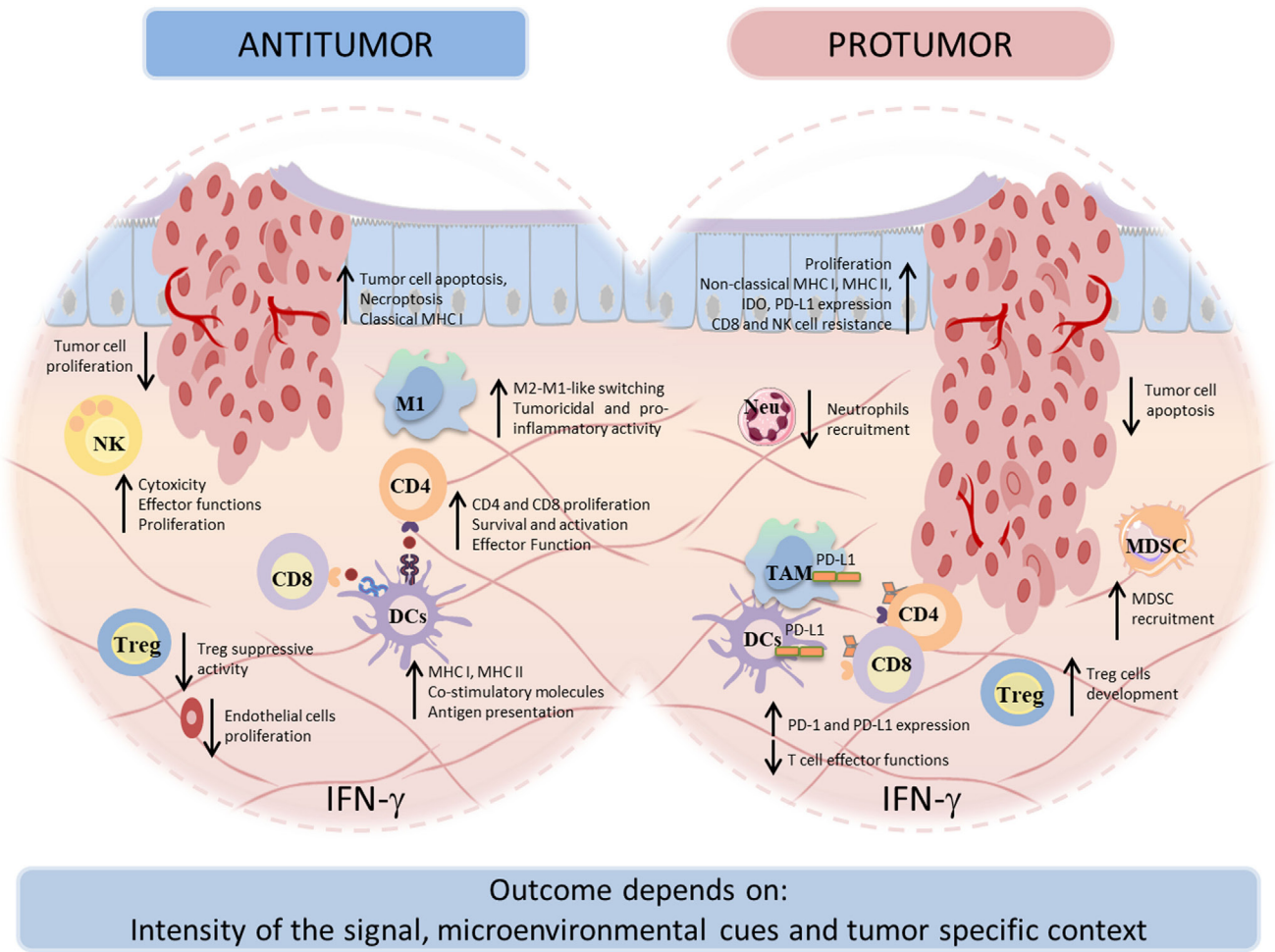

FIGURE 3 | Dual face of interferon-gamma (IFN-γ) in tumor immunity. IFN-γ can display both antitumor and protumor activities. Under both circumstances, IFN-γ influences tumor cells directly and indirectly, by activation of immune cells. The antitumor effects comprise the development, recruitment, and activation of innate immune cells as well as the activation and maintenance of effector T cells. The antitumor effects of IFN-γ result in direct inhibition of tumor proliferation, recognition, and elimination. In other way, the protumorigenic role of IFN-γ involves proliferative and antiapoptotic signals, as well as escape of the tumor cells from recognition and cytolysis by cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells. The broad range of IFN-γ actions depends on the context of tumor specificity, IFN-γ-signaling intensity, and other microenvironment conditions.

determined to be more appropriated in terms of therapeutic efficiency, due to its specific effect at the target site, while simultaneously intensifying the intended cytotoxic effects and immunological stimulation (289). In particular, tumors can be rejected by local IFN-γ expression, but rejection of established tumors was less efficient over time, suggesting that timing of treatment plays a critical role, for transplanted tumors became less susceptible to local IFN-γ treatment the better they are established (206). Another relevant aspect concerns the mode of administration, being it an intermittent or sustained release. Several studies concluded that a sustained release strategy is more efficient by limiting the exposure of other cells and organs to the deleterious effects of high IFN-γ concentrations (290–295). In the particular case of cancer immunotherapy, consistent findings show that a stable and high concentration at the target site is required to elicit an effective response (288, 296), prompting several attempts to promote local delivery of IFN-γ with controlled release. These include liposomes, polymer gels, biodegradable microspheres, gene therapy, and magnetic or albumin nanoparticles (285, 297–301). However, these strategies revealed unsuccessful by failing to maintain a stable and/or bioactive cytokine prior release, an inadequate release rate, a labor intensive and cost ineffective manufacture, and safety issues. Oncolytic viruses have gained interest for immunotherapy due to their ability to selectively destroy tumor cells and to their potential to stimulate antitumor immunity. Oncolytic vesicular stomatitis virus expressing IFN-γ demonstrated greater activation of DCs, higher pro-inflammatory cytokines' secretion, and reduced tumor growth in 4T1 tumor model compared with the parental virus, suggesting that specific production of the IFN-γ within the tumor microenvironment is beneficial for the antitumor immune response (302). Recently, an IFN-γ-delivery system based on chitosan/poly(γ-glutamic acid) polyelectrolyte complexes was described by our group to successfully decrease macrophage-derived stimulation of cancer cell invasion *in vitro* through the modulation of a pro-inflammatory macrophage phenotype (221). In fact, several efforts have been directed to educate APCs toward an immunostimulatory and antitumor phenotype (**Figure 4**) (303–306). In another work, a silk-based hydrogel was designed to regulate cytokine delivery for macrophages, which are actively involved in tissue remodeling and vascularization, with the aim to regulate the microenvironment of biomedical implants (307). Other potential strategy to improve the shorter half-live of IFN-γ is fusing it with antibodies, enhancing its stability in the serum and tumor target specificity and reducing toxic side effects (308). Although promising results have been achieved with some of these strategies, the desired requirements are yet to be accomplished and need further investigation/development.

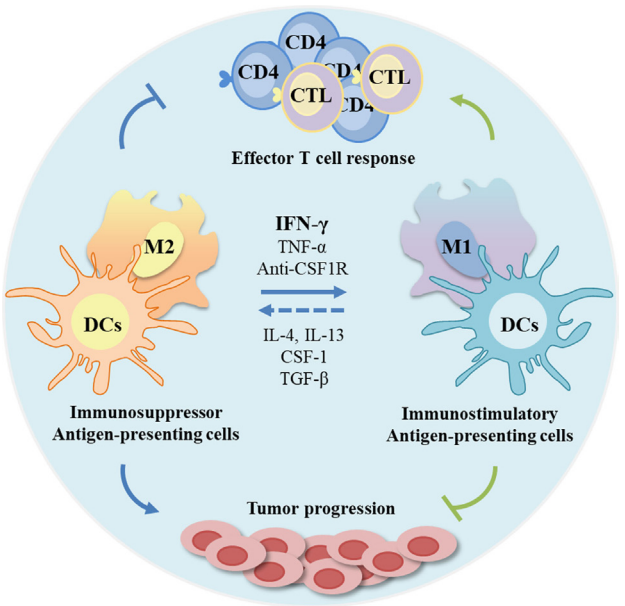

FIGURE 4 | Modulation of antigen-presenting cells (APCs) profile as anticancer therapeutic strategies. The tumor microenvironment is frequently immunosuppressive with APCs functions compromised, and consequently with poor T cell response. As APCs can be modulated by microenvironmental signals, these cells are promising targets. Interferon-gamma (IFN-γ) and other molecules can be used to re-educate tumor-associated macrophages, frequently associated with anti-inflammatory status (M2-like) toward a pro-inflammatory and antitumor profile, while stimulating regulatory dendritic cells (DCs) to an immunostimulatory profile. This stimulation can potentiate effector T cell response and inhibit tumor progression.

CONCLUDING REMARKS

Herein, we discussed the role of IFN-γ on tumor immunity and its potential therapeutic implications. On one side IFN-γ appears as a promoter of tumor immune surveillance and on the other as a supporter of tumor escape. The outcome of IFN-γ signaling depends on the tumor-specific context, the magnitude of the signal, and the microenvironmental cues. Nevertheless, IFN-γ or IFN-γ inducers remain promising agents to include in combined therapies against cancer. We believe that the effectiveness of future IFN-γ-based therapies will involve the development of systems to deliver the appropriate amount of cytokine to target cells, minimizing its side effects. In addition, these strategies would profit from the combination with conventional treatments and with anti-PD-L1 and anti-CTLA-4 therapies to overcome the regulatory effects of IFN-γ. Another important issue is to consider a personalized approach, which takes into account the patient responsiveness to IFN-γ, by using predictive biomarkers, as IFNγR2, SOCS, *APLNR*, STAT1, or STAT3. Thus, a comprehensive understanding of the complex and variable tumor microenvironment, as well as a deeper evaluation of the immune, vascular and stromal profile, will be necessary for the stratification of cancer patients and for the establishment of efficient personalized therapies.

AUTHOR CONTRIBUTIONS

FC performed the initial draft, written the manuscript, and designed **Figures 2–4**. AC written a part of Section "IFN-γ IN Therapy—Where Are We and Where Are We Going?" and performed **Figure 1**. RG, KS, and MO critically revised the manuscript, reorganized ideas, and approved the final version.

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