

Current prospects of type II interferon γ signaling and autoimmunity

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Interferon γ (IFN γ) is a pleiotropic protein secreted by immune cells. IFN γ signals through the IFN γ receptor, a protein complex that mediates downstream signaling events. Studies into IFN γ signaling have provided insight into the general concepts of receptor signaling, receptor internalization, regulation of distinct signaling pathways, and transcriptional regulation. Although IFN γ is the central mediator of the adaptive immune response to pathogens, it has been shown to be involved in several non-infectious physiological processes. This review will provide an introduction into IFN γ signaling biology and the functional roles of IFN γ in the autoimmune response.

According to the National Institutes of Health, autoimmune diseases (ADs)³ are one of the top 10 leading causes of death in female children and women in all age groups up to 64 years of age (1). Excess secretion of IFNs has been associated with development of human ADs (2). Interferons (IFNs) comprise a family of proteins classified as type I (IFN- α , - β , - ϵ , - κ , and - ω), type II (IFN- γ , herein IFN γ), and type III (IFN- λ 1–4) that have pleiotropic roles in immunity, cancer biology, and autoimmunity (2). Here, we aim to provide a basic understanding of the functions of the type II IFN γ and the IFN γ -signaling pathway (hereafter referred to as IFN signaling) in a contextual and timing perspective related to the autoimmune environment and the development of ADs.

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³ The abbreviations used are: AD, autoimmune disease; C/EBP β , CCAAT/enhancer-binding protein- β ; JAK, Janus kinase; APC, antigen-presenting cell; GAS, γ -interferon-activated site; ISG, interferon-stimulated gene; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; EAE, experimental autoimmune encephalomyelitis; ARE, adenylate uridylate-rich element; irAE, immune-related adverse event; mTOR, mechanistic target of rapamycin; IFNGR, IFN- γ receptor; PS, phosphatidylserine; ISRE, interferon-stimulated response element; PIAS, protein inhibitors of activated STAT; NLS, nuclear localization sequence; STAT, signal transducers and activators of transcription; CIA, collagen-induced arthritis.

Biological role of IFN γ on inflammation

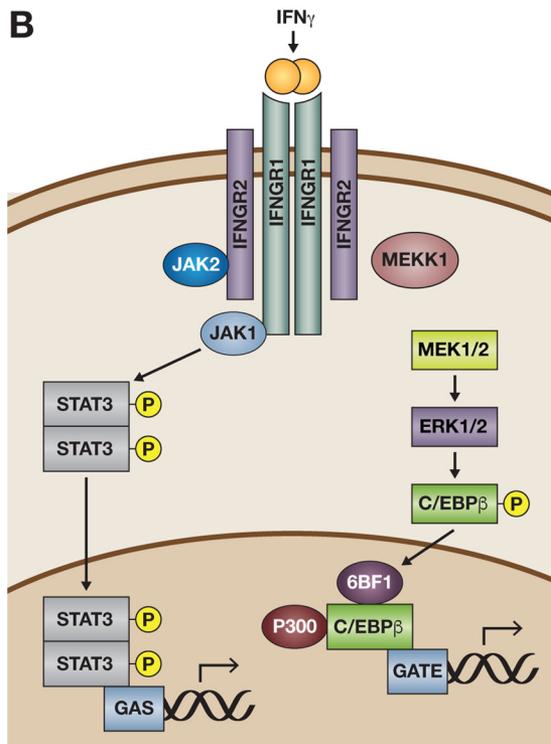
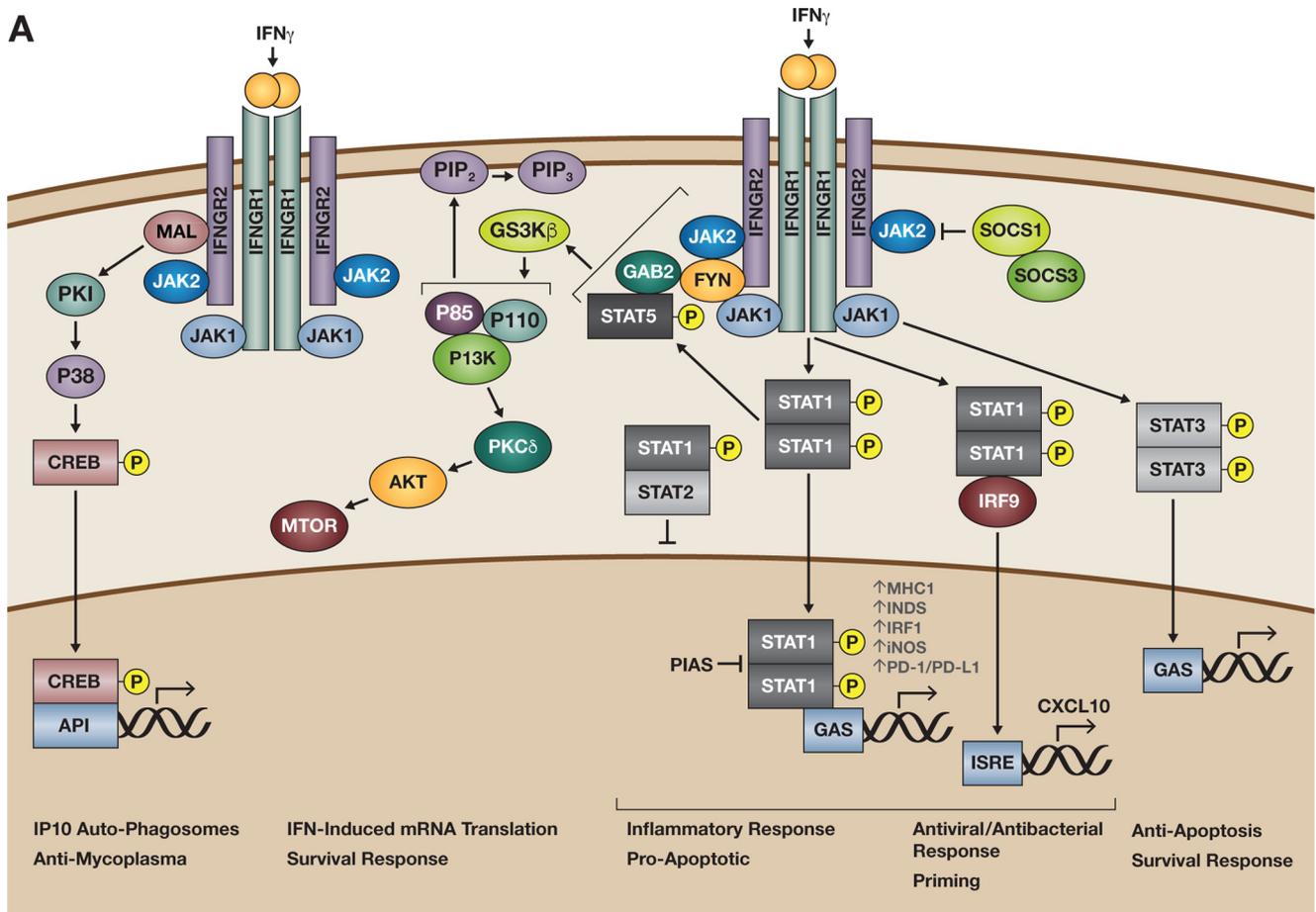
Since Wheelock (3) reported that IFN γ inhibited viral replication, IFN γ has become an essential regulator of several immune processes, including vaccine-mediated responses and pro-inflammatory CD4⁺ T helper 1 (Th1) cell responses (4). As an effector cytokine of Th1 immunity, IFN γ is the key regulator of macrophage activation via the Janus kinase (JAK)/signal transducer and activators of transcription (STAT) pathway (Fig. 1A) (5). Normally, in the early phases of the host immune response, production of IFN γ by natural killer cells, CD4⁺ T helper 1 (Th1) cells, and CD8⁺ T cells aims to improve antigen recognition in antigen-presenting cells (APCs) such as macrophages and dendritic cells. IFN γ activates macrophages toward the "M1" phenotype, which is characterized by the expression of high levels of pro-inflammatory cytokines such as IL-1 β , IL-12, IL-23, and TNF- α ; high production of reactive nitrogen and oxygen intermediates; promotion of Th1 T cell response; and strong inflammatory activity (6). APCs express major histocompatibility complex (MHC) class I and II proteins and activate cross-presentation antigenic pathways. In parallel, IFN γ signaling generates other cytokines and inflammatory factors to sustain inflammation, maintain Th1 responses, and inhibit differentiation of regulatory T cells, CD4⁺ T helper 2 cells (Th2), and Th17 cells (7). Despite these amplification steps, IFN γ signaling is generally short-lived to elicit functional recovery of homeostasis, including tissue repair and reestablishment of tissue physiology.

IFN γ signaling: Canonical and non-canonical pathways

In the context of inflammation, IFN γ induces a rapid response via the JAK/STAT or canonical pathway. However, in the context of ADs, maintenance of chronically high levels of IFNs leads to activation of both canonical and non-canonical pathways, albeit in a cell- and context-specific manner.

Canonical IFN γ -signaling pathways

In the canonical pathway of IFN signaling, IFN γ dimerizes and binds to the two IFNGR1 receptors. The IFNGR is composed of two distinct chains, the high affinity IFNGR1 (α) and a low affinity IFNGR2 (β) (8). The identification of a glycosylation-deficient mutant residue in the IFNGR1 detailed two key steps preceding initiation of IFN γ signaling (9). In the first step, IFN γ binding induces the receptors to undergo a conformational change in lipid nano-domains, whereby the box 1



domains on the IFNGRs are brought into proximity of each other allowing recruitment of two JAKs, JAK1 and JAK2, to the IFNGR1 and IFNGR2 chains, respectively. This recruitment step occurs independently of their enzymatic activities. In the second step, JAK1/2 activation induces a second conformational change that allows STAT1 to associate with the IFN γ -IFNGR complex. In turn, JAK1 and JAK2 phosphorylate the transcription factor STAT1 (pSTAT1) forming a homodimer that translocates to the nucleus (9). At this moment, the released IFNGRs associated with the cortical-actin network and prepared for alternative regulation via receptor trafficking and endocytosis. Importantly, these events demonstrate that receptor internalization may not be required for IFN γ signaling to take place.

In the nucleus, pSTAT1 binds with high affinity to DNA sequences termed the γ -interferon-activated site (GAS) to initiate transcription of interferon-stimulated genes (ISGs) (Fig. 1A) (11). At the same time, acetylation of pSTAT1 sets the timer for STAT1 inactivation via complex formation between acetylated STAT1 and the protein-tyrosine phosphatase T cell protein-tyrosine phosphatase (TCP45) (12). In turn, histone deacetylase 3 (HDAC3) deacetylates STAT1, thus permitting a new cycle of phosphorylation and re-stimulation (13). Hence, the rapid increase of pSTAT1 after IFN γ stimulation elicits a reversible and dynamic response to rapidly restore homeostasis.

Non-canonical IFN γ signaling pathways

The observation that IFN γ is capable of inducing gene expression in STAT1^{-/-} bone marrow-derived macrophages suggested that IFN γ can act independently of STAT-1 or in an alternative non-canonical fashion (14). Generally, the activation of non-canonical pathways appears to be later rather than earlier after STAT1 activation. Nevertheless, there is evidence suggesting that non-canonical pathways could be activated in the absence or presence of Stat1 in a context-dependent manner. In the absence of STAT1 (Fig. 1B), IFN γ can activate STAT3, in a JAK-STAT-dependent process that results in activation of GAS-regulated genes (15, 16). Moreover, the absence of Stat1 in primary fibroblasts or neurons led to enhanced ERK activation following IFN γ addition, implying that the cell-specific availability of signal transducers can diversify the cellular response following IFN engagement (17). STAT-independent IFN γ signaling can occur via activation of other MAPKs, such as PyK2, ERK1/2, and JNK (18, 19); the adaptor proteins CrkL and small G protein Rap1 (20); and the Src homology 2 domain-containing protein-tyrosine phosphatases SHP-1 and SHP-2

(18, 21). Note, IFN γ activation of different kinases such as ERK1/ERK2 (MAPKs) (22) or glycogen synthase kinase 3 (GSK3 β) signaling (23) results in activation of different transcription factors. For example, IFN γ activation of ERK regulates binding of CCAAT/enhancer-binding protein- β (C/EBP β) to a novel IFN γ response element called GATE (22). GATE has little homology with GAS and binds to different transacting factors such as C/EBP β . Recent evidence found that phosphorylation of C/EBP β involves IFN-stimulated proteolytic processing of ATF6, and ERK1 and ERK2 are necessary to control autophagy of several infectious agents (24). Conversely, IFN γ activation of GSK3 β via the phosphoinositide 3-kinase-AKT pathway regulates CREB/AP1-dependent DNA binding to suppress IL-10 production (23).

Conversely, in the presence of Stat1, activation of canonical and non-canonical pathways could happen simultaneously (Fig. 1A). However, the outcomes of such activation are cell- and context-specific. For example, in mice infected with systemic Dengue, Stat1-dependent pathways were required for early viral control, but Stat1-independent pathways were later required for viral clearance (25). Furthermore, in a mouse model of encephalitis, the activation of Stat1-dependent and Stat1-independent pathways advanced IFN γ -induced reduction of myelin sheath thickness in the CNS despite Stat1 knock-down (26). Apparently, initiation of these alternative signaling pathways starts at the JAK activation sites in IFNGR1 with the recruitment of adaptor molecules such as MyD88 adaptor-like (Mal) (27) or the Fyn kinase (28). MAL is encoded by the gene Toll-interleukin 1 receptor domain containing adaptor protein (*TIRAP*). Noticeably, Mal-dependent IFNGR signaling required phosphorylation of the mitogen-activated protein kinases (MAPK) p38, not Stat1, for phagosome maturation and killing of intracellular infectious organisms such as *Mycobacterium tuberculosis*. Moreover, defects in the Mal-dependent IFN γ -signaling pathway due to non-synonymous single nucleotide polymorphism at S180L in the human *TIRAP* gene explains best the increased susceptibility of SLE patients for mycobacterial and pneumococcal infections (27). Similarly, recruitment of the Src kinase Fyn results in the formation of a complex that allows IFN γ to activate Stat5b via PI3K signaling (29). The ability to activate Stat5 while preserving IFN γ activation of STAT1-dependent immune events represents an advantageous adaptation mechanism to regulate macromolecular permeability in enteric epithelium with low STAT1 levels. Moreover, IFN γ activation of AKT and mTOR via PI3K improved mRNA translation of IFN γ -regulated genes comple-

Figure 1. Overview of the STAT-1 canonical and non-canonical signaling pathways elicited by IFN γ . As a dimer, IFN γ (orange) binds the IFNGR, which is composed of the IFNGR1 and IFNGR2 subunits, the kinases Jak1/Jak2. *A*, in canonical IFN γ signaling, phosphorylation of Jak1 and JAK2 results in the phosphorylation of STAT1 (center). A STAT1 homodimer translocates to the nucleus and binds to GAS found in the promoters of IFN γ -regulated genes such as *HLA-A*, *NOS-2*, *IRF1*, *PDCD1*, and *CD274*. Recruitment of adaptor proteins associated with IFNGR2 such as MAL and Fyn results in non-canonical STAT1 signaling. MAL-dependent IFN- γ receptor (IFNGR) signaling elicits signaling via MAPK p38 phosphorylation to up-regulate expression of the chemokine IP-10, antimyoplasma proteins, and formation of autophagosomes (left). GSK3 β activation and Fyn elicit pSTAT5 recruitment to activate PI3K to regulate cell membrane permeabilization. Alternatively, IFN γ activation of GSK3 β via PKC δ activates AKT/mTOR regulation of survival responses. Nevertheless, upon STAT activation, control mechanisms aiming to regulate signaling target either the JAK catalytic sites with SOCS proteins (upper right corner) or blockade of the STAT dimers binding to GAS sites with PIAS or through binding with un-phosphorylated STAT2. Alternatively, IFN priming increases IRF9, which is recruitment to STAT1 dimers for binding to ISRE sites. Antiviral and antibacterial responses benefit from this mechanism. *B*, in cells not expressing STAT1, STAT3 can be phosphorylated by Jak1/Jak2 resulting in translocation of the STAT3 dimer to GAS sites. Moreover, IFN γ activation of ERK results in C/EBP β activation and binding to a novel IFN-response element (GATE). Figure has been adapted and modified from Refs. 17 and 29.

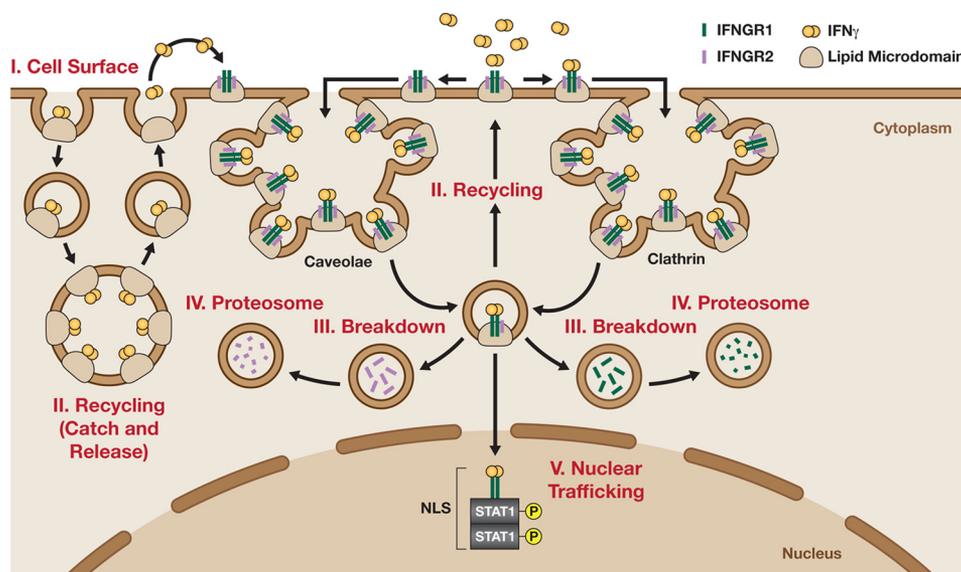


Figure 2. Endocytic mechanisms regulating IFN γ signaling via differential expression of the IFNGR. IFNGR1 (green) and IFNGR2 (purple) are associated in lipid micro-domains on the cell surface. In the presence of IFN γ dimers (orange), the cytokine receptor complex is redirected to clathrin or caveolae sites at the cell surface. Once there, IFN γ either binds the receptor or is recruited to PS-rich domains. Then, the IFNG complexes are endocytosed either through clathrin-dependent events or clathrin-independent events that involve caveolae-dependent mechanisms. IFN γ -PS complexes are slowly recycled back to the cell surface, and IFN γ is released in an autocrine-like manner (left). In contrast, IFN γ -IFNGR complexes are endocytosed and broke down before being recycled or degraded. Noticeably, IFNGR2 is enzymatically cleaved and separated for degradation via proteasomes. Meanwhile, IFNGR1 can be recycled to the cell surface or targeted for degradation. Alternatively, the IFNG-IFNGR-p-STAT1 complex could remain stable and continue translocating to the nucleus using NLS.

menting STAT1-dependent mechanisms (30). Thus, IFN γ can regulate complex processes beyond their known short-term effects. Therefore, the overall biological effect of IFN γ signaling likely results from a well-adjusted combination of Stat1-dependent and Stat1-independent mechanisms activated sequentially during progression of the inflammatory disease.

Alternative mechanisms regulating the IFN γ signaling via endocytosis

As diverse as the IFN γ signaling downstream pathways could be, all actions start generally at the IFNGR. As human IFN γ does not signal in mouse or rat cells, evidence that microinjection of human IFN γ elicited antiviral activity in murine cells suggested that the IFNGR provides species specific responses to IFN γ (31). Further evidence showing that retention of IFN γ within cells also resulted in an IFN γ -dependent signaling led researchers to further claim that the IFNGR drives species specificity responses (32, 33). Thus, the IFNGR holds the key to control the activity of IFN γ among species (34).

The regulation of IFN γ signaling involves essential management mechanisms that regulate the differential expression and cell-surface localization of the IFNGR chains (Fig. 2). IFNGR1 is usually expressed in excess, whereas IFNGR2 is more tightly regulated in most but not all cell types (35, 36). In fact, the absence of IFNGR2 on the surface of Th1 cells supports the model that regulation of its surface expression regulates responsiveness to IFN γ (37). Moreover, several viruses such as the vaccinia virus and myxoma virus encode and secrete IFN γ receptor mimetics, which are peptides with significant sequence similarity to the human and mouse receptors for IFN γ , to neutralize IFN γ activity (38). These data suggest that cell regulation of IFNGR accounts for cell-specific differences in response to IFN γ and how a target cell becomes unresponsive to further IFN γ stimulation.

Recent data showing that the IFNGR1 and IFNGR2 are loosely associated in sphingolipid-rich areas on the cell surface, called lipid micro-domains, provided mechanistic details over their complex formation (39, 40). As shown in Fig. 2, upon IFN γ dimer binding to IFNGR1 and IFNGR2, a ternary complex is formed within the micro-domains in preparation to deliver downstream signaling from the cell surface to the nucleus (41). Note, IFN γ -IFNGR complex readiness is independent of receptor internalization (42). Upon Stat1 activation and internalization, the two IFNGRs are differentially processed within cells using clathrin-dependent or clathrin-independent mechanisms (43). Clathrin-dependent endocytosis utilizes the protein clathrin to mediate endocytosis and is the primary mechanism by which the IFNGR1 is recycled to the cell surface (42). Infectious organisms such as herpesvirus K3-5 or *Trypanosoma cruzi* down-regulate the surface expression of host IFNGR by increasing their endocytosis rates, which leads to suppression of cell-mediated immunity (44, 45). Conversely, clathrin-independent endocytosis uses sphingolipid-rich caveolae, also known as lipid rafts, to regulate IFNGR2 at the cell surface (46). Human T cells use this pathway to limit sensitivity to IFN γ as a strategy for dampening the host immune response (47).

Recently, a novel mechanism explains how membrane cell dynamics modulates cell-to-cell communication via cytokines (Fig. 2). Surprisingly, positively charged regions of IFN γ , IL-12, and IL-23 were proved to interact directly with negatively charged cell-surface phosphatidylserines (PS) on tumor cells (48). The cytokine-PS complexes are endocytosed, possibly via caveolae-dependent mechanisms, to be slowly recycled back to the cell surface in an autocrine-like manner. Once released, cytokines bind their respective receptors. This mechanism explains how tumor cells manage to extend a response after a short-lived cytokine exposure.

Another less understood and controversial regulatory mechanism proposes that the whole IFN γ /IFNGR structure translocates to the nuclei and defines species selectiveness (32). Independent reports showed that such an event is possible due to a putative nuclear localization sequence (NLS) contained both in IFN γ and the IFNGR structures (49). It has been suggested that those NLS allows IFN γ to regulate STAT1 trafficking within cells (50–52). As the concept of a nuclear localization step awaits further verification, its relevance on IFN γ signaling remains incomplete. Together, controlling the IFNGR expression at the cell surface is a straightforward control mechanism available to all cells to regulate responses to IFN γ .

Chronic exposure to IFN γ leads to ADs

In ADs, immune cells are exposed, often simultaneously, to more than one IFN causing integration of IFN signaling (53). Recently, it was determined that such exposure could go back up to 3.0 to 4.5 years before individuals are even diagnosed with ADs such as SLE (54) or rheumatoid arthritis (RA) (55), respectively. In this type of relapsing-remitting ADs, immune cells are constitutively and constantly exposed to waves of significantly “high” (relapse or flare state) or “low” (remission state) levels of type I and type II IFNs during distinct periods of time (56, 57). Pre-exposure to low sub-activating concentrations of IFNs sensitizes cells to produce enhanced responses to extracellular inflammatory stimuli that include IFNs themselves, as well as other cytokines such as tumor necrosis factor α (TNF α) or toll-like receptor activators (58). This process, known as priming, is characterized by the intracellular accumulation of STATs. Contrary to acute inflammation, primed immune cells such as M1 macrophages are the predominant phenotype at sites of inflammation for ADs such as RA, multiple sclerosis, and lupus nephritis (59, 60).

IFN priming elicits post-transcriptional and/or epigenetic changes that promote synergism for gene induction and regulation after subsequent exposure to type I and type II IFNs (Fig. 1). Noticeably, IFN priming involves increased association of similar or different types of IFN receptors (53). Under this mechanism, type I IFNs augment IFN γ signaling via association of type I and II IFN receptor subunits. Conversely, IFN γ priming for IFN α signaling occurs but involves only STAT1 and not STAT2 or STAT3 (61). Priming for production of large amounts of type I IFNs is mediated by an autoamplification loop in which IFN α induces expression of the transcription factor IFN regulator factor 7 (Irf7) that activates IFN α gene promoters (61). This step creates a robust priming effect, where IFN γ enhances positive signaling by recruiting other Irf. For example, the formation of the heterotrimeric transcription factor complex known as ISGF3 between Stat1 and IFN-regulatory factor 9 (Irf9) required type I IFN priming and prolonged IFN γ activation (Fig. 1A) (62, 63). Those complexes elicited Stat1 regulation over gene promoters with GAS and/or interferon-stimulated response elements (ISRE) (64) allowing regulation of genes with either one or both elements such as the IFN γ -regulated cytokine CXCL10/*Cxcl10* (IP-10) (65). Consequently, IFN priming elicits regulation of complex biological process via epigenetic remodeling and enrichment of STAT1-binding motifs in mice (66). For example, microglial reactive

oxygen species production in response to IFNs required simultaneous modification of three mechanisms, including up-regulation of the NADPH oxidase subunit NOX2, up-regulation of NO production, and the reduction of intracellular GSH levels (67).

Recently, it was reported that un-phosphorylated STATs play roles modulating the IFN signaling response. For example, un-phosphorylated STAT1 (U-STAT1) is capable of regulating a set of ISGs that offer some protection against various viruses but rendered cells resistant to chemotherapy and radiation (68, 69). Studies in mouse models of arthritis and experimental autoimmune encephalomyelitis (EAE) determined that U-Stat1 regulated ISGs that are regularly induced later rather than earlier after stimulation with either IFN γ or IFN β (68). Moreover, evidence suggests that IFN γ priming involves regulation of microRNAs, as suppression of miR-3473b limited activation of primed macrophages (70).

To counterbalance the effect of IFN priming and receptor activation, cells also activated precise complementary inhibitory mechanisms at different levels (Fig. 1). At the plasma membrane, suppressor of cytokine signaling proteins block receptor activation by binding to the activated JAK catalytic sites, thus turning off downstream signals. Moreover, at the cytoplasm, an increase in un-phosphorylated STAT2 binds pStat1 to diminish its nuclear translocation during continuous IFN γ stimulation possibly eliciting adaptation to long-term IFN γ stimulation (71). In the nuclei, protein inhibitors of activated STAT (PIAS) associate with activated STAT dimers via their zinc-binding ring finger domain in the center of the molecule, preventing them from binding to the DNA (72). Thus, a primed innate immune system modulates the functions of IFNs and defines the host response to underlying triggers of autoimmune disorders.

Understanding the role of IFN γ in ADs with mouse models

Several mouse models of ADs, such as SLE, RA, collagen-induced arthritis (CIA), and EAE have proven the essential role of IFN γ both in promoting and suppressing different stages during AD progression. For example, it is known that both *ifng*^{-/-} and *stat1*^{-/-} mice are highly susceptible to EAE (73, 74). In contrast, it has been shown that MHC-II induced by IFN γ -hyperproducing T cells is important on the disease course of CIA and RA (75). These data suggest that IFN γ via the JAK/Stat1 pathway modulates AD progression. However, targeting the function of IFN γ at different disease stages is essential to understand its biological role. Indeed, administration of mouse IFN γ applied at very early stages of EAE aggravates the disease because of highly producing IFN γ CD4⁺ T cells (76). However, at a later stage, administration of IFN γ reduces the severity of EAE when it is mediated by CD4⁺ Th17 cells (76). Similarly, IFN γ is required, even in the presence of complete Freund's adjuvant, at the onset and development of severe arthritis following immunization with glucose-6-phosphate isomerase (77). The described evidence indicates that CIA and EAE are more Th17 cell-mediated disease models instead of pure Th1 cell-mediated diseases, where IFN γ exerts a dynamic and context-dependent response. Although IFN γ may be a rea-

sonable target for clinical trials, the lack of biomarkers defining when to start or stop intervention limits the therapeutic value of IFN γ . Specifically, there is a need for markers that determine the availability of IFN γ -producing cells at different time points during the immune stimulus. These limitations probably account for the current narrow therapeutic index and inadequate clinical utility of IFN γ (78). As a result, most of the ongoing preclinical development is concentrated on its inducers, in particular IL-12 or IL-18 (79).

Despite the benefits from mouse models, human ADs are chronic inflammation processes instead of short-term and self-limited diseases induced in preclinical models. To address this issue, our laboratory designed a mouse model carrying deletion of the *ifng* 3'-untranslated region adenylate uridylate-rich element (ARE). The persistent serum levels of IFN γ , due to increased stability of mRNA, result in gradual establishment of SLE-like autoimmunity (80). More importantly, we have provided evidence that heterozygous ARE-del mice share similar levels of autoantibodies and demonstrate a histological score of tissue damage in target organs like that seen in homozygous ARE-del mice that express twice the systemic IFN γ levels (80, 81). This evidence suggests the existence of a threshold level of IFN γ that, when crossed, results in a more severe pathology. Similarly, in another model of lupus, mutation of ring finger and CCCH-type domains 1 (*rc3h1/roquin*) reduced the rate of decay of IFN γ mRNA and increased IFN γ signaling. As a result, germinal center B cells increased their numbers and production of autoantibodies (82). Despite the significant overlap between genes induced by either type I and type 2 IFNs, these models strongly suggest that chronic exposure to IFN γ causes or contributes to SLE-like disease. Hence, development of models for long-term inflammation could be essential for understanding the differences between disease stages.

IFN-mediated chronic inflammation, checkpoint inhibitors, and immunotherapy in ADs

Traditionally, "high" serum levels of IFN γ (referred herein as IFN γ levels) are associated with pro-inflammatory active disease, whereas "low" IFN γ levels are associated with anti-inflammatory inactive autoimmune disease. As levels of IFN γ are compared with healthy individuals, there is no consensus of what "high" and "low" IFN γ levels mean. Data from pre-clinical and clinical studies in mice and humans showed administration of type 1 IFNs (IFN α and $-\beta$) generally exert a linear dose response, whereas exogenous IFN γ exhibits a "bell-shaped" dose-response curve (83). A "bell-shaped" dose response is characterized by induction of stimulatory effects at low doses until it reaches a summit point where additional dosing cause inhibitory activity and deleterious effects (84). These data suggest the function(s) of endogenous and exogenous IFNs are probably defined by the dynamics between systemic and local inflammatory environments. In the local environment, stimulatory and inhibitory pathways are activated to limit inflammation and destruction of self-tissues (85). Recent evidence showed that IFN γ is the main regulator of programmed cell death protein 1 (PD-1) and its two ligands, PD-L1 (B7-H1 or CD274) and PD-L2 (B7-DC or CD273) (86). This fact suggests that chronic inflammation influences the local co-inhibitory

pathways in autoimmunity. In ADs, PD-1 (CD279) is the most studied co-inhibitory receptor, although the role of other checkpoint inhibitors such as cytotoxic T lymphocyte associated protein 4 (CTLA4, CD152) is more controversial (87). PD-1 and CTLA4 have critical, multifaceted roles in regulating the balance among T cell activation, tolerance, and immunomediated tissue damage. Evidence showed that polymorphisms at the mouse *Pdcd1* and *Ctla4* promoters have been associated with susceptibility to develop AD such as in SLE and AR (56). These facts reminded us that insufficient co-inhibition can also promote the development and progression of AD.

Immunotherapy targeting checkpoint inhibitors, such as PD-L1 and CTLA4, is one of the most rapidly growing fields in cancer therapy. Nevertheless, the issue with checkpoint blockade is the occurrence of associated toxicities termed immune-related adverse events (irAEs). Generally, AD patients are thought to be at higher risk of developing hematological malignancies such as Hodgkin's lymphoma or cervical cancer due to the underlying dysregulation of their immune system and anti-inflammatory treatment regime (88). Because of this, therapeutic use of check point inhibitors in patients with ADs developing tumors had mixed results so far. In a small cohort of AD patients that develop melanoma, CTLA4 blockade induces tumor growth inhibition, but 25–50% developed mild to moderate exacerbations of their AD or experienced conventional CTLA4-induced irAEs, respectively (10). Nevertheless, the high risk of irAEs makes the use of checkpoint inhibitors controversial even in patients with certain autoimmune diseases (pernicious anemia, Crohn's disease, ulcerative colitis, SLE, and psoriasis) that develop tumors. Therefore, understanding the regulation, function, and importance of the IFN signaling detailed in this review could help to unravel new therapeutic options for ADs and other chronic diseases, including cancer.

Summary

Overall, there is a consensus that the effect of IFN γ signaling is largely controlled in a multidimensional fashion where timing, exposure levels, target organ, and cellular environment define the outcome of the immune response to IFN γ expression. As such, interventions altering the IFN γ activity will be approached with great attention and thoughtfulness as to both local and systemic effects in a wide variety of disease states. This cautious approach arises because IFN γ impacts the balance between protection and development of autoimmune responses. Hence, regardless of the activated pathways, IFN γ will directly or indirectly determine the dynamics of inflammation in subjects with underlying autoimmunity. Although this review focused on immune cells and autoimmunity, these same principles are starting to be extended to define the role of IFN γ in other chronic conditions such as cancer and immunotherapeutic approaches to treat cancer. Specifically, the functions of IFN γ are being reexamined to better understand how it is impacting defined pathogenic or controlled outcomes. Considering IFN γ priming and cross-regulation as an existing pre-condition during the different stages of autoimmune diseases or in certain types of cancers will help to develop a better understanding of the role of this important immuno-

regulatory molecule in disease initiation, progression, and treatment.

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