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Interferon-gamma (IFN-γ): Exploring its implications in infectious diseases

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Abstract: A key player in driving cellular immunity, IFN-γ is capable of orchestrating numerous protective functions to heighten immune responses in infections and cancers. It can exhibit its immunomodulatory effects by enhancing antigen processing and presentation, increasing leukocyte trafficking, inducing an anti-viral state, boosting the anti-microbial functions and affecting cellular proliferation and apoptosis. A complex interplay between immune cell activity and IFN-γ through coordinated integration of signals from other pathways involving cytokines and Pattern Recognition Receptors (PRRs) such as Interleukin (IL)-4, TNF-α, Lipopolysaccharide (LPS), Type-I Interferons (IFNs) etc. leads to initiation of a cascade of pro-inflammatory responses. Microarray data has unraveled numerous genes whose transcriptional regulation is influenced by IFN-γ. Consequently, IFN-γ stimulated cells display altered expression of many such target genes which mediate its downstream effector functions. The importance of IFN-γ is further reinforced by the fact that mice possessing disruptions in the IFN-γ gene or its receptor develop extreme susceptibility to infectious diseases and rapidly succumb to them. In this review, we attempt to elucidate the biological functions and physiological importance of this versatile cytokine. The functional implications of its biological activity in several infectious diseases and autoimmune pathologies are also discussed. As a counter strategy, many virulent pathogenic species have devised ways to thwart IFN-γ endowed immune-protection. Thus, IFN-γ mediated host-pathogen interactions are critical for our understanding of disease mechanisms and these aspects also manifest enormous therapeutic importance for the annulment of various infections and autoimmune conditions.

Keywords: Cytokine; IFN-γ; Immune Response; Infectious Diseases; Mycobacteria; Host-pathogen interaction; Cytokine therapy.

IFN-γ: An Introduction

The human immune system is evolved to eradicate or contain any pathogenic challenge and eliminate self-altered cancerous cells. In this regard, IFN-γ has a critical role in recognizing and eliminating pathogens. IFN-γ, being the central effector of cell mediated immunity, can coordinate a plethora of anti-microbial functions. It can serve to amplify antigen presentation through antigen presenting cells (APCs) by enhancing antigen recognition via cognate T-cell interaction, increase the production of Reactive Oxygen Species (ROS) and Reactive Nitrogen Intermediates (RNIs) and induce anti-viral responses [1]. Additionally, cancer cells are destroyed by IFN-γ activity via induction of an anti-proliferative state. Immunity to several pathogens is mainly governed by IFN-γ activity. For example, the role of IFN-γ in endowing protection against Chlamydial infections is quite immense [2]. Release of IFN-γ by the CD4+ helper T cell population contributes to necessary effector cell activation and the generation of antibody mediated responses to *Chlamydia* infections [3]. The presence of IFN-γ is of absolute necessity in combating mycobacterial infections through its ability to regulate various protective functions and sustain both CD4+ and CD8+ cell activity [4]. The protective benefits of IFN-γ can also been seen in the context of viral infections, as enhanced survival of neurons infected with varicella zoster virus is observed post IFN-γ treatment [5]. Natural Killer (NK) cell -mediated IFN-γ production can successfully limit Hepatitis C virus proliferation in HIV+ (Human Immunodeficiency Virus) patients [6]. Enhanced anti-bacterial and immune protective effects simultaneous

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with pro-inflammatory responses leading to protection of epithelial monolayers from pathogen mediated injury are also conferred by IFN-γ during *Staphylococcus aureus* infections [7]. Thus, IFN-γ is a robust protective effector molecule mediating protection against a wide array of pathogenic entities.

IFN-γ increases the efficiency of immune system by enhancing its competence to deliver anti-microbial effector functions. Undoubtedly, such an important immune mediator is under stringent regulatory control. Over-activity of IFN-γ has been reported to cause excessive tissue damage, necrosis and inflammation and may contribute to disease pathology. Hyper-activity of both IFN- γ and IL-18 can exacerbate pathogenesis in *Burkholderia* infections [8]. Aberrant production of IFN-γ has been linked with autoimmunity and alterations in gut flora [9,10]. Therefore, IFN-γ activity is a double edged sword and immune regulatory mechanisms strive to strike a delicate balance between infection control and disease pathology in this regard. Foxp3+ (Forkhead box P3) -expressing regulatory T cells (Tregs) and suppressor of cytokine signaling protein-1 (SOCS-1) are critical controllers of IFN-γ activity in this regard.

IFN-γ: Production and signaling

Interferons were initially described as agents interfering with viral multiplication and eliciting potent anti-viral activity. However, with the passage of time, they have been attributed with a vast array of physiological activities and are more than just anti-viral agents. The main producers of IFN-γ include T-cells, NK cells, NKT cells, professional APCs such as macrophages, dendritic cells (DCs) and B cells. The role of IFN-γ secreted by NK cells and APCs has been implicated in early host defense and autocrine regulation [11]. T cells are the major source of IFN-γ during adaptive immunity [12].

IFN-γ responses are an offshoot of receptor-mediated signaling. Its biological effects are manifested through its interaction with the receptor (IFN-γR) present on target cells such as macrophages, DCs and many other cell types. The receptor is a heterodimeric complex composed of a ligand binding alpha subunit (IFNGR1) and a signal transducing beta subunit (IFNGR2). IFN-γ binds to IFNGR1 with relatively higher affinity compared with IFNGR2. Receptor-ligand interaction initiates a signaling cascade that in turn produces a series of protective responses [13]. Binding of IFN-γ crosslinks IFNGR1 and IFNGR2 and activates Janus Kinases JAK1 and JAK2 through phosphorylation. Activated JAKs in turn phosphorylate inactive cytosolic transcription factor STAT1 (signal transducer and activator of transcription 1) [14]. Activated and phosphorylated STAT1 homodimerizes and is translocated to the nucleus where it binds to GAS (gamma activated sequences) elements in the promoter region and mediates transcription of IFN-γ associated genes required for protective immunity. IFN-γ-IFN-γR signaling triggers STAT1 phosphorylation and subsequent activation of transcription factor T-bet which depicts the onset of T cell polarization for commitment to TH1 lineage [15]. Recruitment of an additional factor, Runx3, which binds to the IFN-γ promoter, drives the expression of IFN-γ with concurrent silencing of the *IL-4* gene [16]. Thus, IFN-γ plays a pivotal role in dampening the IL-4 -producing ability of TH1 cells and in maintaining sustained expression of T-bet [17].

An overview of IFN-γ activity

Macrophages represent a class of APCs which are versatile sentinels of the immune system. A pathogen is most likely to encounter a macrophage soon after its entry into the host and IFN-γ-stimulated macrophages show enhanced anti-microbial activity. Thus, IFN-γ activates macrophages and makes them better able to mount an effective immune response, such as enhanced antigen processing and presentation through upregulation of class II MHC, increased ROS and NOS production, induction of autophagy for clearance of intracellular pathogens and increased secretion of pro-inflammatory cytokines. Additionally, it can activate NK cells, increase their tumoricidal activity and also regulate antibody production to modulate B cell responses. Growth and maturation of other cell types and leukocyte migration are also facilitated by IFN-γ. The heightened immune activation ultimately leads to effective clearance of pathogens through enhanced phagocytosis, pro-inflammatory responses and lymphocyte recruitment.

Anti-microbial effector functions of IFN-γ

IFN-γ is endowed with the exquisite ability to promote rapid acidification of phagolysosomes within infected macrophages. This low pH within the phagolysosome ameliorates RNS production and this cooperation leads to elimination of the pathogen [18]. IFN-γ is a potent inducer of autophagy which has now emerged as a novel anti-microbial host response [19,20]. Immunity-related GTPases

(IRGs) comprise another very interesting class of IFN-inducible proteins. IFN-γ stimulation upregulates several IRGs which facilitate recruitment of autophagic machinery onto the bacterial phagosomes. Human IRGM helps in trafficking various components of autophagy pathway to vacuoles containing *Mycobacterium tuberculosis* (*M. tb*) [20]. The IRGs primarily orchestrate vacuolar trafficking and facilitate pathogen delivery to lysosomes for enzyme-mediated degradation. These may function in conjunction with certain other proteins, namely sequestosome (SQSTM1/p62), NDP52 and optineurin. These proteins mainly recognize ubiquitin-bearing bacteria that have successfully escaped the confines of the phagosomal compartment. On the contrary, galactins, another set of IFN-inducible proteins, recognize bacterial glycan moieties. The recognition and subsequent binding recruits the autophagy proteins and directs the bound components towards lysosomes [21]. Activation of *M. tb*-infected macrophages by IFN-γ stimulates the expression of IRGs that eventually coordinate vacuolar traffic and subsequent targeting of bacterial cargo for lysosomal hydrolysis [22]. Orchestration of cell autonomous immune responses by coordinating vacuolar compartmentalization is effective against several intracellular pathogens including *Chlamydia psittaci*, *C. trachomatis* and *Salmonella Typhimurium*. Additionally, Guanylate binding proteins (GBPs), expressed as a functional IFN-γ response also bind escaped bacteria and channelize them towards lysosomes [23]. Their salient structural aspects enable them to identify both phagosomal entrapped bacteria as well as free bacteria. For example, GBP1 can facilitate bacterial binding to lysosomes via interactions with SQSTM1. On the contrary, GBP7 engages autophagy proteins (ATG4B) driving extension of the elongation membrane around bacteria to sequester them completely within the autophagosomal compartment for degradation [21]. Recently, a protective house-keeping role of GBP1 has been observed at the intestinal tight junctions. Being expressed at these places, it serves to control mucosal immunity by exerting regulatory effects on apoptosis [24].

Interestingly, IFN-γ can also deplete tryptophan, touted to be an effective anti-parasitic mechanism in humans [25]. The anti-viral effects invoked by IFN-γ are most notably through the induction of RNA-activated protein kinase R (PKR) and adenosine deaminase RNA specific-1 (ADAR-1). The anti-viral effectors either impact viral multiplication or genomic stability. Interferon-induced transmembrane proteins (IFITMs) and tripartite motif-containing proteins (TRIMs) are another set of effector proteins that also elicit marked anti-viral properties. IFITMs restrict viral uncoating or entry into the host cells and confer protection against a number of viruses including Influenza-A, Flaviviruses, HIV-1, Ebola virus and Corona virus [26]. More recently it was shown that IFITM3 restricts virions inside the endosome to block their access to the cytoplasm [27]. On the other hand, TRIMs are induced in macrophages and myeloid DCs and function to limit viral entry, most notably that of retroviruses [28]. IFN-γ-inducible proteins can also employ components of the autophagic machinery to curb viral replication. For example, IFN-inducible GTPases utilize the microtubule-associated-protein-1-light chain-3 (LC3) protein of the autophagy pathway to arrest murine norovirus replication. The concerted action of IFN-inducible GTPases and GBPs leads to marked inhibition of viral growth in murine cells [29]. IFN-γ also regulates the production of proteins, viz. tethrin or viperin, involved in limiting and interfering with virion egress from cells or viral assembly. Tethrin acts as a restrictive factor against viruses such as Filo virus, HIV-1 or Arena virus by blocking viral exit from the infected macrophages [30].

IFN-γ regulates the production of various lysosomal constituents, granules or exudating substances (β-defensins, α-defensins and cathelicidins) mediating inflammation that display robust killing action against microbes. IFN-γ is a potent inducer of autophagy upon mycobacterial induction by eliciting production of hCAP18/LL-37 cathelicidin in a Vitamin D3-dependent manner [31]. Additionally, IFN-γ also induces of several efflux systems that deprive pathogens of essential cations, consequently limiting their growth within host. NRAMP1, natural resistance associated macrophage protein 1, is one such immune effector protein that is upregulated by IFN-γ. NRAMP1 expels Mn^{2+} or Fe^{2+}, lowering cationic concentration. It can effectively compete with pathogens for essential cations and reduce pathogenic possession of these ions within the phagosome [32]. Alternatively, IFN-γ triggers the upregulation and relocation of P-type Cu^{2+} onto phagosomes facilitating higher inflow of Cu^{2+} cations to ameliorate ROS generation [33]. In a similar mechanism, IFN-γ significantly enhances the expression of iron-exuding ferroprotein 1 with concomitant down-modulation of transferrin receptor to diminish Fe^{2+} concentration, thereby restricting *Salmonella* growth [34].

Implications in infectious diseases

IFN-γ has emerged as an extremely versatile cytokine that can carry out countless biological activities that are non-redundant with other interferons. The literature is inundated with reports that emphasize its importance in

disease pathologies. Consequently, any failure in the IFN-γ-IFN-γR system severely hampers host immune responses to infections. Thus, its relevance with regard to conferring protective immunity in infectious diseases and cancers is undisputable. In this section we discuss and highlight its protective role in mediating immune responses in infectious diseases, cancer or autoimmunity. Numerous evasive strategies have been devised by pathogens to overcome its curbing effects and circumvent the protection conferred by IFN-γ.

Anti-microbial effects of IFN-γ : role in mycobacterial infection

The role of IFN-γ is almost undoubted in mediating protection against tuberculosis (TB) [35]. The hallmark of TB infection is the induction of cellular immunity and inflammation orchestrated by IFN-γ. Quite plausibly, mice lacking functional IFN-γ or the IFN-γR gene are most susceptible to mycobacterial infections. Mice with a disrupted IFN-γ gene show rapid, fulminant mycobacterial growth and disseminated TB [36]. Dysfunctional IFN-γ or IFN-γR signaling increases susceptibility to even mildly virulent strains such as attenuated *Mycobacterium bovis* Bacillus Calmette-Guérin (BCG). Children with non-functional IFN-γR show disseminated infection with BCG [37]. Quite recently, a role of a novel IFN-γR adaptor has been implicated in the killing of *M. tb*. The Mal-dependent IFN-γR-mediated signaling is independent of the Toll-like Receptor (TLR) pathway and initiates a cascade of downstream responses involving phosphorylation by MAPK-p38 (Mitogen activated protein kinase) and induction of autophagy. Mutations in the Mal adaptor diminished its IFN-γR binding capacity which ultimately led to compromised signaling in response to IFN-γ and impaired immune responses to *M. tb* [38].

IFN-γ knock out mice manifest uncontrolled bacterial growth and rapidly develop necrotic granulomas. Such mice have severely hampered cell-mediated immunity and Nitric Oxide Synthase 2 (NOS2) expression. IFN-γ favors ROS, RNI production, revokes the blockade of phagosome-lysosome fusion imposed by *M. tb* as part of its survival strategy, and also induces autophagy to favor clearance of *M. tb* [39]. Apoptosis has been linked with effective anti-microbial host defense mechanisms against intracellular pathogens, especially *M. tb*. IFN-γ also promotes apoptosis in a NO-dependent manner in human macrophages [40]. IFN-γ in combination with TLR2 stimulation also facilitates the production anti-microbial peptides and induction of autophagy in a Vitamin-D-dependent pathway [41,42]. As a counter strategy, *M .tb* has evolved ways to subvert the deleterious effects of IFN-γ. *M. tb* averts the activating effects of IFN-γ on macrophages in a TLR-dependent manner. Many crucial immune protective responses exerted by IFN-γ are inhibited by *M. tb*. Various mycobacterial cell wall lipoproteins abrogate IFN-γ-mediated anti-bacterial effects. For example, the *M. tb* 19 kDa lipoprotein inhibits MHC class II upregulation and antigen processing in IFN-γ treated macrophages to dampen the protective effects of IFN-γ. This occurs due to repressed expression of class II transactivator (CIITA) in a TLR-dependent manner and is believed to be due to inhibitory effects imposed on the chromatin remodeling of CIITA involving the TLR2 and MAPK pathways [43,44]. Prolonged signaling induced by 19kDa protein through the TLR pathway has also been implicated in the downregulated expression of several IFN-γ-induced genes in macrophages [45]. Downstream transcriptional responses induced by IFN-γ are also inhibited by *M. tb*, although the proximal steps involved in IFN-γR signaling such as STAT1 phosphorylation and dimerization are unaffected [46]. Diminished IFN-γ-induced responses are attributed to reduced association of STAT1 dimers with its associated co-activators such as cAMP response element binding protein (CREB) and p300 in *M. tb* infected macrophages. This disrupted association leads to diluted macrophage activity, such as reduced killing of *Toxoplasma gondii* [47]. Interestingly, the role of TLR signaling has been linked with the induction of inhibitory responses to IFN-γ in mycobacterium infected macrophages. It was reported that TLR2 stimulation can abrogate IFN-γ responsive effects through stabilization and expression of a dominant negative form of STAT1β. Higher levels of STAT1β are induced upon *M. avium* infection which is attributed to increased STAT1β mRNA stability. Further, overexpression of STAT1β in macrophages leads to decreased IFN-γ gene expression [48]. STAT1 knock out (KO) mice are highly susceptible to TB and succumb very rapidly to the disease. These KO mice develop multiple necrotic and granulomatous lesions in the lungs with defective Tumor Necrosis Factor (TNF)-α, inducible NOS and IL-12 production [49].

Anti-microbial effects of IFN-γ : role in *Salmonella* infection

IFN-γ is critical in mediating intestinal immunity to *Salmonella typhimurium* [50,51]. Gene KO mice have revealed the importance of IFN-γ in promoting protective immunity. These mice show aberrant immune responses

and disseminated septicemia as compared with wild type (WT) mice which are characterized by high CD4+, CD8+, Class II MHC and Vascular Cell Adhesion Molecule I (VCAM-1) expression [50]. IL-12 dependent IFN-γ production plays a key role in endowing protection against *Salmonella* infections. IL-23 is also a major regulatory cytokine governing IFN-γ production [52].

IL-18 is a major contributor in imparting protective immunity by being a potent inducer of IFN-γ [53]. However, in many instances such as those involving colitis and inflammation, IFN-γ can have a dual role. In such scenarios, IFN-γ can promote inflammation and exacerbate inflammatory responses in the intestines to aggravate *Salmonella*-induced colitis. In this case neutrophils become an important source of IFN-γ. As a corollary, depletion of neutrophils relieves many of the IFN-γ-induced disease symptoms; it curbs excessive inflammation and decreases the severity of intestinal lesions [54]. IFN-γ has also been implicated in generating memory responses essential in vaccine protection against *Salmonella*. Following infection with mutant avirulent *S. typhimurium* Aro strains, mice deficient in IFN-γ are more susceptible than WT mice. These mice elicit highly impaired immunity and widespread septicemia [50]. A host of IFN-γ-induced genes are upregulated in response to *Salmonella*. Significant changes occur in the expression profile of many of the vital players of the immune system, such as chemokines, signaling molecules, transcription factors and surface receptors within the infected macrophage. Amongst the genes upregulated in macrophages or epithelial cells by *S. typhimurium* or *S. dublin* the prominent ones include IRF-1, iNOS and MHC class I and II [55]. This phenomenon can be recapitulated with LPS stimulations in uninfected macrophages. IFN-γ treated cells may exhibit a changed expression profile compared with pathogen-induced gene expression, pointing toward altered host cell responsive and differential activation state. Such profound alterations in the gene expression profile are also evident in in other bacterial infections. IFN-γ also facilitates internalization and promotes early killing of *Salmonella* without employing oxidative burst, accompanied by a decrease in IL-10 levels with a concomitant increase in IL-12 levels [56]. Another novel anti-microbial effect of IFN-γ is the depletion of intracellular iron levels in *S. typhimurium*-infected macrophages. Depleting iron starves the pathogen of this essential nutrient and boosts the bactericidal mechanism of macrophages. This occurs via transferrin receptor 1 which leads to an enhanced iron efflux by increasing the iron exporter ferroprotein. This event ultimately enhances the expression of hemoxygenase I and the siderophore entrapping antimicrobial peptide, lipocalin 2, that further sequesters iron from intracellular pools [57]. The role of hemoxygenase I is pivotal in regulating the initial stages of innate immunity to *S. typhimurium* infections. Iron export from tissue macrophages occurs in conjunction with increased serum levels of IL-12 and IFN-γ [58]. However, relocation of iron from intracellular pools may have negative outcomes as this often leads to anemia pertinent in *Salmonella* infections. Nevertheless, IFN-γ mediated iron scavenging undoubtedly bolsters host survival during acute infections [59].

Anti-microbial effects of IFN-γ : role in *Listeria* infection

Just like in other infections, IFN-γ forms an integral part of immunity against *Listeria* as well. Administration of anti-IFN-γ antibodies reverses the protective effects during *Listeria* infection and increases disease-associated fatality. The literature is replete with evidence stating the requirement of endogenous IFN-γ for the resolution of *Listeria monocytogenes* infection. NK cells and T cells are the key producers of IFN-γ in this regard. IFN-γ helps in bacterial clearance and control of primary infections with *L. monocytogenes* [60]. An essential role of IFN-γ-mediated signaling in inducing protective responses is evident from the fact that mice lacking functional STAT1 exhibit profound propensity to *Listeria* infections and manifest dampened macrophage activity and pathogen clearance [61]. STAT1-mediating signaling was imperative in T cells and DCs only at the level of generating primary adaptive immune responses. The fact that IFN-γ curbs bacterial spread is evident following IFN-γ blockade with neutralizing antibodies resulting in higher bacterial burdens in the mesenteric lymph nodes [62]. The prominent anti-Listerial effects mediated by IFN-γ include the generation of ROS and RNS via induction of NOX2/CYBB (NAPDH oxidase), DUOX (dual oxidase) and iNOS/NOS2. Additionally, induction of inducible GTPases (which facilitate oxidative pathways, autophagy and orchestrate vesicular traffic) and elicitation of mitochondrial ROS through regulated expression of several nuclear genes that encode respiratory machinery through the activity of ERRα (Estrogen related receptor α) are some other examples of IFN-γ-mediated anti-Listerial effects. The role of ERRα has been associated with effective pathogen clearance through concerted mitochondrial ROS production. 63 IFN-γ also regulates the expression of indole 2,3 dioxygenase (IDO), an important enzyme involved in tryptophan metabolism known to limit infection spread and control enormous T cell activity

[64]. Furthermore, in *Listeria* or BCG infected mice, NK cells and T cells show a transient downfall of microRNA, miR29 that is essential for sustained production of IFN-γ. Thus, miR29 negatively regulates IFN-γ expression. Based on these observations researchers were able to devise a unique transgenic mice model (GS29) bearing 'sponge' targets that could compete with miR29 targets. Such mice displayed higher T helper responses, higher serum concentrations of IFN-γ and lower *L. monocytogenes* burden [65]. The pathogen has evolved exquisite evasive strategies to counter immune activation [66]. *Listeria* can modulate expression of many interferon stimulatory genes (ISGs) in epithelial cells. This is accomplished by targeting a chromatin repressing complex BAHD1 at the promoter level which can dampen expression of multiple ISGs and subsequently downregulate IFN-γ induced responses [67]. Interestingly, *L. monocytogenes* can also produce a protein called LntA which is a nucleomodulin. This protein binds to BAHD1 to nullify its effect and functions to ameliorate IFN functions [67,68]. Type-I IFNs are often linked with suppression of innate immune responses in *L. monocytogenes* infection and escalate predisposition to the disease. IFN-α/β production affords some degree of subversion from the macrophage activating effects of IFN-γ which can also be achieved in part through IFN-γR downregulation [69].

Protective role of IFN-γ during parasite infection

IFN-γ plays a pivotal role in host resistance to *Leishmania* infections [70]. Conversely, IL-4 mitigates ROS generation and stifles IFN-γ-mediated anti-pathogenic responses of the host. NK cells are the prime source of IFN-γ production which facilitates CD4+ T cell polarization and differentiation to induce early phases of resistance [70]. In the absence of IFN-γ, TH2 responses are linked to ablated anti-parasitic immunity.

It is reported that both IL-12 and IFN-γ help in limiting visceral leishmaniasis and counteract IL-6, IL-24 and IL-27 activity [71]. Neutralization of TNF-α leads to impaired IFN-γ production by spleen cells in visceral leishmaniasis patients, pointing towards a regulatory role of TNF-α [72]. Another important anti-parasitic mechanism is the degradation of tryptohan. This strategy constitutes an important host defense against obligate parasites such as *Leishmania*, *Toxoplasma*, *Chlamydia* etc. However, some reports have also emphasized on the harmful effects of IFN-γ in having negative repercussions on the disease outcome. For example, IFN-γ exacerbates disease symptoms through recruitment of inflammatory monocytes which diminish iNOS-dependent parasite killing, leading to expansion of the parasite niche. This in turn prolongs host cell survival to support parasite multiplication [73]. Similarly, IFN-γ promotes *L. amazonensis* replication in macrophages *in vitro* by inducing cationic amino acid transporter 2B (CAT2B) that facilitates L-arginine transport, which can be directly utilized by the parasite for its growth and multiplication [74]. Similar to other pathogens, *L. donovani* can also attenuate IFN-γR mediated signaling in macrophages by inhibiting STAT1 phosphorylation at tyrosine residues catalyzed by JAKs. This downregulates IFN-γ-induced genes and consequently, the downstream effector functions are quenched. Furthermore, the *Leishmaina* parasite can effectively thwart STAT1 translocation to the nucleus and also impede JAK activation necessary to achieve full-fledged downstream signaling emanating from IFN-γR [75].

IFN-γ: therapeutic and clinical implications

Administration of cytokines is an important and effective therapeutic strategy to stimulate the immune system and initiate protective responses. This procedure shapes and orchestrates the host cytokine milieu to modulate various facets of immune responses most notably by influencing tumor microenvironment or pathogenesis. Cytokine administration has gained immense popularity in cancer immunotherapy and is beneficial in manipulating tumor behavior. The usability of such an approach is limited by its fatal side effects as systemic administration may lead to "toxic shock" leading to untoward consequences. However, some patients have achieved immense benefits and elicited better prognosis in certain conditions [76,77]. As discussed, owing to the numerous and vital immune-protective functions coordinated by IFN-γ, it is one such potent cytokine which can be administered either systemically or locally. Due to its profound immune modulatory abilities, IFN-γ has become an attractive therapeutic alternative and has been used as a prophylactic measure to cure several infections including cancers, fungal infections and chronic granulomatous disease (CGD) [78-80].

Experimental data point towards myriad protective effects of IFN-γ therapy. IFN-γ administration can successfully impede Ebola virus infectivity and can be an attractive prophylactic alternative. It effectively reduced

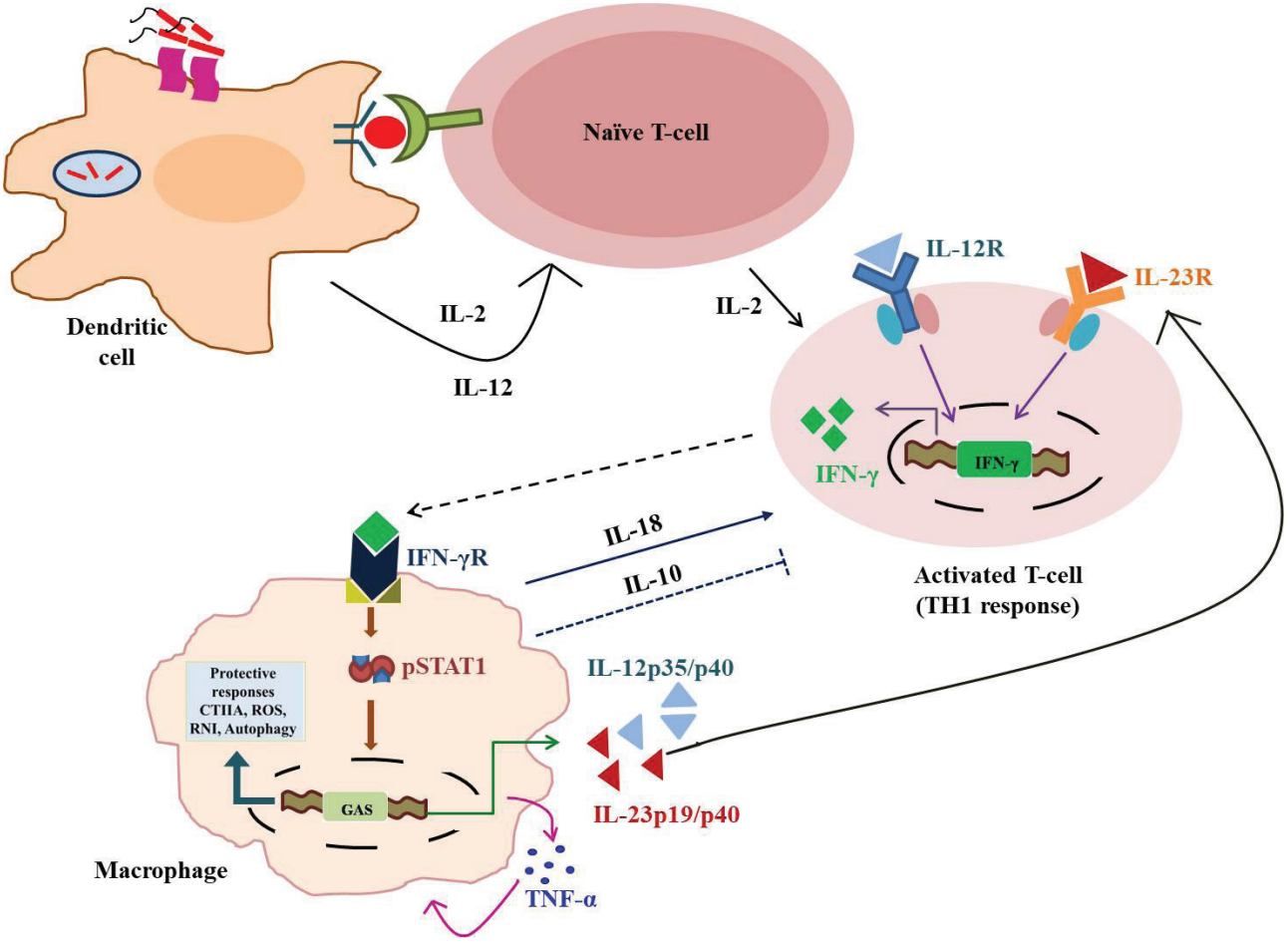

Figure 1: IFN-γ: Production and regulation. A complex interplay between various cytokines determine the transcription and production of IFN-γ. Release of IFN-γ either by NK cells or T cells triggers a series of anti-microbial responses. Following pathogenic recognition and uptake by an APC such as DC, the antigenic peptides are presented to the naïve T-cell population for initiation of an adaptive immune response. The cytokine milieu comprised of IL-12 and IL-2 triggers T cell proliferation to create conditions conducive for a pro-inflammatory phenotype. Exposure to IL-12 aids naïve cells to polarize to a TH1 phenotype. IFN-γ is a predominant cytokine in the TH1 response. Following transcription and production of the IFN-γ protein in the TH1 lineage committed and clonally expanded T cell population, this cytokine primes macrophages to upregulate their protective infections. The activated macrophages in turn produce IL-12 and IL-23 that further ameliorate pro-inflammatory conditions and facilitate IFN-γ production. Thus, IFN-γ production is self-sustained and occurs in a positive feedback manner. Macrophages are also known to produce IFN-γ that leads to autocrine activation further bolstering their anti-microbial functions.

viral viability and serum titers [81]. Similarly, adjunctive immune therapy including IFN-γ as one of the pivotal components also drastically improved the outcome of invasive fungal infections or sepsis. Recombinant IFN-γ administration proved beneficial in restoring immune functionality thereby making IFN-γ as a promising therapeutic contender [82]. In animal infection models, administration of IFN-γ has led to better survival rate and immune responses. For example, enhanced resistance against invasive aspergillosis and disseminated *Candida albicans* infections is elicited in IFN-γ treated mice [83,84]. Similarly, IFN-γ therapy bolstered pulmonary immune responses in corticosteroid-treated rats in experimental legionellosis [85]. Improved survival and decreased pathogenic burden in lungs was observed upon IFN-γ administration in mice infected with *Cryptococcus neoformans* [86]. IFN-γ can prove as an effective therapeutic candidate against as many as 22 infectious agents including bacteria, fungi, protozoa and helminths. Clinically IFN-γ is an FDA approved drug for the treatment of CGD. CGD is a form of immune-deficiency characterized by a series of recurrent infections by pyogenic bacteria. It is majorly an inherited disorder wherein most of the cases arise due to mutatation of the *CYBB* gene (Cytochrome B beta/NAPDH oxidase) encoding gp91phox, the remaining being autosomal recessive. The disease is primarily due

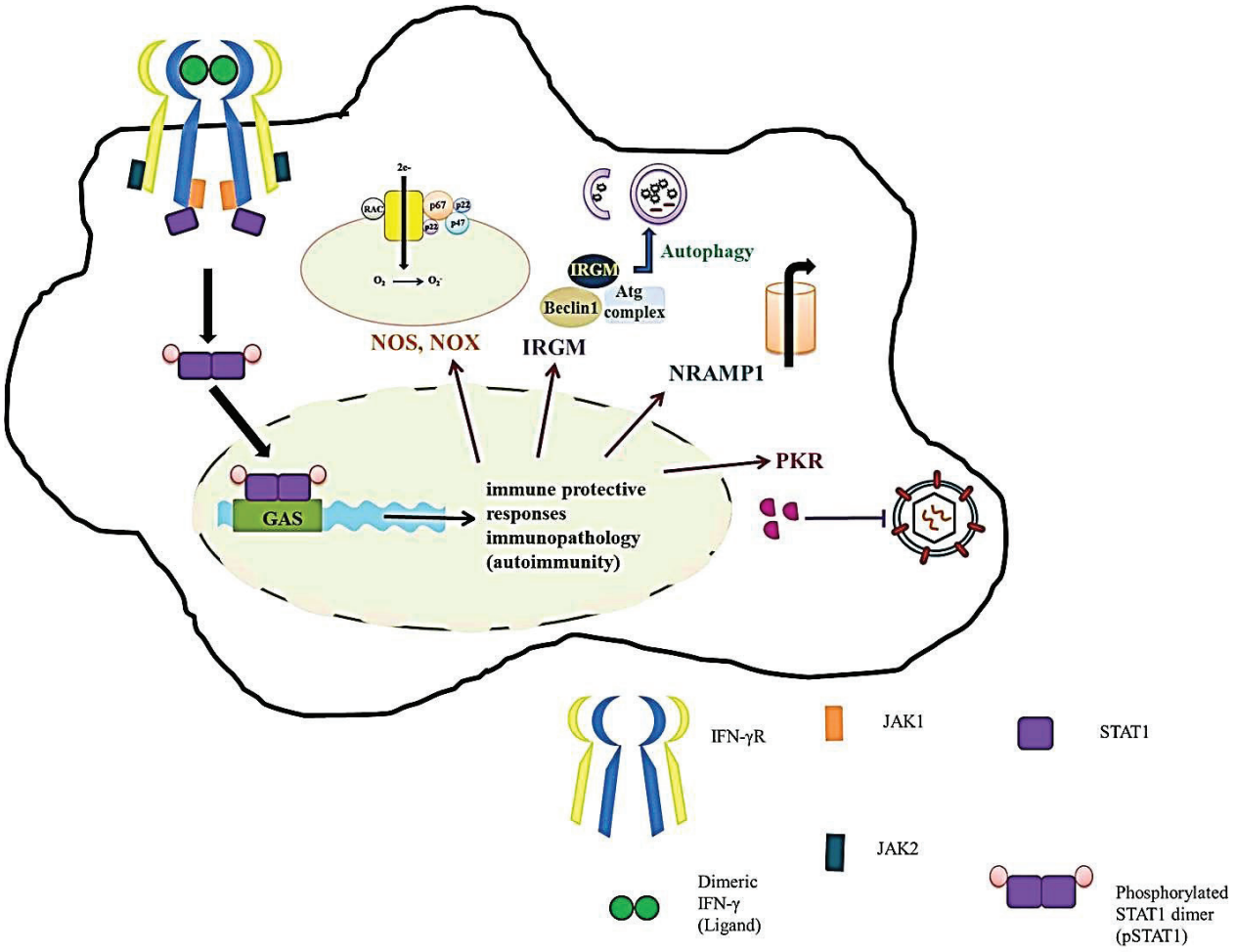

Figure 2: Protective effects of IFN-γ. IFN-γ primes the macrophage to improve its anti-microbial capacity. The biological protective effects commence upon binding of a dimeric IFN-γ to its cognate receptor on the macrophage surface. This binding leads to a series of phosphorylation reactions that ultimately phosphorylate STAT1 proteins. Following phosphorylation of STAT1 molecules, these homodimeric molecules translocate to the nucleus where they bind to GAS elements on the DNA. This triggers a plethora of downstream effector genes that encode products necessary for pathogen containment. IFN-γ -regulated proteins include Reactive Oxygen Species, Reactive Nitrogen Intermediates, autophagy proteins, efflux pumps or antiviral mediators. Besides, controlling infection, IFN-γ can serve as a double-edged sword in an immune dysfunctional state, such as autoimmunity, wherein it can exacerbate disease symptoms due to prolonged pro-inflammatory effects.

to dysfunctional NADPH oxidase machinery caused by defective or missing components of the phagocyte oxidase [87]. IFN-γ promotes respiratory burst by facilitating superoxide release in these patients and partially makes up for the anomalies in the oxidative metabolism. Interestingly, in some cases IFN-γ has been shown to improve splicing efficiency of the *CYBB* gene, which is normally malfunctional in CGD. It augments microbicidal activities and reduces the relative risk of infections by 70% [88]. Thus, IFN-γ is a beneficial prophylactic agent in CGD with minimal side effects such as toxicity, delayed or impaired growth and development.

The therapeutic and immunomodulatory effects of IFN-γ have also been utilized to treat immunodefeciency syndromes. For example, hyperimmunoglobulinemia E (hyper-IgE) is a rare and unique immunodeficiency syndrome which predisposes an individual to severe and recurrent *Stapylococcus aurues* infections. These patients exhibit skin eczema, abscesses, elevated eosinophil production, hyper-production of IgE with limited lymphocyte chemotaxis and reduced responsiveness to IL-12. Systemic administration of recombinant IFN-γ attenuates the course of clinical symptoms in such patients [89]. Efficacy of recombinant IFN-γ as an adjunct increases manifolds when it is used in conjunction with

Table 1: Biological functions of IFN-γ: An overview.

Immunological pathway	Proteins affected	Biological implications	References
Antigen processing and presentation	CTIIA, MHC class II, Cathepsins (L, B, S and H), MHC class I, trimming peptidases (endoplasmic reticulum aminopeptidases, ERAP-1), β2-microglobulin	Mainly targets and boosts the expression of major effector molecules and chaperones involved in the processing and presentation of antigens in both class I and class II pathways. This pathway is central to the inception of adaptive immunity through the priming of naïve T cells by antigenic entities, leading to robust and efficient T cell activation.	[107-112]
Reactive Oxygen Species	gp91phox, p22phox, p67phox and GTPase Gbp7	Upregulates the expression and facilitates the assembly of subunits comprising the NADPH oxidase. The enzyme is critical for the generation of oxidative burst and production of ROS that show potent microbicidal effects.	[21,113]
Reactive Nitrogen Intermediates	NOS2, Argininosuccinate sythetase and GTP cyclohyrdoxylase I	Increases the production of the key enzyme involved in the catalytic production of reactive nitrogen species or acts indirectly via upregulation of accessory molecules that enhance production of substrate or enzymatic cofactors.	[1,114]
Autophagy	LC3-II, IRGM (LRG-47), bioactive Vitamin D3 (via CYP27b1 hydroxylase), antimicrobial cathelicidin and ubiquitin-binding proteins (SQSTM1, NDP52, optineurin and galectins)	A highly coordinated frame work of IFN-γ -inducible proteins including effector molecules, immunity-related GTPases and GBPs proteins trigger and facilitate the assembly of autophagic machinery and delivery of autophagic cargo to lysosomes. Additionally, to counter bacteria that have escaped into the cytosol, IFN-γ may stimulate production of proteins that direct cytosolic ubiquitinylated bacteria to the lysosome for degradation by combining them with the autophagy pathway.	[21,31,115,116]
Apoptosis	Caspases (8, 9 and 1), Fas, FasL, Bax, increased Cytochrome c release, IRF-1 and TNFa	IFN-γ stimulation may trigger both extrinsic and intrinsic death pathways. Concomitantly the levels of pro-apoptotic factors and mediators are increased that facilitate apoptotic death.	[116-118]
Cell migration and leucocyte traffic	CXCL9, MIP-1, MIP-1, IP-10, VCAM-1, ICAM-1, RANTES, CCL2	Expression of many essential chemokines and inflammatory mediators are under the influence of IFN-γ stimulation. These effector and adhesion molecules serve as chemoattractants and aid in immune cell migration or lymphocyte extravasation, thus helping in the initiation of an inflammatory response by recruiting diverse immune cells.	[1,119]
Antiviral mediators	PKR, ADAR, IFITM, TRIM Viperin and Tethrin	Many antiviral proteins induced by IFN-γ help in countering numerous viral infections at several stages like viral entry, uncoating, blocking viral translation or virion assembly. IFN inducible viral RNA editing enzymes may edit RNA bases that may introduce severe genomic instabilities leading to lethal mutations	[21,120]
Lysosome mediated killing/Phagosome maturation	Proton ATPases, IRGM/LRG-47 (via induction of autophagy) GBPs and Rab proteins	IFN-γ elicits lysosomal killing of many intracellular pathogens by facilitating rapid acidification of phagosome and helping it recruit the necessary fusogenic machinery that aids its fusion with the lysosome. Alternatively, induction of several effectors of autophagy by IFN-γ can further facilitate lysosome-mediated enzymatic degradation of pathogens.	[121-124]

Continued **Table 1:** Biological functions of IFN-γ: An overview.

Immunological pathway	Proteins affected	Biological implications	References
Complement pathway	Complement proteins (C2, C3, C4 etc.) and CR3	Complement receptors mediators or proteins (secreted by macrophages or fibroblasts) are upregulated in response to IFN-γ. The surge in complement pathway activity enhances opsonic uptake of extracellular pathogen via receptor mediated phagocytosis.	[125-127]
Fc Receptors	FcγR	IFN-γ-mediated enhanced expression of high affinity FcγR1 on myeloid cells ameliorates antimicrobial defences by facilitating opsonisation of IgG-coated extracellular pathogens via upregulated receptors. Thus, increasing opsonic uptake by phagocytic cells.	[128,129]
Other antimicrobial effectors	Cathelicidin LL-37/hCAP18 Defensins, cationic efflux molecules such as NRAMP1, P type ATPase Cu++ Pump ATP7A and Ferroprotein (SLC40A1), IDO	Many antimicrobial effectors such as antimicrobial peptides, cationic efflux pumps or molecules causing nutrient limitation to the pathogen are under the control of IFN-γ stimulation. These antimicrobial effectors display potent activity and can either directly or indirectly kill the pathogen either by depriving it of essential ions or nutrients or by disrupting its membrane integrity.	[21,130-132]

the conventional anti-microbials. This is especially true in the case of visceral or cutaneous leishmaniasis, atypical mycobacterial infections and lepromatous leprosy [90,91]. Alveolar macrophages exhibit heightened activity and improved effector responses in a limited number of multi-drug resistant TB (MDR-TB) patients who received aerosolized IFN-γ therapy. In a small group of 5 patients, all the individuals who had undergone the therapy (thrice a week for 2 months) showed improved prognosis, reduction in lesion size and tested negative for sputum acid fast bacillus [92]. However, therapy termination led to reversion of sputum smears to positive. Nevertheless, the data indicate that aerosolized IFN-γ may be a potential and effective prophylactic agent for the treatment of MDR-TB patients, who are normally refractory to other lines of treatment. Systemic aerosolic IFN-γ administration also yields promising results for the treatment of respiratory infections caused by atypical mycobacteria [93]. IFN-γ can alleviate symptoms of sepsis by restoring LPS-induced TNF-α production and HLA-DR expression in patients. This recovers macrophage activity and helps in clearance of sepsis [94]. IFN-γ therapy has been shown to successfully contain the damaging consequences of fibrosis. It ameliorates the total lung capacity and partial pressure of lung oxygen with concomitant suppression of pro-inflammatory cytokines in fibrotic disease [95]. As discussed earlier, mycobacteria subvert host immunity for their own benefit, interestingly, many host genes also play a suppressive role during *M. tb* infection and expression of these genes modulates TH1 cytokines including IFN-γ [96]. Our own findings have shown that IFN-γ plays a suppressive role during mycobacterial infection. Infection with *M. tb* induces production of IFN-γ that suppress many host protective functions. Thus, insights into host-pathogen interaction and dynamics of infection would strengthen our understanding of several disease mechanisms. These host-specific preventive therapeutic approaches can emerge as a robust alternative to the conventional pathogen-centric intervention to mitigate the ever-rising incidents of drug resistance. This can aid the development of better drugs and improve our understanding about host immune responses during complex infections.

Recombinant IFN-γ elicits potent anti-proliferative, anti-angiogenic and anti-tumorigenic effects. It is known to trigger apoptotic death in tumor cells and leads to a positive disease outcome. The earliest usage of IFN-γ as a therapeutic agent was for acute leukemia and since then it has been used on and off experimentally to induce anti-proliferative tendencies in multiple cell lines. IFN-γ was successful in limiting carcinogenesis in numerous cancerous cell lines [97-99]. Growth modulatory properties of IFN-γ have also made it an interesting therapeutic option for hematologic conditions and human stem cell (HSC) transplantation. Recent findings have suggested that IFN-γ can negatively regulate the expansion of HSC pool and

lead to progressive loss of such cells in bone marrows and peripheral HSCs in the context of infections [100].

The therapeutic potential of IFN-γ can be limited owing to huge cost of treatments and side effects such as flu, lethargy, suppressed nervous system, cough etc [101]. Although these accompanied adverse effects do minimize the efficacy and potential of such immunomodulatory therapies, IFN-γ administration still proves to be beneficial and an effective adjunct to the existing drug regimen in this era of rapid and formidable drug resistance. Thus, cytokine intervention can control several of the life-threatening and debilitating infections. Targeting IFN-γ has been seen as a new therapeutic approach for combating atherosclerosis, as it is reported to be key player involved in the development and disease manifestation of atherosclerosis [102]. Clinical investigations have further substantiated the role of IFN-γ in progression of cardiac diseases. Patients suffering from chronic heart failure reported to have a higher serum IFN-γ levels as compared to healthy controls [103]. Moreover, myocardial tissues of patients suffering from Chagas' cardiomyopathy displayed significant up regulation of IFN-γ-inducible genes, suggesting a direct link of IFN-γ signaling in this parasite-induced heart problem. Individuals with peripartum cardiomyopathy upon treatment with ACE-inhibitors, beta-blockers and diuretics resulted in improved cardiac function along with lowering down of serum IFN-γ levels which was high otherwise [104]. Additionally, levels of IFN-γ in serum and cerebrospinal fluid have also been found up-regulated in the neurodegenerative disease, Amyotrophic lateral sclerosis (ALS). Hence, linking this key pro-inflammatory cytokine with the progression of the disease [105].. Elevated levels of serum targeting IFN-γ has been seen as a new therapeutic approach for combating atherosclerosis, as it is reported to be key player involved in the development and disease manifestation of atherosclerosis [102]. Clinical investigations have further substantiated the role of IFN-γ in progression of cardiac diseases. Patients suffering from chronic heart failure are reported to have a higher serum IFN-γ levels as compared to healthy controls [103]. Moreover, myocardial tissues of patients suffering from Chagas' cardiomyopathy displayed significant up regulation of IFN-γ-inducible genes, suggesting a direct link of IFN-γ signaling in this parasite-induced heart problem. Individuals with peripartum cardiomyopathy upon treatment with ACE-inhibitors, beta-blockers and diuretics resulted in improved cardiac function along with lowering of serum IFN-γ levels which was high otherwise [104]. Additionally, levels of IFN-γ in serum and cerebrospinal fluid have been found to be up-regulated in ALS, linking this key pro-inflammatory cytokine with the progression of the disease [105]. Elevated levels of serum IFN-γ has also been reported in patients with Parkinson's disease [106]. Thus, IFN-γ can be a relevant disease marker in a clinical set-up, and therapeutic targeting of IFN-γ may emerge as a potential alternative strategy to alleviate disease pathologies.

Conclusion and future perspective

Owing to a plethora of biological activities mediated by IFN-γ, this cytokine remains an imperative and obligate protective factor in the host immune system. This versatile immune factor directly or indirectly regulates the expression of numerous genes that supposedly confer protection and mediate anti-microbial activities. Thus, comprehending its biological functionality or implications in infections or immune dysregulatory conditions, such as autoimmunity, becomes critical. Deeper understanding of the molecular basis of these IFN-γ-mediated mechanisms vis-à-vis diseases and associated pathologies would unravel valuable information about intricate host-pathogen encounters. A more in-depth understanding of the complex pattern of host-pathogen interactions would facilitate research in the areas of vaccine and drug development. Deciphering mechanisms of host mediated responses can also present a possibility of devising host-directed therapies that would alleviate the scourge of widespread drug resistance and limited drug usability. Likewise, the protective effects of IFN-γ have been harnessed for therapeutic purposes as well. This further reiterates its protective role in the host system. Comprehending the evasive mechanisms and tolerance to IFN-γ in various immune compromised conditions and cancers would further delineate complex patterns of pathogenesis and increase our understanding about disease mechanisms.

Abbreviations

SNP: Single-nucleotide polymorphism, NF-κB: Nuclear factor kappa B, cAMP: Cyclic adenosine monophosphate, IRF: Interferon regulatory factor, IDO: Indoleamine 2,3 dioxygenase, CXCL: Chemokine (C-X-C motif) ligand, MIP: Macrophage inflammatory protein, IP-10: Interferon-gamma-induced protein 10, CCL: Chemokine (C-C motif) ligand.

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