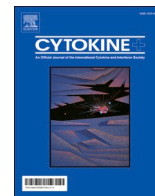ELSEVIER

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Review article

Between immunomodulation and immunotolerance: The role of IFNγ in SARS-CoV-2 disease

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ABSTRACT

Interferons have prominent roles in various pathophysiological conditions, mostly related to inflammation. Interferon-gamma (IFNγ) was, initially discovered as a potent antiviral agent, over 50 years ago, and has recently garnered renewed interest as a promising factor involved in both innate and adaptive immunity. When new disease epidemics appear such as SARS-CoV (severe acute respiratory syndrome coronavirus), MERS-CoV (Middle East respiratory syndrome coronavirus), IAV (Influenza A virus), and in particular the current SARS-CoV-2 pandemic, it is especially timely to review the complexity of immune system responses to viral infections. Here we consider the controversial roles of effectors like IFNγ, discussing its actions in immunomodulation and immunotolerance. We explore the possibility that modulation of IFNγ could be used to influence the course of such infections. Importantly, not only could endogenous expression of IFNγ influence the outcome, there are existing IFNγ therapeutics that can readily be applied in the clinic. However, our understanding of the molecular mechanisms controlled by IFNγ suggests that the exact timing for application of IFNγ-based therapeutics could be crucial: it should be earlier to significantly reduce the viral load and thus decrease the overall severity of the disease.

1. IFNγ overview

IFNγ is a pleiotropic cytokine with roles in a variety of biological responses including protection from viral and bacterial infections, anti-tumor effects, and regulation of effector cells in both innate and adaptive immunity. It was discovered in 1965 and described initially as an interferon-like virus inhibitor in cultured human leukocytes following exposure to the mitogen phytohemaglutinin [1]. In the 1970s, it became recognized as being distinct from classical virus-induced interferons, leading to its designation as immune interferon or Type II interferon, and eventually IFNγ [2].

IFNγ is structurally unrelated to Type I IFNs, binds to a different receptor, and is encoded by a separate chromosomal locus. Moreover, multiple conserved noncoding sequences (CNS) were discovered at the mouse Ifng locus with enhancer activity and unique, necessary functions [3]. These distinct CNSs are required for IFNγ expression at each stage of the differentiation pathway and they differ between immune-reactive cell populations. As such, cell-type selective expression of human IFNγ depends upon the noncoding segment of the genome indicating a tight and very complex mechanism of its activation and functioning. During infection, IFNγ is predominantly produced by natural killer (NK) cells but also arises from other specialized cells of the immune system (T cells, cytotoxic lymphocytes and antigen-presenting cells, such as monocyte/macrophage and dendritic cells). Its production is controlled by positive (interleukins IL-12 and IL-18) and negative (IL-4, IL-10, transforming growth factor-β) regulators secreted by antigen-presenting cells [4]. IFNγ signals through the IFNγ receptor (IFNGR) and further activation of the JAK signal transducer/STAT transcription activator pathway. However, IFNGR is expressed on most cells meaning that IFNγ can bind to nearly all cell types. The IFNγ related gene interaction network contains 1060 genes with 26,313 interactions among them. These genes are implicated in various immune mechanisms such as response to the extracellular stimulus, lymphocyte activation, and regulation of apoptosis [5].

A broad range of IFNγ host-defense mechanisms has been identified in many published studies. In summary, IFNγ promotes several aspects of immunity such as enhancing antigen presentation through class I and class II major histocompatibility complex molecules, cell trafficking, cell differentiation, stimulation of phagocytes, orchestration of leukocyte-endothelium interactions, effects on cell proliferation and apoptosis,

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as well as the stimulation and repression of a variety of genes [6]. It also directs regulation of the differentiation, activation, and homeostasis of T and NK cells, activation of the classic M1 subtype of inflammatory macrophages, and induction of cyto/chemokines for recruitment of specific effector cells to different inflammatory microenvironments [7]. It is considered as a key link between the innate and adaptive immune response [8] and as a master switch of a cytokine cascade that contains large numbers of separate molecules, each operating through different receptors [9].

While IFNγ plays a certain role in viral infections, this review provides a unique perspective on the complexity of its action, with emphasis on the SARS-CoV-2 virus, incorporating recent data related to the current epidemic from both basic science and the clinic.

2. IFNγ in viral infections

IFNγ function has been strongly conserved throughout evolution and across multiple species indicating its crucial role in immunity The structural and functional/biological conservation of IFNγ during speciation, indicates the resilience of a gene subjected to strong selective forces [10,11] but moreover, its importance as a first-line defense in the survival of species. As innate immunity arises as a consequence of synergistic evolutionary cohabitation between viruses and hosts, it is not unusual that in different pathophysiological states (autoimmunity, cancer, and microbial/viral infections) IFNγ performs the same biological actions. All of these conditions involve complex reactions of the host immune system. During viral infections, IFNs are involved in numerous immune interactions as inducers, regulators, and effectors of both innate and adaptive antiviral mechanisms [12]. The controversial role of IFNγ is reflected in part by the fact that it increases production of pro-inflammatory cytokines leading to clinical manifestations of disease while also helping clearance of the virus, by provoking defensive mechanisms through increased chemokine production important for infiltration of antibody-secreting B cells [13].

Several IFNγ-orchestrated mechanisms contribute to the host antiviral response. (i) IFNγ is a potent inducer of nitric oxide (NO) in surrounding cells, such as macrophages. NO is an important mediator in intracellular inhibition of viral replication, which results in lower viral load and more efficient host clearance of the infection [14]. NO also modulates local vascular reactions at inflammatory sites allowing increased extravasation of recruited immune cells to the site of infection and inflammation [15]. (ii) IFNγ has specific roles in the stimulation of innate immune responses through classical macrophage activation and release of reactive oxygen species [16], increased macrophage receptor-mediated phagocytosis, and promotion of polarization of macrophages to an M1 phenotype [17]. Maintaining a balance between M1 (classically activated, allegedly pro-inflammatory) and M2 (alternatively activated, allegedly anti-inflammatory) macrophage phenotypes influences the immune homeostasis in the host. (iii) IFNγ stimulates an adaptive response via enhancement of the antigen presentation process during T-cell priming [18]. IFNγ priming induces post-transcriptional and/or epigenetic changes, which are responsible for subsequent Toll-like receptor (TLR) and ligand-triggered inflammatory responses [19]. IFNγ-induced downstream transcriptional network changes result in extensive remodeling of the epigenome that further alters gene transcription, thus providing an attractive potential explanation for the priming and silencing effects that occur in a stable and gene-specific manner in IFNγ-polarized macrophages [20].

2.1. Role of IFNγ in immunomodulation

In normal conditions, the immunological response to infections is under tight control and capable of avoiding the undesirable effects of excessive activation. It is important to note that even suboptimal concentrations of IFNγ, which do not activate immune cells, make them prepared for subsequent response to stimuli and ensure a rapid

induction of the inflammatory response, as well as a state of refractoriness against viral proliferation in the surrounding tissues, as a part of important antiviral effector mechanisms [21]. Discrepancies and contradictions of numerous studies are a reflection of the complex role of IFNγ during various pathophysiological conditions. During the co-evolution of viruses and host-defensive mechanisms a variety of evasive adaptations arise that allow viruses to circumvent or inactivate host antiviral mechanisms. Such mechanisms are strikingly similar in the co-evolution of cancer and the host and it is therefore no suprise that the main IFNγ actions seen in cancer progression match those during virus–host cohabitation and consequent disease progression. As for malignant cells, viruses must survive and multiply in the host, overcoming innate and adaptive immune mechanisms. During this process, viruses have evolved multiple strategies to escape the IFN system such as inhibition of IFN synthesis and binding, inactivation of secreted IFN molecules, blocking IFN-activated signaling or disturbing the action of IFN-induced antiviral proteins [22]. The molecular mechanisms involved range from a broad shut-off of the host cell metabolism to fine-tuned elimination of key components of the IFN system or direct suppression of antiviral effector proteins of the IFN-induced antiviral state directed against them [23]. In addition, viruses are capable of producing specialized proteins with IFN-antagonistic functions or expressing virulence genes that target members of the IFN family or components of the JAK-STAT signaling pathway, adaptations directed toward potentiating a viral replication speed that surpasses the IFN response [24].

IFNγ is also associated with an interesting phenomenon called immunoediting that is typically linked to malignant cells. Cancer immunoediting (i.e. modulations of tumor immunogenicity) has three phases: elimination (immunosurveillance during which the innate and adaptive mechanisms cooperate to recognize and destroy any transformed cells), equilibrium (due to acquired capabilities some transformed cells survive the elimination phase but are prevented from outgrowth due to immunological checks and lay dormant under the influence of adaptive immunity), and escape (a final outgrowth of tumor cells that have surpassed immunological restraints of the equilibrium phase with consequent development of a clinically evident tumor) [25,26]. Hypothetically, if we put this phenomenon in the context of viral infections, a striking resemblance is imposed: "malignant cell dormancy" can be equated to "virus latency" while cancer immunoediting as modulation of tumor immunogenicity can be equated to modulation of virus immunogenicity. It could be considered like this: selection pressure exerted on the virus by the immune system (elimination and equilibrium) results in removal of highly immunogenic viruses and favors the generation of virus variants that exhibit reduced immunogenicity, which would render them "invisible" to the immune system. Such evolutionarily-selected viruses would be capable of evading the host immune system, thus increasing viral replication, and resulting in a clinical presentation (escape). The characteristics of the IFNγ-induced antiviral response (induction and action) could differ quantitatively if not qualitatively and are dependent on the specific combination of the three key components: the virus, the host cell, and IFNγ itself [27]. Moreover, different immunoregulatory events that influence the expression of some cytokines during and after viral infection could explain the complexity of disease pathology.

2.2. Role of IFNγ in immunotolerance

As a consequence of this immunoregulatory potential of IFNγ, another phenomenon arises within a host microenvironment that could influence the outcome of the virus-host relations. This refers to the development of immunotolerance. This is mainly observed in chronic pathogen/viral infections, where the balance exists between clinical disease and the excessive reaction of the immune system. Tolerance refers to the capacity of a host to limit the damage caused by both, immune-related infection-associated pathology, and the specific pathogen itself [28]. The most interesting mechanism of virus-host relation is

discovered in certain species (such as bats) which, instead of mounting a highly pro-inflammatory immune response, rely on mechanisms of tolerance as a peculiar form of evolutionary adaptation. Enhanced infection tolerance may be an antiviral defense strategy [29]. However, the interactions that reduce immune-related pathology are also tightly controlled with the same effectors. For example, earlier studies provide evidence that SARS is an innate immune-regulated disease. Although in many viral infections the IFNs act not only to control viral infections, but also to program the adaptive immune response to promote viral clearance, in patients with severe SARS disease, aberrant IFN, Interferon Stimulated Genes (ISGs), and cytokine responses were observed [30]. Antagonism of IFNs and IFN-stimulated gene expression could also be a crucial constituent of the immunotolerance mechanism during viral infections. As Crespi explained, from the evolutionary medicine point of view, this is a mismatched conflict, with dynamics and pathology driven by three main factors: (i) bat immune systems that rely on low inflammation but high efficacy of IFN-based defenses; (ii) viral tactics that differentially target the human interferon system leading to substantial asymptomatic and pre-symptomatic transmission; and (iii) high mortality caused by hyper-inflammatory phenotypes, that represent dysregulated tradeoffs whereby collateral immune-induced damage becomes systemic and severe [31]. In general, paradoxical and seemingly contradictory IFNγ effects are evident in various pathophysiological states. IFNγ actions are greatly dependent on the specific timeline of the immune response and cooperation with other factors of both the adaptive and innate immune system and opposing interplay with other cytokines [32].

Additionally, a possible role for host defensive strategies that are involved in achievement of virus-host cohabitation is supported by the theory of virus evolution within hosts. Co-evolution is often observed, when the virus and the host reciprocally affect each other's evolution, perpetually struggling to maintain a constant fitness level [33]. According to the Red Queen Hypothesis, the relentless struggle to seek evolutionary advantages drives perpetual cycles of adaptation and counter-adaptation [34]. As Lauring & Hodcroft assume, separating cause from consequence is important, meaning that sometimes a mutation is part of an adaptive mechanism that enhances one viral property (for example binding to a receptor) but that also reduces another property (such as escaping host defensive strategies) [35]. Still, they also point out that in most cases, the fate of newly-arising mutations crucial for virus adaptations is determined by natural selection. Those that confer a competitive advantage with respect to viral replication, transmission, or escape from immunity will increase in frequency, while those that reduce viral fitness tend to be culled from the population of circulating viruses. The influenza pandemic that emerged in 2009 (and spread in three temporally distinct waves between 2009 and 2011) provided an unprecedented opportunity to study adaptation of a virus. Notably, phylogenetic analysis of complete A(H1N1)pdm09 influenza virus genomes showed that new variants had accumulated several mutations in second- and third-wave viruses. *In vitro* studies on mouse and human cells showed that recombinant viruses in the third wave induced less interferon in infected mouse lungs and human cells, suggesting that changes to internal viral proteins, occurred during evolution of the third wave viruses and caused adaptation of virus for increased replication in human cells [36]. These adaptations, however, provided successful cohabitation between virus and the host that falls basically under the purview of immunotolerance. Regarding SARS-CoV-2, analysis of mutation patterns in 34 human SARS-CoV-2 isolates and a closely related RaTG13 virus isolated from *Rhinolophus affinis* (a horseshoe bat), showed that high frequency C > U transitions reflected virus adaptation processes in their hosts, and that SARS-CoV-2 could have been evolving for a relatively long period in humans following the transfer from animals before spreading worldwide [37].

3. Patterns of IFNγ action in influenza A virus infection

Previous IAV epidemics could inform us about IFNγ actions in seemingly similar infections. Lessons from such epidemics could be helpful for understanding mechanisms and directions of future therapeutic opportunities in similar epidemic diseases, including targeted manipulation of IFN signaling pathways. Infection with influenza viruses is usually self-limited, though the severe cases, especially those caused by highly virulent strains (e.g., the 2009 pandemic H1N1 virus, and the 'bird flu' viruses H5N1 and H7N9) are characterized by severe pulmonary disease and lethal acute respiratory distress syndrome (ARDS) that involves damage to the epithelial-endothelial barrier of the pulmonary alveolus, fluid leakage to the alveolar lumen, and respiratory insufficiency [38]. However, as in other diseases with similar clinical manifestations (although not etiology), progression of the disease is dependent on finely regulated antiviral immunity, which in a case of excessive immune response could cause damaging inflammation.

Virus infection often activates the interferon IFNγ-inducible gene NOS2 (Nitric oxid synthase) which, although limiting viral replication, may also contribute more significantly to the development of influenza pneumonitis, as observed in mice via suppression of another IFNγ-dependent antiviral mechanism. Genetically deficient NOS2−/− mice survived after infection with IAV, with little histopathologic evidence of pneumonitis, and they cleared IAV from their lungs by an IFNγ-dependent mechanism that was not evident in wild-type mice [39]. As this was an experimental model, it was concluded that during infection, IFNγ-inducible NOS2 probably displays all three of its major effects (antimicrobial, inflammatory, and immune-suppressive) but to variable degrees. IAV could subvert the antiviral host defense mediated by IFNγ through effects on the intracellular signaling pathways. Induction of pulmonary inflammation (associated with translocation of antigen from the lung to lymphatic tissue) is related to IFNγ [40]. Increased production of IFNγ is detected during the acute stages of illness in influenza A virus-infected individuals in the upper respiratory tract secretions and the serum [41]. Moreover, recent results suggested that IFNγ could play an important role in acute lung injury induced by severe influenza A (H1N1)pdm09 infection, and monoclonal antibodies against IFNγ could be useful as a potential therapeutics for future influenza pandemics [42]. Modeling such interference with IFNγ, by infection of mice lacking the IFNγ receptor (IFNγR-/-) at a dose that caused severe disease in wild-type 129 Sv/Ev mice, resulted in milder clinical symptoms and significantly lower lung virus titers, lower levels of inflammatory cytokines, and less infiltration of monocytes and macrophages than in wild-type mice [43]. The authors concluded that although lack of an IFNγ response is rare in humans, this may be particularly important if the infective dose is low, as may occur in a natural infection, and the virus must be rapidly amplified to establish an infection before secondary immune responses are activated. The lack of an IFNγ response leads to an attenuated IAV infection and early control of virus spread which is virus dose-dependent. According to Califano *et al.* influenza-induced IFNγ production contributes to increased susceptibility to influenza virus infection via suppression of ILC2 lymphoid cell group-mediated protection in the lung, and consequent restriction of the production of IL-5 [44]. Taken together, these data suggest that blocking IFNγ function could have therapeutic benefit, at least in influenza infections.

On the other hand, there are opposite findings that indicate that shaping the cellular and cytokine profiles in the early stages of infection may favorably change the fate of influenza pathogenesis (rapid clearing of the virus and reduction of inflammation). For example, sequential administration of IFNγ - and not its interference as described - above in the early stage after influenza infection protected mice from death in an NK cell-dependent manner. IFNγ treatment stimulated NK cell proliferation and function and increased their number in the bone marrow, blood, spleen, and infected lungs, keeping viral clearance intact. It significantly reduced the number of T cells and NKT cells in the lungs during the inflammatory phase following infection [45]. This may have

important implications for the development of improved IAV vaccines. In a study aimed at evaluating the phenotype and function of NK and T lymphocytes, before and following vaccination with the routinely administered trivalent IAV vaccine [46], the authors demonstrated increased innate and adaptive cellular immune responses and showed that NK cells are a significant source of IFNγ following IAV vaccination. An increase in the frequency of IFNγ-producing NK cells was observed in many subjects postvaccination. Understanding the precise mechanisms by which IAV interacts with the innate immune system, particularly in the context of low, physiological doses of the virus, will aid in designing new prophylactic and therapeutic strategies for the prevention and control of IAV infections. According to the previously mentioned IFNγ immunomodulatory role, an interesting possibility to consider is that the virus has found ways to exploit the IFNγ response to enhance infection as an integral part of viral pathogenesis.

3.1. Implications for SARS-CoV-2 infection

Contradictory findings related to IFNγ actions in IAV are indicators that could help in understanding its importance for SARS-CoV-2. A recent study uncovered the possibility of differences in the antiviral immune response between IAV and SARS-CoV-2 based on characteristic cytokine patterns in the circulation that have not been previously suspected [47]. Examination of temporal cytokine patterns in various patient groups (nonhospitalized versus hospitalized patients - both critically and noncritically ill) showed that nonhospitalized IAV patients with mild disease exhibited similar production of pro-inflammatory cytokines such as IFNγ (and TNF, IL-6, IL-7, IL-8) compared to either noncritically or critically ill hospitalized patients. On the contrary, all patient groups with SARS-CoV-2 produce pro-inflammatory cytokines such as IFNγ (and TNF, IL-6, IL-8, IL-10), with critically ill patients exhibiting also the significant tendency for higher IFNγ, consistent with the increased hyper-inflammatory state at specific time intervals. This reveals a major imbalance in the induction of antiviral and pro-inflammatory responses of patients with SARS-CoV-2 that does not occur in IAV. Perhaps the main difference between IAV and SARS-CoV-2 comes from the fact that the SARS-CoV-2 genome contains open reading frames (ORFs) that encode for accessory proteins important for the modulation of the host's infected cell metabolism and innate immunity evasion. Among those, ORF8 is a hypervariable gene rapidly evolving in SARS-related coronaviruses, with a tendency to recombine and undergo deletions that are deemed to facilitate virus adaptation to the human host [48]. The ORF8 gene encodes for the homonymous multifunctional, highly immunogenic, immunoglobulin-like protein that was recently found to inhibit the presentation of viral antigens by class I major histocompatibility complex, suppress the Type I IFN antiviral response, and interact with host factors involved in pulmonary inflammation and fibrogenesis [48]. Rapid mutations and evolution of the SARS-CoV-2 virus make ORF8 the important immune evasion virulence factor. ORF8 acts as a contributing factor to the cytokine storm during infection and an important inhibitor of the IFN system [49] and in that context it is not suprising that there is atypical, delayed induction of the host immune response to SARS-CoV-2 infection which favors virus replication in the absence of typical IFN responses and simultaneously increases chemokines induction [50].

4. Patterns of IFNγ action in SARS-CoV-2 virus infection

SARS-CoV-2 is the seventh zoonotic coronavirus known to infect humans, similar to the previous SARS-CoV and MERS-CoV. It causes severe acute respiratory syndrome and fatalities [51] and has had a massive impact on the world over the last year, far beyond the effects of those previous epidemics. Based on clinical criteria and available serological and molecular information, the new disease was called 'coronavirus disease of 2019′ (COVID-19), and the novel coronavirus was called 'SARS Coronavirus-2′ (SARS-CoV-2), emphasizing its close

relationship to the 2002 SARS virus (SARS-CoV). What is known so far is that following viral infection, the infected cells promote the secretion of large amounts of pro-inflammatory cytokines and this "cytokine storm" causes injury in lung epithelial and microvascular endothelial cells, ischemia, hypoxia, pneumonia, pulmonary fibrosis, systemic inflammation, hyperferritinemia, hemodynamic instability, and multi-organ failure at the clinical level [52]. The 2002–2003 SARS-CoV outbreak had higher pathogenicity and higher mortality rates, while SARS-CoV-2 infection appears to be much more contagious. Moreover, many SARS-CoV-2 infected patients are reported to develop low-titer neutralizing antibodies and usually suffer a prolonged illness, suggesting a more effective SARS-CoV-2 immune surveillance evasion than SARS-CoV [53].

Differences and similarities between earlier and current SARS-CoV viruses are becoming clear from *in vitro* and *in vivo* studies and in time the exact mechanism of disease progression and the role of key effectors of the host defense will be defined more precisely. However, some apparent discrepancies could be simple consequence of different study designs regarding *in vitro* and *in vivo* approaches. For example, earlier *in vitro* studies on SARS-CoV viruses suggested that IFNs could be effective as an exogenous modulator of virus reproduction. Treatment of Vero E6 cells with either IFNβ or IFNγ marginally reduces viral replication, while treatment with both significantly inhibited SARS-CoV plaque formation and viral replication [54]. A recent study found that only the combination of TNF-α and IFNγ induces a cytokine-mediated inflammatory cell death signaling pathway (via JAK/STAT1/IRF1 axis, inducing NO production and caspase-8/FADD) associated with acute lung damage and that treatment with neutralizing antibodies against TNF-α and IFNγ protected mice from mortality during SARS-CoV-2 infection [55]. Treatment of cells with IFNγ shows antiviral activity when administered immediately after SARS-CoV infection. The binding of recombinant SARS-CoV spike protein to these cells was diminished on cells treated with IFNγ (and IL-4) because of downregulation of cell surface expression of angiotensin-converting enzyme 2 (ACE2), the SARS-CoV receptor [56]. A recent *in vitro* study on airway epithelial cells revealed that ACE2 is a human IFN-stimulated gene and that IFNs can enhance the expression of host ACE2, implying that SARS-CoV-2 could exploit species-specific IFN-driven upregulation of ACE2, to enhance infection [57]. All IFN types inhibited SARS-CoV-2 replication in a dose-dependent manner, in both a human lung cell line model and primary human bronchial epithelial cells, but type-specific mechanisms or kinetics shape the IFN-enhanced ACE2 transcript and cell surface levels. IFNγ enhanced surface expression of ACE2, influencing indirectly the severity of the disease. An imbalance in IFNγ actions is reflected through the fact that antiviral action of IFNs against SARS-CoV-2 could be counterbalanced with proviral effects of ACE2 induction [58]. Virus infection in BALB/c mice (genetically susceptible to coronavirus retinopathy) was associated with increased levels of systemic IFNγ [59]. Moreover, IFNγ mRNA was detected within the retinas of infected animals during the acute phase of the disease at the time of T-cell infiltration, associated with the upregulation of MHC class I and II molecules and temporally related to the clearance of the infectious virus. In contrast to that, in IFNγ-deficient (IFNγ gko) mice, the virus was unchecked, and the infection was lethal. These results could have implications for the current pandemic since potential ocular manifestations of the new coronavirus disease such as retinopathy, viral conjunctivitis, immune conjunctivitis, and oculomotor palsies are described [60].

The occurrence of pulmonary fibrosis among SARS-CoV-2 patients is a serious complication of coronavirus infections and usually associated with specific cytokine profiles. IFNγ is often mentioned as a discriminatory factor in the occurrence of lung fibrosis. However, the results are contradictory. Circulating levels of IFNγ (together with IL-6 and IL-10) are increased in patients with a severe type of SARS-CoV-2 compared to those with mild disease [61]. Other studies revealed the inverse relationship, for example Hu *et al.* suggested that the risk of developing lung fibrosis is inversely associated with basal circulating IFNγ and that

decreased circulating IFNγ levels could be the main factor for the occurrence of lung fibrosis [62]. Such an inverse relationship between endogenous IFNγ levels and disease severity would indicate that treatment with IFNγ could be beneficial as prophylaxis and treatment of SARS-CoV-2 disease at the early stage of infection and perhaps substantial in the inhibition of fibrosis for better functional recovery. In a randomized controlled clinical trial, it was shown that IFN-α2b (Heberon Alpha R, CIGB, Havana, Cuba) and IFNγ (HeberFERON, CIGB, Havana, Cuba), in addition to standard antiviral therapy, demonstrate a favorable pharmacodynamic response of genes underlying antiviral activity that might be involved in host defense from a SARS-CoV-2 infection. Both treatments were safe, had a positive impact on viral clearance and the resolution of symptoms and no patients developed severe disease [63]. Concerning SARS-CoV antigen-specific cellular and humoral immune responses and therefore the potential persistence of SARS-CoV-2-specific memory T cells for long periods, a study was conducted involving SARS-recovered individuals several years after initial natural infection [64]. In their blood samples low levels of specific memory T cell responses to SARS-CoV S, M, E, and N peptides were detected, and IFNγ was the predominant cytokine. The majority of memory CD8(+) T cells produced IFNγ, whereas memory CD4(+) T cells produced IFNγ, IL-2, or TNF-alpha. Although it is expected that in the absence of antigen immune response gradually decreases, the persistence of stand-by defense effectors for several years is not negligible and could have an impact on the rational design of vaccines against SARS-CoV infections. Measuring immunological markers and correlating them with disease severity during follow-up of the disease brought out contradictory findings. Chen *et al.* showed a decrease in IFNγ production by CD4+ T cells which tended to be lower in severe compared to moderate SARS-CoV-2 cases, that could be caused by the fact that infection may affect primarily T lymphocytes, particularly CD4+ and CD8+ T cells resulting in a decrease in their numbers [65]. In contrast, evaluation of early inflammatory responses in the upper airway (by measuring IFNγ, TGF-β1, and RANTES) at the mRNA level in SARS-CoV-2 infected patients (asymptomatic and symptomatic) and controls revealed higher IFNγ expression in SARS-CoV-2 infected patients in comparison with controls and in increase in symptomatic patients [66]. In a prospective study by Gadotti *et al.* higher levels of IFNγ were related to a poorer prognosis. IFNγ was higher in the early stages of the disease but these levels were not sustained after ten days of symptoms [67]. In those patients with sustained IFNγ levels, the mortality increased. IFNγ increased over time in critically ill patients, but with decreased levels in moderate patients, higher levels of IFNγ were found in patients who died in contrast to the survival group. These results suggest that the immune responses (increase in inflammatory mediators) developed by the host at the beginning of infection may have been one of the causes for the moderate to severe progression of the disease. Regarding the role of IFNγ, this involves the complex interplay between the capacity of NK and NKT cells to produce IFNγ (before the specific Th1 adaptive immune response), the possibility of blocking IFNγ gene transcription by other inflammatory cytokines, IFNγ-driven differentiation of T cells to the Th1 profile, or IFNγ-driven inhibition of the proliferation of Th2 cells. This implies that some kind of control of IFNγ production with combined therapies could be crucial for future clinical treatment of SARS-CoV-2 infection.

With so many contradictory findings it is difficult to understand the potential of endogenous levels of IFNγ for disease monitoring or its exogenous administration as a treatment option. So far though, it is clear that the molecular mechanism behind disease progression (and hence, severity of disease) depends at least partly on activation of IFNγ (and other IFNs) during infection. Its effect could differ (as pointed out earlier) and depend on prevailing mechanisms, varying on a scale between a more immunomodulatory role to maintenance of immunotolerance. A simple explanation for these discrepancies was postulated by Yang *et al.* [68]. The first task of the host immune system is inhibition of viral replication through the stimulation of IFNγ-stimulated genes. Failure to achieve an IFNγ response leads to suboptimal activation of the adaptive immune response and increased viral load. The increased viral load causes severe tissue damage, inducing a late wave of IFNs and an exacerbated inflammatory response. Taken together, an early deficient IFN response and the following hyper-inflammatory state are the hallmarks of severe SARS-CoV-2 infection. The authors therefore suggested that IFN-based therapy could potentially be beneficial as prophylaxis and treatment at the early stage of infection. The picture, however, appears more complicated because a new study has revealed an increase in IFNγ (and IL-2) production in asymptomatic compared to symptomatic individuals after activation of SARS-CoV-2-specific T cells in the blood [69]. This was associated with a proportional secretion of IL-10 and pro-inflammatory cytokines (IL-6, TNF-α, and IL-1β) only in asymptomatic infection, while a disproportionate secretion of inflammatory cytokines was triggered by SARS-CoV-2-specific T cell activation in symptomatic individuals. Thus, they concluded that in the case of SARS-CoV-2, asymptomatic individuals are not characterized by a weak antiviral immunity; on the contrary, they mount a robust and highly functional virus-specific cellular immune response that protects the host without causing any apparent pathology. This is the exact mechanism described as immunotolerance during virus-host adaptation. In a study that investigated the immune response of patients with long-term SARS-CoV-2 infections extending to 50 days from onset, clinically cured with an absence of related symptoms, it was shown that decreased IFNγ production by NK cells and low neutralizing antibodies might favor SARS-CoV-2 long-term existence together with low viral load and weak viral pathogenicity [70]. Accordingly, NK cells may contribute to SARS-CoV-2 clearance, and the decrease in IFNγ-expressing NK cells might delay viral clearance in these patients favoring viral persistence for a long time in a latent form. However, only continual measurement of the IFNγ levels (and other cytokines) during the whole course of the disease, in different patients groups, could provide insight when approximately "too low" becomes "too much" and vice versa.

It is also unknown if the severity of the disease is a consequence of a dysfunctional immune response or virus evasive actions against the host. Possible causes for the dysfunctional immune response are polymorphisms of cytokine genes (such as IFNγ). In a study by Chong et al. the IFNγ + 874A allele was significantly associated with SARS-CoV infection [71]. This polymorphism was suggested as a genetic risk factor for SARS-CoV associated with susceptibility to this infection in a dose-dependent manner. The mechanism by which the IFNγ + 874A/T allele influences the susceptibility to SARS may depend on its role in the regulation of IFNγ production. The T allele of IFNγ + 874A/T provides a binding site for the transcription factor nuclear factor-κB (NF-κB), which can regulate IFNγ expression. On the other hand, recent *in vitro* evidence suggests that SARS-CoV-2 can be more efficiently antagonized by IFNs than SARS-CoV-1 and that there is a difference in IFN susceptibility between SARS-CoV-2 and SARS-CoV-1. Systematic analysis of the impact of 29 SARS-CoV-2 encoded proteins on the major branches of the cell-intrinsic innate immune system such as IFN induction, IFN/pro-inflammatory cytokine signaling, and autophagy, revealed that IFNγ (and IFNλ1) pathways are antagonized the least, and consequently treatment with these two cytokines is most potent against SARS-CoV-2 [72]. According to the authors, these results provide a plausible explanation for why SARS-CoV-2 is more susceptible to IFN treatment than SARS-CoV-1 and indicate that a combination of IFNγ and IFNλ1 is an effective anti-SARS-CoV-2 approach. In combination, IFNγ and -λ act synergistically and drastically reduce SARS-CoV-2 replication at exceedingly low doses in infected cells.

5. Conclusion and therapeutic implications

Since the initial discovery of IFNγ as an antiviral macromolecule, its immunomodulatory potential has been much investigated in different pathophysiological states, such as cancer, autoimmune diseases and infections. IFNγ triggers multifaceted inflammatory responses that are uniquely universal in these pathophysiological conditions. Based on

current knowledge, it seems to be an important endogenous regulator of immune responses although it is still not recognized as a universal biomarker, nor has its potential as an exogenous immunomodulatory factor been realized. IFNγ clinical trials using recombinant derived protein, adenovirus vectors that express IFNγ cDNA or neutralizing antibodies against IFNγ have been conducted for the treatment of tuberculosis, hepatitis, chronic granulomatous disease, osteoporosis, scleroderma, cutaneous lymphoma, malignant melanoma, and autoimmune diseases [73]. There are now approved recombinant IFNγ therapeutics in use for some conditions and diseases, such as long- and/or short-term prophylaxis in T cell-deficient states or immune suppressed patients, in patients with a reversible host defense defect or immune response suppression, as adjunctive treatment along with conventional antibiotic therapy, opportunistic infections in general, or for infections that typically respond poorly and require prolonged therapy [74]. Typically, there are tolerable side effects. These agents have not however, been widely indicated as prophylactic or treatment options for the recent pandemic and/or epidemic respiratory syndromes (the current SARS-CoV-2 infection, ongoing Influenza A virus (H1N1)pdm09 and earlier SARS, and MERS infections).

The safety of IFNγ has already been demonstrated but its efficacy needs to be confirmed. Encouragingly, the prophylactic effect of IFNγ intranasal administration was demonstrated in high-risk volunteers: medical workers and personnel in the SARS-CoV-2 "red zones" [75]. As there are clinical protocols available for dosage and treatment regimens of IFNγ-based therapeutics, their use in virus pandemics has been suggested particularly for at-risk, vulnerable patients [76]. In a recent open-label, randomized, study that included patients with moderate SARS-CoV-2 infection, addition of IFNγ to complex therapy resulted in more favorable changes in the stabilization of patient's vital signs, as well as in reduced length of fever and hospital stay [77]. More important, patients who received recombinant IFNγ experienced no progression of respiratory failure and required no transfer to intensive care unit. The advantages of administering IFNγ are its efficacy against viral infections in general (not specific for particular viruses), safety (with minimal side effects), and the availability of clinical data, clinical protocols, and therapeutics [78]. In the context of the current pandemic, these advantages should not be underestimated. Indeed, we agree that its use should be seriously considered and even recommended.

The understanding of IFNγ actions - whether immunomodulation and immunotolerance are independent or overlapping phenomena - is more complicated by the fact that during disease progression, there is a time-dependent subtle balance of immunity cascades when "less is more" and/or vice versa. A broader approach in perception is needed especially in the context of the recent pandemic if researchers want to define the specific roles of each particular performer in virus-host interplay, a show in which IFNγ deserves a "highly ranked" position.

CRediT authorship contribution statement

Nataša Todorović-Raković: Writing - original draft. **Jonathan R. Whitfield:** Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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