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New Insights into Type I Interferon and the Immunopathogenesis of Persistent Viral Infections

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Abstract

Most viruses generate potent T cell responses that rapidly control infection. However, certain viruses can subvert the immune response to establish persistent infections. The inability to clear virus induces an immunosuppressive program leading to the sustained expression of many immunoregulatory molecules that down-regulate T cell responses. Further, viral persistence is associated with multiple immune dysfunctions including lymphoid disorganization, defective antigen presentation, aberrant B cell responses and hypergammaglobulinemia. Although best known for its antiviral activity, recent data has highlighted the role of type I IFN (IFN-I) signaling as a central mediator of immunosuppression during viral persistence. It is also becoming increasingly apparent that many of the immune dysfunctions during persistent virus infection can be attributed directly or indirectly to the effects of chronic IFN-I signaling. This review explores the increasingly complex role of IFN-I in the regulation of immunity against persistently replicating virus infections and examines current and potential uses of IFN-I and blockade of IFN-I signaling to dampen chronic inflammation and activation in the clinic.

IFN-I Production and Signaling

IFN-Is, which comprise multiple subtypes of IFN α and a single IFN β , are central components of the host's antiviral response. IFN-Is are rapidly induced by viral infection through pattern recognition receptors [PRRs; e.g., toll-like receptors (TLRs)] and intracellular proteins that recognize direct cellular infection (1). There is considerable controversy as to which of the molecular recognition pathways and cell types are the dominant IFN-I producers in persistent infections (2-6), and likely many combine to produce the IFN-I induced effects observed. In general, plasmacytoid dendritic cells (pDCs) are considered the dominant early IFN-I producer, although TLR signaling pathways in pDCs can become refractory to stimulation shortly after infection (7). Conventional DCs and macrophages are now also being acknowledged for their IFN-I production throughout

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persistent infection (5). Whether they too become refractory to TLR stimulation is not known, but residual TLR activity can be induced experimentally (4) and it may be sufficient to induce biologically relevant levels of IFN-I. In addition, infection of non-immune cells can also augment the level and composition of IFN α/β , further regulating the antiviral immune response.

Not only are IFN-Is a critical component of the innate antiviral response, but they also have important immune modulating capacity and are associated with chronic inflammation in many virus-induced disease states (1), indicating the broad functionality of the IFN-I system. Although the entire IFN-I cytokine family signals through a single dimeric receptor (IFNAR), there is evidence that individual subtypes of IFN-I can differentially interact with the IFNAR1 or IFNAR2 subunits of the receptor, potentially resulting in their unique downstream effects (8, 9). Upon binding its receptor, “classical” IFN-I signaling triggers Janus kinase 1 (JAK1) and tyrosine kinase 2 (TYK2) activation, followed by recruitment and phosphorylation of signal transducer and activator of transcription (STAT)1 and STAT2 transcription factors. Together with interferon regulatory factor (IRF)9, STAT1 and STAT2 form the IFN stimulated gene factor 3 (ISGF3) complex that activates hundreds of IFN-stimulated genes (ISGs), many directly antiviral, that together make up what is termed the “IFN-I signature” (10). Yet, IFN-I signaling is highly complex, and in addition to this classical pathway, IFN-I can elicit a multitude of different signaling pathways (10). The determination of the specific pathways induced is dependent upon the particular cell type stimulated, its differentiation state and differential expression of signaling molecules, the timing within infection, and the co-modulation of signaling by other stimuli and cellular interactions within the immune environment (11). Further, IFN- γ -derived signaling can overlap with certain IFN-I pathways, feeding into and enhancing their effects (10). Together, this diversity in signaling leads to an incredibly vast array of temporal and contextually different biological outcomes, encompassing antiviral, immune stimulatory and immunosuppressive effects.

The Contrasting (Yet Simultaneous) Antiviral and Immunosuppressive Roles of IFN-I

Using the LCMV system, our group and Michael Oldstone's group recently identified IFN-I signaling as a sensor translating viral replication kinetics (i.e., clearance or persistence) into immunologic outcomes (12, 13). Serum IFN-I levels peak 1-2 day after LCMV infection and then decline (5, 13) and are required to limit viral replication of both acute LCMV-Armstrong (Arm) and persistent LCMV-Clone13 (Cl13) (14) as well as other virus infections (15-17). Although it has been postulated that the inability to initially resolve LCMV-Cl13 is due to diminished IFN-I production at the onset of infection, in most studies early IFN-I levels are as high or higher in persistent infection as they are in acute LCMV infection (7, 13). However, serum IFN- α levels may decline more quickly in persistent LCMV infection (5), or more specifically, extended IFN-I production beyond the initial burst may be required for more aggressive virus infections. In this vein, recent data deleting OASL1, a negative regulator of IFN-I signaling, suggest that sustained IFN-I signaling may be beneficial to control what will become a persistent infection (18). Similarly, early

administration of IFN- α beginning at the time when serum levels normally decrease was effective to control persistent LCMV infection, whereas administration after a week of infection had no noted effects (5). Therefore, prolonging IFN-I signaling within a designated timeframe early in infection against viruses prone to persistence (or perhaps not allowing for the decrease in IFN-I signaling after the initial burst of infection) may help to enhance viral clearance.

Although the ability to measure IFN-I proteins wanes after the first few days of infection, viral persistence promotes IFN-I signaling as evidenced by the sustained IFN-I signature and the ability to abolish this signature and increase virus replication by blocking IFNAR signaling (12, 13, 19, 20). How a vastly decreased level of IFN-I that is undetectable in the serum can have such a substantial effect on the immune response remains unknown. It is possible that the cells become sensitized to IFN-I and require less to maintain effective signaling. Alternatively, IFN-I may function at high concentrations in localized niches or directly between cells at the site of infection. Regardless of how IFN-I mediates its effects, blockade of IFNAR in the LCMV system demonstrated that sustained IFN-I signaling in persistent virus infection drives expression of the immunosuppressive factors PDL-1 and IL-10. In addition, chronic IFN-I signaling was responsible for many of the immunologic dysfunctions associated with persistent virus infections including lymphoid disruption and the suppression of antiviral CD4 T cell responses (12, 13) and (Figure 1). Although blockade of IFNAR at the onset or during an established persistent LCMV infection generated an initial increase in viral titers (due to the loss of IFN-I's antiviral effects), once removed, the blockade ultimately led to enhanced viral clearance (12, 13). The mechanism by which this is achieved is incompletely defined, but is dependent on CD4 T cells and IFN- γ (12, 13). Interestingly, many of these dysfunctions (e.g., regulation of IFN- γ , suppression of CD4 T cells) can be directly linked to the effects of suppressive factors IL-10 and PDL-1, suggesting their role as an important mechanism stimulated by IFN-I to facilitate persistent LCMV infection. Thus, these data suggest that IFN-I is a central component of an immunologic surveillance system through which the host "senses" and translates virus replication dynamics (i.e., whether the virus or the immune response is winning the battle) into distinct immunologic outcomes.

IFN-I Mediated Control of the Immune Response during Viral Persistence

Targeting of APCs to alleviate immunosuppression

Simultaneously, but in contrast with its antiviral and immune stimulatory functions, chronic IFN-I signaling also induces many of the immunosuppressive mechanisms known to inhibit antiviral immunity during viral persistence. IFN-I enhances DC and macrophage expression of IL-10 and PDL-1 (12, 13, 21, 22) generating specific subsets of immunoregulatory DCs and macrophages with concentrated expression of many suppressive factors (including PDL-2 and indoleamine 2,3 dioxygenase; IDO) that can inhibit T cell responses (23, 24). However, it is still unknown whether IFN-I induces the generation of these suppressive DC and macrophage subsets as a distinct differentiation program or whether IFN-I acts on stimulatory DCs and macrophages to endow them with suppressive capacity. In the case of the former, it will be important to determine the IFN-I receptive DC/macrophage precursor

and in the case of the latter, it will be interesting to determine why IFN-I only acts on specific groups of DCs and macrophages and whether this is a result of differential IFNAR expression and regulation. Interestingly, in the first 48 hours after infection with persistent LCMV-C113, IFN-I can act on monocytes to promote increased engulfment of apoptotic red blood cells (hemophagocytosis) that induces release of IL-10 from these cells (25). Whether these mechanisms are sustained after the initial burst of IFN-I production leading to long-term IL-10 production or the expression of other suppressive factors remains to be determined. In addition to promoting APCs with suppressive activity, IFN-I during viral persistence also blocks new development/expansion of conventional stimulatory DCs from the bone marrow (26), thus tipping the balance toward a suppressive antigen presenting cell environment (Figure 1).

IFN-I induced IL-10 production by DCs and macrophages has been observed in multiple systems and chronic diseases (26-30) and a link between IFN-I and PDL-1 upregulation has also been established (27-29), indicating it as a conserved mechanism underlying immunosuppression during viral persistence. Consistent with this notion, ongoing IFN-I production in mouse models of persistent *Mycobacterium tuberculosis* (*Mtb*) infection is linked to emergence of IL-10 expressing macrophages and DCs that limit *Mtb*-specific immunity (30, 31). In addition, a population of macrophages was recently identified in persistent LCMV infection resembling the myeloid derived suppressor cell (MDSC) observed in cancer (32). These MDSC-like cells did not express IL-10 or PDL-1 and although they possessed *in vitro* suppressive activity their deletion *in vivo* had minimal effect on the antiviral immune response or virus control. However, this does indicate that multiple different types of potentially suppressive APC populations are generated in response to persistent virus infections with cellular and mechanistic parallels to those observed in other chronic diseases. It will be interesting to determine if the suppressive DC and macrophage subsets that emerge in persistent LCMV infection share common mechanisms of generation and origin across distinct persistent virus and *Mtb* infections, and whether these cells are a hallmark of chronic inflammation induced by IFN-I across other tumor models and diseases. It is enticing to speculate that targeting and killing these specific APCs could lead to a simultaneous decrease in many suppressive factors (while maintaining the stimulatory APCs that enhance T cell activity). Ultimately, many questions remain as to how IFN-I leads to the induction of immunosuppression and globally regulates the antiviral immune response during viral persistence (See Figure 1 legend).

Multiple direct and indirect effects of IFN-I on T cell activation, differentiation and exhaustion

The generation and maintenance of robust T cell immunity is crucial to counter viral persistence, and IFN-Is play a key role, both directly and indirectly, in the regulation of these responses. Upon activation, virus-specific T cells require direct IFN-I signaling for maximal expansion and survival, (33-36) although other survival factors can partially compensate in certain viral models (36). Absence of IFNAR specifically on LCMV-specific CD8 T cells leads to an almost complete loss of these cells (34). Interestingly, anti-IFNAR antibody blockade at the onset of persistent LCMV infection (i.e., such that all IFNAR signaling is abolished in all cells systemically) leads to unchanged or only slightly

diminished virus-specific CD8 T cell responses, despite an overall increase in CD8 T cell numbers (12, 13). Thus, virus-specific CD8 T cells require IFNAR signaling for optimal expansion/survival, particularly when in competition with cells able to receive IFNAR signals, but are capable of generating nearly maximal responses during persistent LCMV infection when all cells lack IFNAR signaling. On the other hand, what mediates the increase in non-virus-specific CD8 T cell numbers when IFNAR is blocked systemically is unclear, but may be due to a lack of early IFN-I induced attrition of non-specific T cells (37). Further, how IFN-I continues to modulate virus-specific CD8 T cells throughout persistent infection remains to be determined; however, the fact that exhausted virus-specific CD8 T cells maintain a high IFN-I signature suggests active regulation (19, 38, 39).

Sustained and properly directed CD4 T cell responses are a strong correlate of control of persistent viral infections (40), and IFN-I shapes both the magnitude and type of CD4 T cell help generated. In contrast to CD8 T cell responses, anti-IFNAR blockade at the onset of persistent LCMV infection increases total virus-specific CD4 T cell numbers (12, 13, 35). This IFN-I mediated CD4 T cell repression is an indirect effect, and like CD8 T cells, when only virus-specific CD4 T cells lack IFNAR they exhibit severe defects in expansion (35). Therefore, the accumulation of virus-specific CD4 T cells when IFN-I signaling is systemically abolished may be due to the modulation of APC function, loss of suppressive APC subsets and/or a consequence of enhanced cellular interactions resulting from maintenance of lymphoid architecture upon IFN-I blockade. Recent evidence indicates that IL-27 is critical for maintaining virus-specific CD4 T cells during persistent LCMV infection (41). Since IL-27 can be induced by IFN-I in APCs (42), it is possible that by acting on APCs, IFN-Is functionally suppress virus-specific CD4 T cells via production of IL-10, and PDL-1, while also maintaining their physical presence through IL-27 elicitation.

Like CD8 T cells, CD4 T cells exhibit an increased IFN-I signature at the onset and throughout persistent infection (12, 19, 20). Yet, how IFN-I regulates CD4 T cell differentiation is complex and appears to be dependent on the stage of viral infection when the cells are primed (35). Persistent viral infections primarily induce virus-specific CD4 T cell help in the form of Th1 cells (critical for sustaining residual CD8 T cell immunity) and Tfh cells (crucial for promoting B cell immunity and antiviral antibody responses) (40). Interestingly, anti-IFNAR blockade at the onset of persistent LCMV infection leads to increased Tfh differentiation and responses (35, 43), but sustained overall Th1 numbers (35). In stark contrast, chronic IFN-I signaling severely inhibits *de novo* virus-specific Th1 differentiation when virus-specific CD4 T cells are primed in the midst of an established persistent LCMV infection, whereas *de novo* priming of Tfh cells is not affected (35). This IFN-I mediated Th1 suppression is independent of IFN-I signaling on T cells (35), and although the mechanism remains to be determined, it may occur through modulation of DC function. The decreased capacity to generate new CD4 Th1 cells after viral persistence is established could have important implications for overall immune direction, the ability to balance attrition and to counter virus escape mutants. Not only is *de novo* Th1 priming suppressed, but Th1 responses initially primed and supported at the onset of persistent infection are also gradually lost and converted to Tfh upon viral persistence (44), leading to a narrowing of the CD4 T helper cell subsets present as persistent infection progresses.

Similar to the sustained IFN-I signature in SIV, HIV and HCV, an accumulation of Tfh cells in these persistent infections has also been reported (45-47). Although the humoral responses promoted by Tfh cells are critical for virus control (44, 48), the combined loss of pre-established, as well as *de novo* primed Th1 responses upon viral persistence could further diminish adequate help to CD8 T cells, contributing to their exhaustion and the inability to control virus. Thus, IFN-I signaling throughout viral persistence likely plays an important role in both sustaining as well as regulating differentiation of the antiviral T cell response.

In addition to effects on viral-specific T cells, chronic IFN-I signaling also elicits bystander activation in many persistent viral infections, and is thought to be a major driver of the elevated nonspecific T cell activation, apoptosis and senescence characteristic of HIV infection. Although likely due to multiple mechanisms, IFN-I induced upregulation of TRAIL (TNF-related apoptosis inducing ligand) on monocytes and pDCs can cause the activation induced death of bystander uninfected T cells which express TRAIL's receptor DR5 (49). Recently, abortive HIV infection in CD4 T cells has been linked to caspase-1 mediated pyroptotic death (50). As pyroptosis is highly inflammatory, this then perpetuates a cycle where enhanced inflammation promotes further CD4 decline (50). Ultimately, understanding the direct and indirect, positive and negative regulation of CD4 and CD8 T cells by IFN-I will provide critical biologic insight into the mechanisms and factors involved in their differentiation, as well as potential novel strategies to modulate T cell function to fight infection.

New Insights into Chronic IFN-I signals in HIV infection

Chronic IFN-I signaling, as well as increased expression of IL-10 and PDL-1 is conserved amongst persistently viremic infections (HIV, HCV, HBV, SIV, LCMV), (40) suggesting that a similar IFN-I mediated mechanism of suppression may also diminish immunity to these infections. In humans and animal models of HIV, elevated IFN-I signaling correlates with worsened disease, independent of viral load (51, 52). As a result, chronic IFN-I signaling has emerged as a prime suspect in driving HIV disease progression. Interestingly, an engineered high affinity IFN- α 2 mutant (IFN-1ant) (9) used for short-term IFN-I blockade at the time of SIV infection diminished some of the chronic immune activation associated with infection, but led to increased virus replication (due to the loss of IFN-I induced antiviral control) and accelerated AIDS progression (53). Although in this study the effect on suppressive factors was not measured, similar to the LCMV system, these data indicate that IFN-I simultaneously regulates the negative (non-specific immune activation) and the positive (antiviral) aspects of SIV infection. However, and as a cautionary note, in the case of SIV the positive effects of decreased immune activation were outweighed by the loss of antiviral activity, leading to increased virus replication and enhanced CD4 T cell death.

Combination antiretroviral therapy (cART) decreases HIV infection and IFN-I levels, however, often a degree of inflammation remains, particularly in patients whose initial CD4 numbers were lower or where cART is less effective (54). Although cART is highly effective, the ongoing smoldering inflammation is thought to underlie some of the non-

resolving non-specific T cell and B cell activation, apoptosis and senescence, correlating with progressive blood-based conditions (54, 55). Although the exact role of IFN-I toward the continued inflammation in cART treated patients is unclear (56), its continued presence suggests that it may potentiate this inflammation (54). Thus, understanding the mechanisms underlying chronic IFN-I induction, signaling and consequent immune activation will enhance our understanding of how HIV causes disease and could lead to targeted therapies to halt disease progression.

Therapeutic Uses of IFN-I or anti-IFN-I Blockade to Treat Persistent Viral Infections

IFN-I, in combination with ribavirin, is a key component of current anti-HCV therapies (57). However, in instances when this therapy is not effective, although likely for many reasons, a common thread is a high IFN-I signature prior to treatment (58, 59). As in other persistent infections, a link between the level of IFN-I, IL-10/PDL-1 expression and immune exhaustion was recently demonstrated in HCV infection (60), suggesting a potential mechanism for this treatment non-responsiveness. Thus, efficacy of IFN-I treatment may in part be related to pre-existing IFN-I levels, with chronic signaling de-sensitizing downstream pathways and diminishing or nullifying further IFN-I effects. A recent study suggested this idea, demonstrating that administration of pegylated IFN- α 2a to several HIV patients whose viral loads were controlled by combination antiretroviral therapy (cART) allowed prolonged time to viral rebound after cART interruption (61). These data suggest that IFN-I may be more potent in combination with effective cART that reduces viral loads and thus, down-regulates the IFN-I signature. It is possible, therefore, that in patients where cART is less effective, or where abnormal levels of immune activation and inflammation remain, IFN-I treatment would be less potent.

Therapeutically blocking IFN-I signaling has been proposed as a strategy to halt chronic inflammation, restore immune competence and fight HIV infection. However, the beneficial effects of loss of IFN-I mediated immunosuppression and chronic activation must be weighed against the deleterious effects of the loss of its potent antiviral activity. It is becoming a common misconception that IFN-I only elicits antiviral function at the beginning of viral infection, subsequently giving way to immunosuppressive activity. Although changes in the balance between antiviral, immune stimulatory and immunosuppressive functions may occur as persistent infection progresses, mounting evidence clearly demonstrates that IFN-I continues to provide critical antiviral function throughout persistent infection (12, 13, 54, 62). In fact, the antiviral effects of IFN-I may be crucial for limiting the re-emergence of persistent viruses in response to the anti-inflammatory and immunosuppressive effects of IFN β therapy in multiple sclerosis patients.

Critical clinical questions that remain are the potential differences in IFN-I mediated functions induced by distinct IFN α subtypes and IFN β ; how different intracellular signaling pathways and STAT usage may contribute to distinct IFN-I mediated effects; as well as how varying levels of IFN-I expression (regardless of subtype) quantitatively alter the type of response driven by IFN-I (e.g., antiviral at low concentrations, antiviral plus immunosuppressive at higher levels) (11). Since the antiviral activity of IFN-I is critical

throughout infection, strategies to uncouple the antiviral and the suppressive mechanisms of IFN-I signaling (if possible) would be an important biologic and therapeutic advance to specifically ablate the negative while maintaining the critical positive IFN-I functions.

Conclusions

It is becoming increasingly apparent that in addition to its critical ongoing antiviral activity, prolonged IFN-I signaling in persistent viral infection can elicit immunosuppressive effects and chronic immune activation leading to disease progression. While the use of IFN-I as an antiviral agent is well established, IFN-I blockade is currently under investigation as a mechanism to dampen inflammation and restore immune responses. However, it is critically important that these effects be weighed with the simultaneous loss of IFN-I's antiviral potency. Uncovering mechanisms to uncouple these opposing positive and negative arms of IFN-I mediated functions will be invaluable for exploiting the IFN-I pathway to treat persistent viral infections and other chronic diseases.

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Box 1. Important future questions

1. How does initial and/or chronic IFN-I signaling potentiate viral persistence? What are the critical immunologic processes and cellular mechanisms restored by blocking IFN-I that facilitate control of persistent infection?
2. How do different levels and compositions of IFN α and IFN β contribute to distinct global and cell-specific IFN-I mediated effects? Are distinct pathways utilized to induce antiviral, immune stimulatory and immune suppressive functions of IFN-I; is there a threshold effect that drives the distinct outcomes and/or is there a temporal effect in how cells respond to the same IFN-Is at different times?
3. How does IFN-I drive expression of immunosuppressive factors (e.g., PDL-1 and IL-10) and cell types? How does chronic IFN-I continue to regulate CD4 and CD8 T cell responses throughout persistent viral infection and what is the relationship with the enhanced IFN- γ that enables virus control?
4. What is the actual role of chronic IFN-I signaling toward the smoldering inflammation and non-specific immune activation in HIV infection?
5. What are the mechanisms by which chronic IFN-I signaling interferes with the antiviral effects of IFN-I treatment, and can these be overcome? Can initially lowering the IFN-I signature in patients improve the treatment efficacy of IFN-I?
6. Does chronic IFN-I signaling induce conserved pathogenic (and beneficial) effects and biologic outcomes across disease states or is the effect of IFN-I disease specific?
7. Based on the critical antiviral role of IFN-Is, will it be possible to uncouple IFN-I signaling to modulate its specific functions and maintain its critical antiviral and immune stimulatory actions, while inhibiting its immunosuppressive mechanisms?

Highlights

1. IFN-I simultaneously drive critical antiviral and immunomodulatory functions during viral persistence
2. IFN-I drives many of the immune dysfunctions associated with persistent virus infection
3. Blocking IFN-I in a mouse model enhanced control persistent virus infection
4. Uncoupling distinct IFN-I functions could help treat multiple disease states

Figure 1. Broad spectrum immune modulation and antiviral activity mediated by IFN-I during viral persistence

IFN-I production has widespread modulatory effects during acute and persistent virus infections. During viral persistence, the antiviral effects of IFN-I are critical to maintain control of infection and loss of this control leads to enhanced virus replication. T cells require IFN-I signals for their initial activation/differentiation and continue to demonstrate a high IFN-I signature throughout persistent infection, suggesting that ongoing IFN-I signals are important for regulating their responsiveness (although the positive survival signals are likely balanced by the chronic immune activation through IFN-I). Simultaneously, but in contrast with its antiviral and immune stimulatory functions, chronic IFN-I signaling also induces many of the immunosuppressive mechanisms known to inhibit antiviral immunity during viral persistence. IFN-I enhances dendritic cell and macrophage expression of IL-10 and PDL-1 while blocking new development/expansion of conventional stimulatory DC from the bone marrow, thus tipping the balance toward a suppressive antigen presenting cell environment. However, despite what we know, many questions remain: ?1) what is the relationship between virus replication and induction of IFN-I throughout viral persistence and what are the molecular recognition pathways involved. ?2) IFN-I signals lead to enhanced IL-10 and PDL-1 expression from a specific subset of DCs and macrophages, but it is unclear whether IFN-I specifically leads to the genesis of these cells that as part of their program have suppressive activity or whether IFN-I acts upon existing DCs/macrophages to endow them with suppressive activity. In the case of the latter, then it will be important to understand what leads to the induction of these cells and why are only subsets of cells acted upon to produce suppressive factors? ?3) How does IFN-I prevent the differentiation of new stimulatory DCs during viral persistence and is this linked to the induction of the suppressive subsets? ?4) What are the direct effects of IFN-I during viral persistence and

how does modulation of these signals lead to changes in T cell differentiation and function. Answering these questions will ultimately provide insight into how IFN-I positively and negatively regulates the immune response to both control and potentiate viral persistence.

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