

## Whence Interferon? Variety in the Production of Interferon in Response to Viral Infection

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The type I IFNs (IFN- $\alpha$  and IFN- $\beta$ ) were first characterized as cytokines capable of inducing an antiviral state in sensitive target cells (1). They were originally classified as leukocyte IFN and fibroblast IFN, respectively, to designate their distinct presumptive cellular origins. This designation has been replaced by a more precise nomenclature, based on molecular characterization after the isolation, cloning, and sequencing of the IFN multigene family. The originally detected IFN- $\alpha$  activity is encoded by a multigene family of closely related and clustered genes, while IFN- $\beta$  is encoded by a single, somewhat more distantly related gene. It has also become clear that type I IFNs can be synthesized by many, if not all, nucleated cells, just as virtually all nucleated cells have the capacity to respond to secreted IFN to induce an antiviral state. This ability of most cells to secrete and respond to IFN makes the IFN system a powerful first line of defense against pathogens and an essential component of innate immunity (2, 3).

IFNs induce more than just antiviral functions, also possessing potent immunomodulatory activities (4). These immunomodulatory effects allow IFNs to serve as a link between innate and adaptive immunity. There is also increasing evidence that they function in a constitutive mode to maintain homeostasis, particularly in the hematopoietic system (5–7). This latter function appears to be a priming effect that maintains target cells in a state of readiness (8). The response to continuous, low-level basal IFN production increases the abundance of a variety of cytokine and pathogen signaling components, potentiating a more rapid and robust response to subsequent activating signals, including the production of IFN itself.

*Transcriptional Regulation of IFN Gene Expression.* One of the molecular hallmarks of the IFN system is its precise regulation of expression (9). Basal IFN is expressed at very low levels, while high levels of expression can be rapidly induced by viral infection. Induction of IFN- $\beta$  has been most intensely studied (10), and its transcriptional induc-

tion relies on the activation of three distinct transcription factor complexes, NF- $\kappa$ B, ATF/c-jun, and IFN regulatory factors (IRFs). Each of these factors is activated by serine phosphorylation in virus-infected cells, either directly or of associated inhibitory proteins, and while the kinases involved and the viral signals important for kinase activation are not completely defined, viral replication in many cases is critical, especially in the case of negative-sense RNA viruses (11). Existing evidence suggests that virus-encoded double-stranded RNA (dsRNA) provides one of the inducing signals for IFN induction, but additional virus-encoded components also appear to be necessary (12–15). For viruses capable of inducing IFN in the absence of replication, the presumption is that they deliver a payload to the cell upon infection of preformed inducing molecules.

The IFN- $\beta$  enhancer contains binding sites for all three transcription factors necessary for its expression, and their cooperative interaction and concerted recruitment of coactivator proteins allows efficient transcriptional induction of IFN- $\beta$  expression. The IFN- $\alpha$  genes are also transcriptionally induced in response to viral infection (16), and similar to IFN- $\beta$ , they require serine-phosphorylated IRF proteins for their expression (17). Recently, it was found that IFN- $\beta$  and the multigene family of IFN- $\alpha$  proteins are not uniformly regulated during viral infection (18), and that their differential expression is at least partially regulated through a positive-feedback loop involving induction of IRF proteins (18, 19). IFN- $\beta$  and the IFN- $\alpha$ 4 isotype of mouse IFN- $\alpha$  are induced with immediate early kinetics through the action of the constitutively expressed IRF-3 protein. However, the enhancers of other members of the IFN- $\alpha$  gene family cannot bind IRF-3, and are instead activated by IRF-7 (20). IRF-7, unlike IRF-3, is not constitutively expressed in most cell types, but rather its expression is induced by IFN signaling through the Jak-Stat pathway. Thus, in response to early secretion of IFN- $\beta$  and IFN- $\alpha$ 4 through the action of IRF-3, induction of IRF-7 makes cells permissive for induction of additional IFN- $\alpha$  subtypes (referred to as the nonIFN- $\alpha$ 4 subset), leading to robust production of multiple IFN- $\alpha$  species and potent antiviral activity. Significantly, the robust expression of the complete complement of IFN- $\alpha$  genes occurs only in IFN responsive cells, due

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to the requirement for IRF-7 induction in response to IFN signaling (11).

*Cellular Origin of IFN In Vivo.* Given the many cell types potentially producing IFN, the high levels of local and circulating IFN observed after viral infection raises the question of the cell-type of origin of this abundant cytokine. Some years ago, a cell with dendritic characteristics was identified as the major human IFN-producing cell and was designated the natural IFN-producing cell, or NIPC (21, 22). These are rare cells, but are factories for IFN production on a per-cell basis. These cells display surface markers characteristic of dendritic cell (DC) precursors, and due to morphological criteria are called plasmacytoid DCs. Recently, a similar cell population was identified in the T cell zones of spleen and inflamed lymph nodes of mice (23, 24), greatly enhancing the ability to conduct physiologic and genetic studies. The mouse NIPC appears to be the primary IFN-producing cell population in response to virus infection, both in vitro and in vivo.

The identification of plasmacytoid DCs as the major source of IFN in vivo raises some significant questions concerning the relevance of the extensive in vitro data demonstrating IFN production by diverse cell types. Even more perplexing are data from Barchet and colleagues in this issue that question the relevance of positive feedback for IFN production, as well as some of the molecular mechanisms of IFN transcriptional control previously defined in cell culture and in vitro studies (25). However, also in this issue, Dalod and colleagues provide intriguing insights into the cellular plasticity of cytokine production that suggest possible resolutions to these apparent paradoxes (26).

Barchet et al. measured the spectrum of IFN- $\alpha$  subtypes produced in response to vesicular stomatitis virus (VSV) infection of mice. Unlike a similar infection of fibroblasts, the nonIFN- $\alpha 4$  subset of IFNs predominated the response in vivo. Moreover, production of IFN was more rapid in vivo and was remarkably resistant to the block of positive feedback imposed by deleting the IFN- $\alpha$  receptor (IFNAR), though positive feedback was clearly manifested, especially at late times of infection. The major cell type producing IFN in virus-infected animals was the plasmacytoid DCs in the marginal zone of the spleen, but while these cells were among the splenocytes capable of inducing IRF-7 production in response to IFN, significantly reduced levels of IRF-7 mRNA induction in IFNAR<sup>-/-</sup> mice only modestly reduced IFN production. Coupled with the finding that replication-defective viruses were potent IFN producers, these data suggest a very different mechanism for induction of IFN from that operating in fibroblasts and epithelial cells in vitro.

Dalod et al. also found major IFN production by plasmacytoid DCs in virus-infected mice, and these cells also produced IL-12, another key cytokine for controlling viral infections. By looking somewhat later during the infectious process during the time of peak serum IFN titers, Dalod and colleagues detected all major isoforms of IFN and a significant contribution of positive feedback. Interestingly, their results highlight some of the diversity of the response to infection, as well as the plasticity of the responding cell popu-

lations, and some of the complexity of interpreting data from cytokine-deficient mice. While infection with murine cytomegalovirus (MCMV) fit the emerging picture of the mouse NIPC, infection with lymphocytic choriomeningitis virus (LCMV) was anomalous. IFN production in response to LCMV infection did not require T or NK cells, but it also did not appear to arise from plasmacytoid DCs. Maximal IFN production in response to LCMV depends on positive feedback through an IFN-STAT-1-IRF-7 pathway, but this virus also stimulates an alternative loop involving IFN- $\gamma$  (26a). Thus, two different pathogenic viruses stimulated IFN production from distinct cell subsets in vivo.

Regulation of IL-12 synthesis during viral infection may give a hint of the complexity of the response to pathogens. Plasmacytoid DCs also produced IL-12 during MCMV infection, although a nonplasmacytoid DC also contributed to IL-12 production. IFN negatively regulates IL-12 induction in response to MCMV, and Dalod and colleagues have defined the mechanism for this cross-inhibition. IFN significantly limits IL-12 production from nonplasmacytoid DCs. In the absence of IFN responsiveness (IFNAR<sup>-/-</sup> mice) or in the absence of IFN production (NIPC-depleted mice), nonplasmacytoid DCs became the major site of significant IL-12 production, revealing considerable plasticity in cellular cytokine response profiles dependent on the local cytokine milieu. Ablation of the IFNAR gene thus had the unexpected consequence of altering the type of cell producing a major immunomodulatory cytokine. Coupled with the increased viral load in IFN-unresponsive mice, comparisons between strains otherwise isogenic except for a single locus become quite complex.

So what can these data teach us about the regulation of IFN production? First, it is clear from both studies that positive feedback contributes significantly to total IFN production during viral infection, and is thus critical for controlling and clearing infection. Moreover, while induction of IRF-7 remains responsible for positive feedback, the initial production of virus-induced IFN by plasmacytoid DCs is largely independent of IRF-7 induction. How, then, are the nonIFN $\alpha 4$  subset genes induced, given their promoter structure containing bindings sites for IRF-7 but not IRF-3? First, it is possible that other IRF family members could substitute for IRF-7 in a cell-type specific manner. It was recently shown that IRF-5 is also capable of regulating at least some IFN- $\alpha$  subtypes in response to infection (27), adding to the potential complexity of IFN gene regulation. Second, IRF-7 was originally characterized as a hematopoietic-restricted protein (28), and its abundance is significantly higher in lymphoid organs, even in the absence of IFN feedback, and is constitutively expressed in DCs (unpublished data). Coupled with a mild induction of IRF-7 in response to infection by an IFN-independent mechanism (18), it is possible that sufficient levels of IRF-7 exist in NIPCs to induce nonIFN- $\alpha 4$  gene expression, even in IFNAR<sup>-/-</sup> mice. It will be of interest to determine if NIPCs selectively express significant levels of IRF-7 in the absence of IFN signaling, rather than needing to hypothesize a distinct molecular mechanism for IFN- $\alpha$  transcription in these cells.

**Cellular Complexity of Virus Infection.** Comparison of MCMV and LCMV infections demonstrates that IFN production is not a uniform response since different viruses stimulate IFN from distinct cell types. Even for a virus such as CMV that stimulates IFN production largely from NIPCs, there may still be a role for IFN production by other cell types. CMV establishes a latent infection, and at least in fibroblasts, its control appears to be dependent on IFN- $\beta$  induction through a lymphotoxin-dependent mechanism (29), highlighting the importance and complexity of cytokine interactions. Route of infection may also significantly alter the picture. Many experimental studies in the mouse have used an intravenous or intraperitoneal route of viral entry. However, other than nosocomially derived infections, most natural viral infections are acquired through respiratory or mucosal routes, including infections by VSV, LCMV, and CMV. The initial cellular site of viral replication and the major IFN-producing cell type during bronchial-alveolar infection remains to be determined. As a case in point, VSV is a relatively innocuous pathogen for mice. However, mice become highly susceptible to VSV infection in the absence of a single IFN antiviral response protein (PKR), but only after respiratory infection (30). PKR-deficient mice are fully capable of controlling VSV infection by most routes, but they are exquisitely sensitive to respiratory infection due to a failure to control viral replication and to impaired autocrine IFN production. Again, while the IFN-producing cell remains unknown, it is likely that cells resident to the respiratory tract are critical for the early control of inhaled VSV.

Diversity in the cellular response to viral infection is of clear evolutionary advantage in the battle between host and viral pathogen. To be successful, viruses develop strategies to evade the IFN system at multiple levels (3). Cellular mechanisms that circumvent these viral evasions maintain the balance required for coexistence of pathogen and host. It is now the challenge to define this diversity in cellular and molecular terms, including the cells that produce IFN, the virus-encoded inducing signals, and the cellular response factors. An intriguing family of cellular proteins that might contribute to this diversity consists of the toll-like receptors (TLRs). Toll is a *Drosophila* protein essential for microbial resistance, and the related mammalian TLRs function similarly, each with selectivity for a distinct microbial product (31). Interestingly, the different TLRs also display distinct patterns of cell-type expression (31–33), and several are also differentially expressed in response to IFN (34). Recently, TLR3 was shown to be a receptor for dsRNA, a component of the virus-encoded IFN activating signal, and TLR-3 was required for induction of IFN, at least in macrophages stimulated with dsRNA (35). Among DC subsets, TLR-3 is expressed exclusively in CD11c<sup>+</sup> immature DCs, and these cells produce IFN in response to dsRNA. In contrast, TLR-9, the receptor for CpG oligonucleotides that also stimulate IFN production, is expressed on plasmacytoid DCs (33).

It is unclear whether TLR-3 is truly a viral receptor for IFN production since dsRNA represents only a subset of

viral activating signals (12–15) and since its cell-type distribution appears to be distinct from the primary IFN producer during viral infection. In fact, TLR-3 may serve instead as a sensitive detector of virus-induced apoptosis, responding to the dsRNA released from dying cells rather than to primary infection. TLR-9, on the other hand, appears to be involved in IFN production in response to bacterial infection (33, 36). Significantly, the expression of TLRs changes during DC maturation (32) and is also modulated in response to cytokine priming, such as the induction of TLR-7 in response to IFN (34), providing increased plasticity to pathogen recognition. TLR-7, like TLR-9, is also uniquely expressed on plasmacytoid DCs (33). Given the current data on the importance of these cells during viral infections, it will be of great interest to explore the ligands for TLR-7 and the phenotype of mice deficient in this gene. TLR-7-deficient mice display impaired IFN production in response to small molecular weight immunomodulatory compounds, but their response to viral infection has yet to be determined (37). Different TLRs mark distinct cell populations involved in innate immunity, enabling them to respond to distinct microbial signals. TLR proteins, or perhaps a yet to be discovered family of pathogen-responsive cellular receptors, are ideal candidates to modulate the diverse cellular response to virus infection.

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