Current therapy of chronic hepatitis C virus (HCV) infection is based on type I interferon (IFN) preparations. The IFNs comprise a class of related cytokines that induce antiviral and immunomodulatory actions of their target cells. The type I IFNs include multiple IFN-α species and a single IFN-β species. The type I IFNs bind to a common receptor that is largely used for HCV therapy consist of IFN-α2a or IFN-α2b subtypes. The type I IFNs bind to a common receptor that is expressed on the surface of target cells. Receptor engagement leads to the activation of the Jak-STAT signaling pathway through the actions of the Jak1 and Tyk2 protein kinases, which catalyze phosphorylation events leading to the activation and heterodimerization of the signal transducer and activator of transcription (STAT) proteins STAT1 and STAT2. STAT3 has also been shown to respond to type I IFN receptor signaling and is proposed to link IFN signaling events with cell growth regulation and the phosphatidylinositol 3-kinase pathway. The STAT1/2 heterocomplex translocates to the cell nucleus, where it associates with p48/IRF-9 to form interferon-stimulated gene factor 3 (ISGF3). ISGF3 binds to the IFN-stimulated response element (ISRE) of cellular genes, known as IFN-stimulated genes (ISGs), leading to their induced expression and synthesis of the ISG products. Sequence motifs within the ISRE also serve as target sites for interferon regulatory factors (IRFs), whose actions and ISRE-binding properties contribute to define the overall spectrum and duration of ISG expression.

It is the biochemical actions of the ISG products that impart the primary therapeutic actions of IFN against HCV infection. However, IFN therapy continues to be problematic, because even under the best conditions, contemporary treatment protocols show limited efficacy such that only about one half of all treated patients exhibit sustained viral clearance. The reasons for this limited efficacy are unclear, but are likely to involve both viral and host factors that ultimately affect the level and extent of ISG expression and function induced during the course of IFN therapy. Results from microarray expression studies indicate that the human genome encodes hundreds, if not thousands of functionally diverse ISGs, some of which are known to direct antiviral actions. However, the complete spectrum of ISGs has not been defined nor have the ISGs that impart control of HCV replication been identified. The refinement and further improvement of IFN therapy for chronic HCV infection will no doubt come from a better understanding of how IFN affects the infected hepatocyte and other target cells. This requires defining the spectrum and kinetics of ISG expression under in vivo conditions of IFN therapy and categorizing ISG expression patterns within patients who respond to therapy and in those patients who fail IFN therapy. In vivo studies that couple analyses of viral decay kinetics with microarray profiling of ISG expression during specific phases of IFN therapy will provide the opportunity to correlate ISG expression patterns with antiviral effect. Such an approach should facilitate the identification of an ISG subset or “short list” whose expression patterns positively correlate with viral decay and whose actions are likely to influence HCV replication. Further mechanistic studies to evaluate protein levels, biochemical actions, and anti-HCV potential of ISG products encoded in this “short list” could then define those ISGs that are the important players of IFN action against HCV. Results from such studies could lend to further refinement of IFN dosing regimens that are biased to maximize the expression and extent of action of these specific anti-HCV effector ISGs.

Studies published in the current and March issues of Hepatology demonstrate the feasibility of these approaches. Ji et al. utilized the microarray approach to profile gene expression patterns in patients during the initial phases of IFN therapy, and results are presented to
show the extent and duration to which IFN-α induced overall ISG expression in vivo. In a second study, Zhu et al. utilized the HCV replicon system, which consists of the Huh7 human hepatoma cell line harboring an autonomously replicating and selectable HCV subgenomic RNA to examine ISG expression and the antiviral effects of IFN-α against HCV RNA replication. In the study by Ji et al., the investigators focused on evaluating the effect of Rebetrion combination therapy (IFN-α2b with ribavirin) upon global gene expression within a cohort of patients with chronic HCV infection. Microarray technology was used to profile the expression of over 38,000 human genes before IFN administration and 3 or 6 hours after IFN administration. Peripheral blood mononuclear cells (PBMCs) served as the tissue source for the gene profiling studies, which included parallel experiments of PBMCs isolated from the pretreatment time point but cultured for 3 or 6 hours in vitro in the presence of IFN-α2b. Overall, the gene expression profiles obtained from in vivo and ex vivo exposure to IFN were remarkably similar, although the kinetics of ISG induction varied somewhat between conditions. On the average, more than 500 ISGs were identified after in vivo or ex vivo IFN treatment, while only a small number of genes were identified whose expression was suppressed in response to IFN.

Consistent with previous analyses, the ISGs defined by Ji et al. represent diverse functional groups, ranging from genes encoding immunomodulatory proteins to those encoding metabolic regulators. Using stringent statistical analyses, a “short list” of 123 ISGs were identified whose expression increased in vivo and in vitro within 3 hours of IFN treatment, thereby preceding the onset of viral decline that first follows IFN administration. Assuming a temporal relationship in which ISG expression and action precede the decline of HCV load, it is likely that ISGs within this “short list” contribute to the therapeutic effects of IFN on HCV. The results presented in this study mark a positive step toward identifying the spectrum of ISGs in human cells that respond to therapeutic levels of IFN in vivo, and toward understanding the dynamics of ISG expression in hematopoietic cells. However, because PBMCs rather than liver tissue were examined, one cannot directly extend these results to the hepatocyte and thus cannot ascertain what ISGs are actually relevant to control HCV replication in the liver. Moreover, it should be noted that PBMCs represent a heterogeneous population of cells, any one type of which may influence the ISG signals obtained by microarray analysis. The task now will be to evaluate the expression and action of the “short list” ISGs inasmuch as they are expressed in hepatocytic cells and pose a blockade to viral RNA replication.

Because native HCV cannot be efficiently propagated in cultured cells, HCV replicon systems have proved valuable for the study of virus-host interactions that influence viral RNA replication. Several studies have shown that treatment of HCV replicon cell cultures with IFN-α2a or -α2b could limit HCV RNA replication in a dose-dependent manner, although the host cell signaling pathways and the ISGs that mediated the anti-HCV effect were not defined. Towards addressing this issue, Zhu et al. examined STAT signaling events and ISG expression within IFN-α2b-treated Huh7 cells harboring an HCV genotype 1b subgenomic replicon. In accordance with the previous studies, IFN treatment resulted in a reduction in the levels of the HCV replicon RNA, and this occurred concomitant with the induction of ISG expression. Examination of STAT phosphorylation levels that were induced as a result of IFN treatment revealed a paucity of phospho-STAT1 and comparably excessive levels of phospho-STAT3, to which the investigators concluded that STAT3 represents the major triggering mechanism of ISG induction in Huh7 cells. Microarray analyses were then used to characterize the spectrum of ISG expression within IFN-treated Huh7 cells alone and those harboring...
the HCV replicon. In the replicon system HCV RNA replication had little overall impact on ISG induction except to influence an apparent potentiation or increase in the expression levels of certain ISGs. The reason for this is not clear, but could be related to a priming effect rendered by a low level of IFN production within the replicon cells, as has been shown in other studies.17

The gene lists presented by Zhu et al.13 show that, overall, IFN induced a pattern of ISG expression in Huh7 HCV replicon cells that is consistent with the actions of ISGF3. What the investigators may have overlooked is that known STAT3 target genes, such as p21(waf1), myc, pim-1, cyclin D1, and others5 were not part of this list. The overall representation of ISGF3 target genes in the ISG list, coupled with the lack of STAT3 target genes indicates that the major effector by which IFN signaled ISG induction was actually ISGF3, rather than STAT3 as was concluded by the investigators. On closer look at the list of genes induced in general during HCV RNA replication in cells that were not treated with IFN, one finds that viral RNA replication was associated with the stimulation of interleukin 6 (IL-6) expression. IL-6 is a potent inducer of STAT3 activation,5 and the expression of IL-6 by HCV replicon cells could possibly have contributed to the overall abundance of phospho-STAT3 and its rapid accumulation upon IFN treatment. These items raise the possibility that the increased abundance of phospho-STAT3 within the HCV replicon cells was not due to IFN alone, but may have involved the actions of IL-6 and/or potential cross-talk between IFN and IL-6 signaling pathways. A technical limitation of this study was that microarray experiments represented only a single 6-hour time point post-IFN treatment, which may not have been temporally optimal for observing the complete spectrum of ISG expression or the STAT3 response. Moreover, the microarray platform that was used consisted of only 832 genes and, thus, likely did not represent the global spectrum of possible ISGF3 or STAT3 target genes. However, the overall study design provides a framework for future work focused on evaluating ISG expression and antiviral potential against HCV RNA replication.

By the classic definition, ISGs are the genetic component of the cellular response to IFN. However, recent work in the IFN field has shown that during virus infection the overall complexity of the ISG repertoire expressed by the host cell is heavily influenced by the activation state of IRFs that are regulated independently of IFN.3 In particular, double-stranded RNA (dsRNA) and other products of virus infection and replication are potent inducers of IRFs, which cooperate with other transcription coactivators to stimulate the expression of specific IRF target genes.2 Importantly, many known ISGs are among the list of identified dsRNA or IRF-3 target genes,18,19 showing that expression of the ISG repertoire is influenced through cellular pathways that can be signaled independently of the type I IFN receptor. As depicted in Fig. 1, virus infection triggers host cell pathways that activate IRF-3, NF-κB, IRF-1, ATF/c-jun, and other factors that promote gene expression through IFN-independent signaling events, the integrity of which is critically important for mediating innate resistance and immunity to virus challenge.20 The convergence of these signaling pathways ultimately initiates the production and secretion of IFN-β, and triggers the synthesis and action of IRF-7 to impart the expression of the different IFN-α subtypes and the ISGs that that establish the antiviral state.21 A comprehensive definition of ISGs relevant to the control of HCV infection will therefore require approaches that examine overall gene expression in the context of virus infection and replication within IFN-responsive host cells.

This issue has been partially addressed through microarray studies of liver-specific gene expression in chimpanzees that were experimentally infected with HCV.22,23 This work showed that HCV infection and replication triggers ISG expression. Importantly, the resolution of acute HCV infection corresponded with a “short list” ISG profile that included a vigorous induction of known dsRNA or IRF-3 target genes followed by the expression of IRF-7 and the subsequent induction of a broad array of ISGF3 target genes.22 The involvement of dsRNA and IRF pathways in ISG signaling events that limit HCV RNA replication has also been shown in studies of the HCV replicon system.10,17,24 Thus, control of HCV infection and replication is likely to involve a spectrum of ISGs whose expression is determined through complex virus-host interactions that regulate IFN-dependent and IFN-independent signaling pathways.

The use of IFN/ribavirin combination therapy and pegylated versions of IFN have led to marked improvements in the outcome of IFN-based therapy for chronic HCV infection.1 Despite these improvements IFN therapy for HCV remains inadequate for the more than 170 million people with chronic infection. Although new generations of HCV antiviral compound are being developed or are soon to be introduced into the clinic,4 IFN therapy remains the cornerstone for the treatment of HCV infection. Further improvements to IFN-based approaches to treat HCV will undoubtedly require a deeper understanding of how IFN interacts with HCV to suppress virus infection. A central focus should be placed on understanding the role of ISGs as the effectors of the antiviral response induced during virus infection and IFN therapy. Which ISGs are induced during the course of HCV in-
fection, and how might these vary in patients who respond to therapy versus those who do not respond? What are the molecular mechanisms of therapeutic IFN action against HCV, and what specific ISGs are involved in this action? What are the relevant signaling pathways within the host cell that mediate innate resistance to HCV infection, and how might these pathways contribute to overall ISG expression? In summary, a greater understanding of ISG function will support new approaches to refine and improve IFN-based therapies for chronic HCV infection.

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References