Hepatitis C Virus Resistance to Antiviral Therapy

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Research produces highly efficient antiviral drugs. At the same time, viruses develop sophisticated strategies to evade their actions. Resistance to antiviral therapy has become a major issue in the management of patients with chronic viral infections. The best known example is human immunodeficiency virus (HIV), but cytomegalovirus, herpes simplex virus, and hepatitis B and C viruses exhibit similar capabilities. About 60% of patients with chronic hepatitis C do not clear hepatitis C virus (HCV) infection with the currently approved therapeutic regimen and are considered "resistant" to therapy. The resistance of HIV to antiretroviral drugs has often been used as a model for HCV resistance. However, the two drugs currently used in the treatment of HCV, interferon alfa (IFN-α) and ribavirin, have complex and indirect actions, making the mechanisms of HCV resistance generally different from those of viral resistance to specific antiviral agents. Molecular mechanisms of resistance to IFN-based therapy have recently been proposed on the basis of *in vitro* experiments but have not been validated in patients, in whom treatment failure appears to be multifactorial. The aim of this review is to clarify what is known, what is hypothesized, and what remains unknown in the field of HCV resistance to IFN-based antiviral therapy.

MECHANISMS OF THE ANTIVIRAL ACTION OF IFN-α

Treatment of chronic HCV is aimed at cure. Unlike drugs for HIV, which specifically target viral protein functions, IFN-α alters host-virus interactions in a very complex way, and the drug-induced host responses to the virus are central to the antiviral effect. IFNs are natural cellular proteins with various actions, including induction of an antiviral state in their target cells and cytokine secretion, recruitment of immune cells, and induction of cell differentiation.[1-3] After subcutaneous administration, IFN-α is specifically fixed onto high-affinity receptors at the surface of target cells. IFN-receptor fixation triggers a cascade of intracellular reactions leading to activation of numerous IFN-inducible genes.[4-8] The products of these genes are the mediators of the various cellular actions of IFN-α.[9] They are responsible for the antiviral effects of IFN-α through two distinct but complementary mechanisms: the induction of an antiviral state not specific to the virus in infected cells, which results in a direct inhibition of HCV replication; also, induction of immunomodulatory effects that enhance specific anti-HCV immune responses of the host.

Numerous IFN-induced proteins and enzymatic pathways are involved in the establishment of an antiviral state in infected cells, but only a few have been identified so far.[9] Three have been extensively studied: the 2′-5′ oligoadenylate synthetase (2′-5′ OAS) system, the Mx proteins, and the double-strand RNA-dependent protein kinase (PKR).

2′-5′ OAS is a cellular enzyme synthesized in response to IFN-α stimulation. In infected cells, 2′-5′ OAS enzymatic activity is induced by double-stranded RNAs, such as the intermediates of replication of RNA viruses or folded single-stranded RNAs.[10] 2′-5′ OAS catalyzes polymerization of adenosine triphosphate into 2′-5′ oligoadenylate that, in turn, activates a cellular endoribonuclease, RNase L, at subnanomolar concentrations.[11] RNase L degrades cellular and viral single-strand RNAs. Thus, viral replication is inhibited as a result of protein synthesis inhibition in a totally non–virus-specific way.

The Mx proteins (MxA and MxB) belong to a recently discovered guanosine triphosphatase family. They are produced in response to IFN-α stimulation and to several viral infections.[12,13] Their antiviral actions may be related to a direct inhibitory effect on viral polymerases, the inhibition of messenger RNA synthesis, or the blocking of viral polymerase transfer to the nucleus.[13] The Mx proteins do not appear to play an important role in HCV infection.[14,15]

PKR is a natural cellular serine-threonine kinase, the synthesis of which is induced by IFN-α.[16] Its activation by double-strand RNAs results in its autophosphorylation.[17] Activated PKR in turn phosphorylates the α subunit of eukaryotic translation initiation factor eIF-2. eIF-2α phosphorylation inhibits protein synthesis, thus blocking viral replication in a non–virus-specific way.[18-20]

Many other IFN-inducible genes are probably involved in the intracellular action of IFN-α. These include genes that could be more or less specific for the cells into which HCV replicates.

Besides its direct nonspecific antiviral effect, IFN-α also exerts immunomodulatory effects as a result of its binding onto receptors at the surface of immune cells. IFN-α induces expression of class I major histocompatibility complex antigens. IFN-α also activates effector cells, such as macrophages, natural killer cells, and cytotoxic T lymphocytes.[21] Finally, IFN-α interacts with the cytokine cascade in a complex way[22]: it stimulates the production of type 1 T-helper (Th1) cells, which synthesize mainly IFN-γ and interleukin 2 (IL-2), and reduces that of Th2 cells, which synthesize mainly IL-4 and IL-5.[22] IFN-α also has anti-inflammatory properties through the inhibition of peripheral production of IL-1, IL-8, and tumor necrosis factor α and the stimulation of IL-10 production.[23-28]

MECHANISMS OF ACTION OF RIBAVIRIN

Ribavirin is a synthetic guanosine analogue used principally in the past for the treatment of severe respiratory syncitial virus infections in infants. Given alone, ribavirin has not proved to be efficient in chronic HCV infection.[29] When

Abbreviations: HIV, human immunodeficiency virus; HCV, hepatitis C virus; IFN-α, interferon alfa; 2′-5′ OAS, 2′-5′ oligoadenylate synthetase; PKR, double-strand RNA-dependent protein kinase; Th, T-helper; IL, interleukin; ALT, alanine aminotransferase; HVR1, hypervariable region 1.

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added to IFN-α, ribavirin increases the initial response, *i.e.*, the ratio of patients who clear HCV RNA during therapy, and markedly reduces relapse rates in these patients.[30-33] The mechanisms underlying these effects are not yet fully understood.

Ribavirin selectively inhibits viral RNA polymerases *in vitro*. It has been reported to inhibit *in vitro* the replication of bovine viral diarrhea virus, a pestivirus close to HCV, and to synergize the antiviral effect of IFN-α in this model.[34] We recently showed that ribavirin alone exerts a moderate (*i.e.*, less than 0.5 $\log_{10}$ copies/mL reduction) and transient (*i.e.*, occurring at days 2 and 3 of administration) but significant inhibitory effect on HCV replication in infected patients *in vivo*. This effect is additive to that of IFN-α administered 3 times per week.[35] Ribavirin competition with the inosine monophosphate dehydrogenase enzyme was suggested to contribute to its antiviral activity by inhibiting viral DNA and RNA synthesis through depletion of intracellular guanosine triphosphate pools.[36] Nevertheless, VX-497, a novel potent inosine monophosphate dehydrogenase inhibitor, does not appear to exert any effect on HCV replication in infected patients.[37]

Ribavirin also appears to potentiate the immunomodulatory properties of IFN-α. The underlying mechanisms are poorly known. It has been suggested that ribavirin could alter the Th1/Th2 balance by causing a shift towards Th1 responses.[38] The greater efficacy of combination therapy with IFN-α plus ribavirin might also be related to ribavirin ability to suppress HCV-specific IL-10 production.[39]

Overall, whatever its actual mechanisms of action, ribavirin appears to act mainly, or exclusively, by potentiating the various antiviral actions of IFN-α. This, together with the fact that the predictors of the response to IFN-α monotherapy and to IFN-ribavirin combination are the same,[31-33,40-43] suggests that the mechanisms underlying HCV resistance to the combination of IFN-α plus ribavirin are probably not different from those underlying HCV resistance to IFN-α monotherapy.

DEFINITIONS: INCIDENCE AND PATTERNS OF HCV RESISTANCE TO ANTIVIRAL THERAPY

A sustained virologic response to antiviral therapy is defined by normal alanine aminotransferase (ALT) activity and negative HCV RNA detection in serum 6 months after treatment withdrawal. Long-term follow-up studies showed that the vast majority of the sustained virologic responders are probably cured from HCV infection.[44-46] HCV resistance is defined as HCV RNA persistence in serum after therapy. The incidence of HCV resistance to IFN-based therapies varies according to the administered regimen. Almost 60% of patients fail treatment after receiving the currently approved regimen, based on 3 megaunits recombinant IFN-α 3 times per week plus ribavirin, 1.0 to 1.2 g per day for 24 to 48 weeks.[31,32] Based on preliminary results, weekly administration of pegylated IFN-α appears to be associated with a 60% to 75% incidence of HCV resistance according to the therapeutic schedule and the treated population.[47-49] The incidence of HCV resistance in patients receiving the combination of pegylated IFN-α and ribavirin will be of the order of 50%. It should be stressed that resistance is more frequent in certain subgroups of patients, such as those infected with HCV genotype 1 and/or those with high pretreatment viral loads, as well as those with one or more other causes of treatment failure.[31,32,40-43,47,48]

Several different virologic response patterns can be observed in the patients who fail to clear HCV RNA, according to whether or not the initial, induction phase of therapy is successful. HCV RNA clearance during the induction phase may reflect inhibition of virus production or of *de novo* infection of new cells, death of infected cells, or a combination of these events. The "responder-relapser" clears HCV RNA during therapy but relapses after treatment withdrawal, whereas the "responder with breakthrough" clears HCV RNA during therapy but relapses before the end of treatment. In both groups, treatment induction achieves HCV RNA clearance, but the subsequent maintenance phase fails to ensure the elimination of infected cells and thus allows relapse. The "partial responder" experiences HCV RNA load decreases of more than 1 $\log_{10}$ during therapy but fails to reduce HCV RNA to undetectable levels, whereas the true "nonresponder" has no significant HCV RNA load change during therapy. In the latter groups, virus persists because the induction phase has failed to clear HCV RNA from serum. As a result, there is no subsequent maintenance phase.

CAUSES OF HCV RESISTANCE TO IFN

IFN failure to clear HCV infection is multifactorial. Its causes are often combined in the same patient and may relate to the treatment regimen, the host, the presentation or the severity of HCV-related disease, or viral factors.

Treatment-Related Factors. At the chronic stage of infection, HCV replication kinetics are at a steady state.[49-51] This means that virus production by infected cells is compensated by peripheral virion degradation, whereas *de novo* infection of noninfected cells is compensated by the infected cell death. This steady state is characterized by an estimated half-life of free HCV virions of approximately 3 hours, and by a daily virion production-clearance rate of about 10^{12} viral particles per day.[50]

The current IFN-α administration schedule (*i.e.*, 3 million units 3 times per week) in the induction phase of therapy is not suitable for such rapid viral kinetics.[35,49,50] Indeed, higher doses of IFN-α induce sharper decreases of HCV viral load 24 hours after a single injection.[49,50] In addition, intermittent IFN administration is associated with a rebound of viral replication at day 2 (*i.e.*, before the second IFN injection), hampering the second slope of viral decrease and HCV RNA clearance during treatment in most patients.[35,49] The addition of ribavirin appears to prevent this rebound, leading to a second very slow but significant slope of viral decrease and to HCV RNA clearance in about half of the patients.[35] Daily IFN-α administration and weekly pegylated IFN-α administration induce a typical biphasic decline of viral replication in most cases (Fig. 1): the first rapid slope at day 1 is related to direct IFN inhibition of virus production, whereas the second slope of viral decrease starts at day 2, is slower, appears to be related to infected cell death in the context of efficient inhibition of virus production, and leads to HCV RNA clearance in a large proportion of patients.[35,49,50,52] Future therapies, especially specific anti-HCV drugs (such as HCV protease, helicase or polymerase inhibitors, ribozymes, or antisense oligonucleotides, . . .) will probably permit further increases in the ratios of HCV RNA clearance, with a steeper decline during induction.

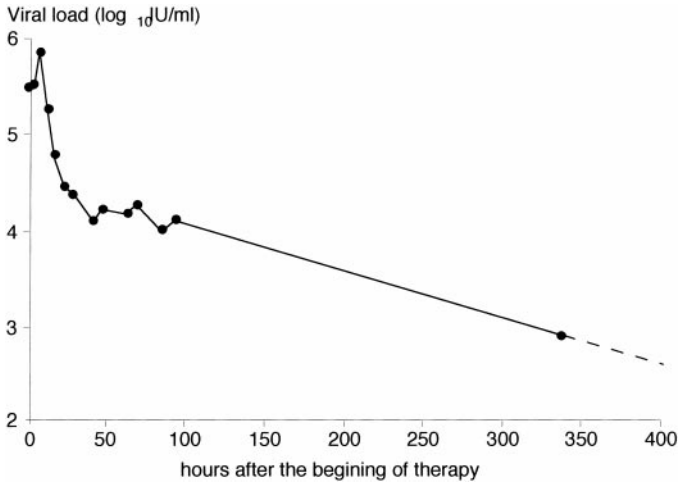

FIG. 1. Example of a patient receiving 3 megaunits of IFN-α daily. Blood samples were taken before therapy and every 4 hours for 12 hours, every 6 hours for 36 hours, every 12 hours for 48 hours, and at day 14. HCV RNA was quantified with the reverse transcription-polymerase chain reaction-based Amplicor HCV Monitor assay version 2.0 (Roche Molecular Systems, Pleasanton, CA). The HCV RNA loads are expressed in $\log_{10}$ international HCV RNA units (IU)/mL. Viral load decrease was biphasic, with a first rapid slope of viral decrease at day 1, followed by a second, slower slope of viral decrease starting at day 2.

Treatment duration significantly influences the results of IFN-based therapy. Indeed, longer treatment significantly decreases the incidence of relapse in the patients clearing HCV RNA during treatment.[31,32,53,54] In most of the reported studies, both the induction and maintenance phases of therapy used the same regimen, given for a fixed period. Thus, the duration of the maintenance phase may vary considerably, depending on the duration of the induction phase (*i.e.*, the time needed to clear HCV RNA from serum). This might at least partly explain that early HCV RNA clearance from serum, *i.e.*, a shorter induction phase followed by a longer maintenance phase, is predictive of a sustained virologic response in the patients receiving a fixed treatment duration.[31,32] Improved maintenance phase strategies will be needed to prevent relapses in order to increase the ratios of sustained virologic response. New drugs or therapeutic strategies, especially those based on immunologic approaches (such as molecules with immunomodulatory properties and therapeutic vaccines) might prove to be particularly useful. In addition, increasing overall treatment duration and tailoring individually the duration of the maintenance phase to the duration of the induction phase in patients who initially clear HCV RNA might significantly decrease the incidence of relapse, especially in the groups of patients with low ratios of sustained virologic response.

Patient-Related Factors. Patient characteristics such as an older age, male gender, and ethnicity (African or Hispanic versus Caucasian or Asian) have been shown to be associated with poorer ratios of sustained virologic response, which point to genetic, hormonal and/or immunologic determinants of HCV sensitivity to IFN-α.[31-33,55] Body weight may also influence the response ratio by modifying the volume of distribution of the drug and its final concentration at the receptor level.[42,56] In the patients who initially respond to therapy, induction of neutralizing anti-IFN antibodies by recombinant IFN appears to be responsible for up to 50% of the breakthroughs occurring during treatment.[57-60] A switch or retreatment with another IFN-α molecule may rescue the response in some cases.[61,62] Various behaviors are associated with lower response ratios, such as active alcohol or intravenous drug intake.[63-65] Finally, compliance to therapy appears to be a major determinant of its outcome.

Disease-Related Factors. Certain forms of HCV-related liver disease, such as advanced fibrosis and compensated cirrhosis, are associated with lower ratios of response to therapy.[31,32,66] In patients with decompensated cirrhosis, treatment is contraindicated.[67,68] Patients with HCV-HIV coinfection also have lower response ratios, especially those with low CD4-positive cell counts.[69,70] This may be caused by HIV-induced immunosuppression, by direct viral interactions, or by drug interactions. Large-scale clinical trials currently underway will establish the ratios of sustained virologic response to IFN plus ribavirin combination in HCV-HIV coinfected patients.

Patients with extrahepatic manifestations of HCV infection, such as mixed cryoglobulinemia or membranoproliferative glomerulonephritis, have been reported to have lower ratios of sustained viral clearance than patients without extrahepatic manifestations, but this is still unclear.[71] Finally, nonresponders to a previous course of IFN or combination of IFN plus ribavirin have low ratios of sustained virologic response after retreatment.[72]

Patients with repeatedly normal ALT levels and those with histologically mild chronic hepatitis are often considered difficult-to-treat. In fact, their chances of achieving a sustained virologic response are the same as for patients with elevated ALT activity and moderate-to-severe chronic hepatitis based on liver biopsy.[73-75] They should therefore be considered difficult-to-decide-to-treat rather than difficult-to-treat because no clear benefit on the long-term outcome of their liver disease has been identified.

Viral Factors. The existence of intrinsically IFN-resistant HCV strains is unlikely, because virtually all of the patients with chronic hepatitis C receiving a sufficiently high initial dose of IFN-α experience, at least within the first hours of treatment, a significant decrease of viral replication because of the nonspecific antiviral effect of IFN.[50,51] In addition, specific genome mutations conferring IFN-α resistance to the corresponding HCV variants are unlikely. In the vast majority of, if not all, patients who do not achieve a sustained virologic response, IFN administration and subsequent IFN withdrawal are associated with significant qualitative changes in the composition of HCV quasispecies.[76-80] Both the mechanisms of action of IFN-α and recent clinical and virologic observations suggest that the selected HCV quasispecies variants are not intrinsically resistant to IFN-α but just become fit in the environment created by IFN-induction of host responses. This is supported by the following arguments:

In contrast to the effect of antiretroviral therapy on HIV, IFN-α does not specifically target an HCV gene but enhances host antiviral responses that, in turn, exert selection pressures on various viral genome regions, proteins, and epitopes. Thus, the targets of IFN-α actions are numerous and located over the entire genome. They may vary from one patient to the next, according to the characteristics of the infecting strain and to the genetic and immunologic background of the host. This means that, if viral escape plays a role in HCV resistance to IFN, HCV variants escape specific host responses induced by IFN, not IFN itself.

Nonresponders or partial responders and, more often, responder-relapsers to a first course of IFN-α may achieve a sustained virologic response after a second identical course of IFN-α therapy.[79,81,82] In such cases, we observed significant qualitative HCV quasispecies changes after the first course of IFN.[79] The selected HCV variants were not intrinsically resistant to IFN-α, because they were definitively cleared after the second course of IFN. They just became fit in the environment created by the administration and subsequent withdrawal of IFN during and after the first course of treatment. They became nonfit and could be cleared during the second course of IFN because the nature of the host-virus interaction was different several months after the first course and could be tipped toward sustained viral clearance by IFN at that time.[79]

Viral factors, nevertheless, appear to play an important role in HCV resistance to IFN-based therapy, probably conferring HCV quasispecies and infected cell properties that allow them to survive in the context of IFN administration. High quasispecies genetic complexity (*i.e.*, a large size of the quasispecies sequence repertoire) at the beginning of therapy is an independent predictor of treatment failure.[78,83,84] In contrast, the sustained virologic responders generally have a small size of their pretreatment quasispecies sequence repertoire.[78,83,84] The meaning of this finding is unclear. A large pretreatment quasispecies sequence repertoire could make it more likely that at least some variants have properties that enable them to escape the IFN-induced responses of the host.

The role of viral factors in HCV resistance to IFN-α is strongly suggested by the fact that HCV genotype is a strong and independent predictor of the sustained virologic response to recombinant or pegylated IFN-α monotherapy and to IFN-α plus ribavirin combination. Indeed, HCV genotypes 1 and 4 are significantly less sensitive to IFN therapy than HCV genotypes 2 and 3 in all instances.[31-33,40-43,47,48] In addition, among patients infected with the same genotype, some respond more quickly than others, some clear infection, and some do not. HCV strains belonging to different genotypes differ from each other by their nucleotide and, as a result, their protein sequences.[85] This is also true for different HCV isolates in the same genotype, although the overall sequence difference is smaller. HCV genome sequence differences may be responsible for differences in viral protein structures and functions. Given that certain viral protein functions could at least partly inhibit the action of certain IFN-induced antiviral effectors in infected cells, sequence differences might be associated with different HCV quasispecies fitnesses in the presence of IFN. This could account for the differences in sensitivity to therapy observed among different HCV genotypes or isolates.

The nature of the viral protein(s) and genome or protein function(s) involved in HCV resistance to IFN therapy remains purely speculative at the present time. The role of NS5A protein has been suggested. Certain NS5A sequences were reported to be more frequent before treatment in HCV-resistant than in HCV-sensitive patients.[78,86-91] Nevertheless, the numerous published results conflict,[86-99] probably because of methodologic biases in the definition and selection of sensitive and resistant HCV strains and the generation of NS5A sequences. Recently, NS5A gene expression was shown to be able to antagonize the antiviral action of IFN-α in transfected cells *in vitro*,[100,101] a strong argument for NS5A involvement in HCV resistance to IFN-α. This effect appeared to be independent of any "interferon sensitivity determining region."[100] It has been suggested, on the basis of *in vitro* experiments, that inhibition of the IFN-induced PKR by NS5A (and, possibly, by the E2 envelope glycoprotein) is sequence dependent and could be the principal mechanism underlying viral resistance to IFN-α.[101-104] Nevertheless, the existence of such interactions *in vivo* and their role in HCV resistance to IFN therapy are not convincingly supported by epidemiologic or biologic observations.[105-108] Viral polyprotein inhibition of the Jak-Stat pathway (the main pathway responsible for IFN-α signal transduction) upstream of PKR has also been reported *in vitro*, but the involved mechanisms remain unknown.[109]

Overall, several genome or viral protein functions, or both, and their interactions with numerous host cell functions are probably involved in HCV resistance to the nonspecific antiviral action of IFN-α in infected cells. More work is needed to elucidate these very complex mechanisms.

CONSEQUENCES OF HCV RESISTANCE TO IFN

HCV Quasispecies Evolution. Failure to clear HCV RNA during therapy is associated with significant qualitative HCV quasispecies changes in most, if not all, cases.[76-80] Although viral escape could be one of the mechanisms involved in treatment failure, most of these changes appear to be the consequence of the inability of the host to clear infection. They result in the accumulation of mutations on HCV genome during ongoing replication and selection of the corresponding variants. The nature of the changes varies from one genomic region to the next, according to the type of IFN-induced selection pressures on the region and to conservatory constraints related to viral RNA and/or protein structure and/or functions, as illustrated by the following examples.

Hypervariable region 1 (HVR1) encodes a 27-amino acid stretch located at the N-terminus of the E2 envelope glycoprotein. It is tolerant to amino acid substitutions, although we recently observed that HVR1 physicochemical properties and conformation are conserved, pointing to structural and probably functional conservatory constraints (Penin et al., unpublished observations, July, 2000). HVR1 contains one of the principal HCV-neutralizing epitopes and also appears to be a target for cytotoxic responses.[110-113] We showed that IFN-α administration and subsequent withdrawal result in shifts of HVR1 nucleotide sequences in more than 70% of patients infected with the most frequent HCV genotypes (*i.e.*, 1a, 1b, 2a, 2c, 3a, and 4a) receiving IFN-α in monotherapy.[79] Similar changes are observed in patients receiving the combination of IFN-α and ribavirin.[114] HVR1 quasispecies changes during and after therapy are evolutionary. They are characterized by amino acid changes that appear to be driven by positive selection pressures (most likely IFN-enhanced neutralizing responses and, eventually, cytotoxic responses).[79] Approximately 1 of 4 patients has no apparent HVR1 quasispecies change over time, meaning that the major pretreatment variants remain fit when IFN is given and withdrawn.[79] These patients might be unable to mount an efficient response against HVR1 or HVR1 might be protected from antibody binding, for instance through lipoprotein binding as recently suggested.[115] The patients without HVR1 changes generally belong to the "nonresponder" group,[79] making it unlikely that selection of neutralizing escape mutants plays a major role in HCV resistance to IFN. Interestingly, quasispecies changes are observed in genomic regions other than HVR1 in most of the patients without HVR1 quasispecies changes.

Although the function of the NS5A protein is not precisely known, it is assumed to play an important role in regulating viral replication and interacting with host cellular functions.[116] Evolutionary quasispecies changes are also observed in the central region of the NS5A gene in a large proportion of patients infected with HCV genotype 1b or 3a receiving IFN-α alone or in combination with ribavirin and followed-up for up to 5 years (Castera et al., unpublished observations, July, 2000).[78,114] In contrast with HVR1, these changes are not related to positive selection pressures. Indeed, most of the mutations are synonymous, *i.e.,* do not induce amino acid changes, suggesting random accumulation of nucleotide mutations over time in the context of ongoing viral replication in patients who failed to clear HCV RNA (a genetic evolution similar to that seen in untreated patients) (Castera et al., unpublished observations, July, 2000).[78,114] Structural constraints caused by NS5A functional properties might partly explain that amino acid mutations are rarely observed.

The 5′ noncoding region of HCV genome contains a stem-loop structure located immediately upstream of the open reading frame and acts as an internal ribosome entry site. The internal ribosome entry site plays a major role in cap-independent translation of the HCV polyprotein.[117,118] It is therefore highly conserved and undergoes no significant quasispecies change during therapy (Soler et al., unpublished observations, July, 2000).

Changes in the Outcome of HCV-Related Liver Disease. In the vast majority of the patients who relapse during or after treatment with IFN-α or the combination of IFN-α plus ribavirin, the relapse is characterized by a transient peak of HCV replication associated with a transient ALT peak. Subsequent evolution is characterized by a return back to lower values, followed by ALT fluctuations and roughly constant viral load (Fig. 2).[79,119] Interestingly, this pattern is identical to that usually observed during acute HCV infection progressing to chronicity[120,121] and to that observed during liver graft reinfection after transplantation for HCV-related end-stage cirrhosis.[122,123] It probably reflects acute reinfection of the liver by the new treatment-selected HCV quasispecies and its subsequent evolution towards chronicity.[79]

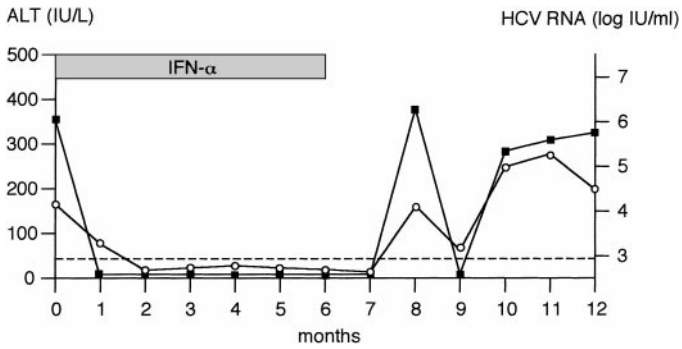

FIG. 2. Typical example of a patient with a virologic response to IFN therapy followed by a relapse. ALT levels are expressed in international units (IU)/mL. HCV RNA was quantified with the reverse transcription-polymerase chain reaction-based Amplicor HCV Monitor assay version 2.0 (Roche Molecular Systems). The HCV RNA loads are expressed in $\log_{10}$ international HCV RNA units (IU)/mL. The relapse was characterized by concomitant peaks of ALT and HCV replication, followed by ALT fluctuations and roughly constant HCV RNA loads in relation to chronic reinfection of the liver by the new selected HCV quasispecies.

In most of the patients who do not achieve a sustained virologic response, pretreatment and posttreatment diseases are thus due to qualitatively different HCV quasispecies, that probably have qualitatively and quantitatively different interactions with host responses. Because the hepatic lesions of chronic HCV are mostly due to the local anti-HCV immune response,[124-126] IFN-induced quasispecies changes could be at least partly responsible for the frequent posttreatment modifications of the outcome of HCV-related liver disease. Short-term outcome changes have been well characterized in the patients who do not achieve a sustained virologic response: they include improvement and stability but also aggravation of liver disease in which qualitative and quantitative quasispecies changes could actually be involved. The role of quasispecies changes in the long-term outcome of HCV disease after therapy (including prevention of cirrhosis and, eventually, of hepatocellular carcinoma[127-130]) needs more definitive evaluation.

CONCLUSIONS

Because of the complex mechanisms of antiviral action of IFN-α and of IFN-α plus ribavirin, the mechanisms underlying HCV resistance to antiviral therapy remain largely hypothetic. The only certainties are (1) that IFN failure to clear HCV is multifactorial, (2) it is characterized by significant qualitative and quantitative changes in the populations of replicating viruses, and (3) these changes can have consequences on the outcome of liver disease after therapy. Lack of a clearly identified viral mechanism precludes development of diagnostic assays to predict and characterize HCV resistance during therapy, such as those currently used to monitor antiretroviral therapy. Detecting HCV RNA and measuring HCV replication remain the only tools to demonstrate treatment failure. Nevertheless, a better knowledge of the pretreatment parameters predicting greater sensitivity or resistance to antiviral therapy has already made it possible to tailor combination treatment duration to HCV genotype and pretreatment viral load.[68] Progress in our understanding of HCV replication dynamics during therapy should enable us to tailor therapy to its actual effect on viral replication in the near future. Finally, development of new drugs that potently inhibit viral replication and maintenance schedules that efficiently prevent viral relapses in patients who cleared HCV RNA during the induction phase of therapy will lead to progressively increasing response ratios, especially in the subgroups of patients with poor therapeutic prognosis.

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