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Review

Mechanisms of antiviral treatment efficacy and failure in chronic hepatitis C

Jean-Michel Pawlotsky*

Department of Virology (EA 3489), Hôpital Henri Mondor, Université Paris XII, 51 Avenue du Maréchal de Lattre de Tassigny, 94010 Créteil, France

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Abstract

The treatment of chronic hepatitis C is currently based on a combination of pegylated interferon (IFN)-α and ribavirin. When successful, this treatment leads to sustained HCV clearance which, in virtually all cases, signifies viral eradication. However, approximately 20% of patients with hepatitis C virus (HCV) genotype 2 or 3 infection, and 50% of patients with genotype 1 infection, fail to eradicate the virus. The risk of treatment failure is related to multiple factors, including the treatment schedule, adherence of therapy, host factors, and the severity of HCV-associated disease. Viral factors can also lead to true "HCV resistance". The mechanisms underlying this resistance are unknown, but indirect evidence suggests that chronic infection is associated with phenomena that protect HCV from the antiviral action of IFN-α and hinder the clearance of infected cells. This article discusses current knowledge of the mechanisms of action of IFN-α and ribavirin, the virological characteristics of chronic hepatitis C treatment success and failure, and possible underlying mechanisms.

Keywords: Hepatitis C virus; Interferon-α; Ribavirin; Resistance; Viral kinetics; Quasispecies

1. Introduction

Over 170 million individuals worldwide are infected by hepatitis C virus (HCV). HCV infection can lead to chronic hepatitis, cirrhosis and hepatocellular carcinoma (Consensus Conference on Hepatitis C, 2002b). HCV has become the first cause of liver cancer in Japan, and the leading indication for liver transplantation in industrialized countries (Consensus Conference on Hepatitis C, 2002b). The biology of HCV, its epidemiology, and the natural history and pathogenesis of infection and associated disease have been recently reviewed (Hoofnagle, 2002; Kim, 2002; Marcellin et al., 2002; Nelson, 2001; Pawlotsky, 2003; Penin et al., 2003; Seeff, 2002). The treatment of chronic hepatitis C is currently based on a combination of pegylated interferon (IFN)-α (an IFN-α molecule linked to a polyethylene glycol molecule to ensure sustained IFN concentrations after single weekly injections) and ribavirin, a synthetic guanosine analog (Consensus Conferences on Hepatitis C, 2002b). When successful, this treatment leads to sustained HCV clearance which, in virtually all cases, signifies viral eradication (McHutchison et al., 2001). In two recent pivotal clinical trials in treatment-naive patients receiving pegylated IFN-α2a or pegylated IFN-α2b combined with ribavirin, treatment failure, defined as persistent HCV replication after the end of treatment, occurred in respectively 24 and 18% of patients infected by genotype 2 or 3, and in 54 and 58% of patients infected by genotype 1 (Fig. 1) (Fried et al., 2002; Manns et al., 2001). Treatment failure is even more frequent in everyday practice: in contrast with large-scale clinical trials, patients are not initially selected according to their lack of comorbidity or the likelihood of adherence to treatment (Falck-Ytter et al., 2002). Failure of IFN-α-based treatment appears to be due to multiple factors, and the underlying mechanisms are still largely unknown. This article discusses current knowledge of the mechanisms of action of IFN-α and ribavirin, the virological characteristics of chronic hepatitis C treatment success and failure, and possible underlying mechanisms.

2. Antiviral mechanisms of interferon-α and ribavirin

2.1. Hypotheses based on viral kinetics

Studies of viral kinetics during antiviral therapy, based on frequent viral load measurements and mathematical

* Tel.: +33-1-4981-2827; fax: +33-1-4981-4831.
E-mail address: jean-michel.pawlotsky@hmn.ap-hop-paris.fr (J.-M. Pawlotsky).

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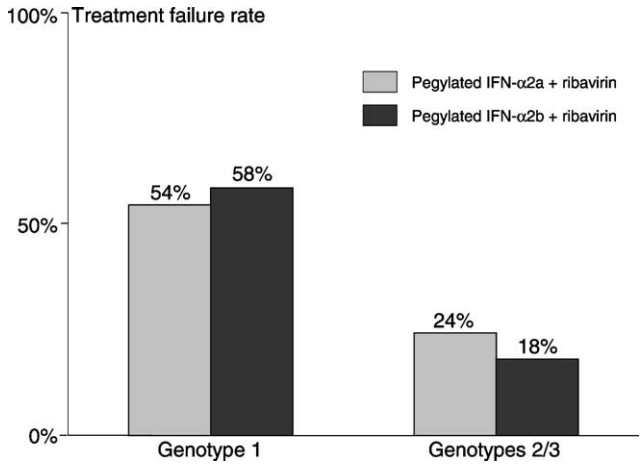

Fig. 1. Incidence of treatment failure (defined as a lack of sustained HCV RNA clearance) according to the HCV genotype in two pivotal clinical trials including 2651 treatment-naive patients with chronic hepatitis C receiving a combination of ribavirin and pegylated IFN-α2a or pegylated IFN-α2b (Fried et al., 2002; Manns et al., 2001).

modeling, have generated hypotheses on the mechanisms of action of antiviral drugs in chronic hepatitis C (Lam et al., 1997; Neumann et al., 1998; Zeuzem et al., 2001). During IFN-α-based treatment, HCV RNA levels generally fall in a biphasic manner (Lam et al., 1997; Neumann et al., 1998). The first, rapid phase of viral suppression starts a few hours after the first IFN-α injection as it lasts until the end of the first day. Mathematical modeling suggests that this first phase is related to an inhibition of viral replication by a direct, non-specific action of IFN-α (Lam et al., 1997; Neumann et al., 1998). The second, slower phase of viral suppression starts on day 2 and may gradually lead to HCV RNA clearance from serum. Mathematical modeling suggests that this second phase is related to gradual clearance of infected cells by the patient's immune system (possibly stimulated by IFN-α), while HCV replication is efficiently inhibited (Lam et al., 1997; Neumann et al., 1998).

2.2. Mechanisms of action of IFN-α

Interferons are natural cellular proteins with a variety of actions, including induction of an antiviral state in their target cells, cytokine secretion, recruitment of immune cells, and induction of cell differentiation. After subcutaneous administration, IFN-α binds specifically to high-affinity receptors at the surface of its target cells. IFN receptor ligation triggers a cascade of intracellular reactions that activate numerous IFN-inducible genes. The products of these genes mediate the cellular actions of IFN-α (Katze et al., 2002). They are responsible for the antiviral effects of IFN-α through two distinct but complementary mechanisms: (1) induction of a non-virus-specific antiviral state in infected cells, resulting in direct inhibition of viral replication; and (2) immunomodulatory effects that enhance the host's specific antiviral immune responses and may accelerate the death of infected cells (Sen and Ransohoff, 1993).

The hypothesis that IFN-α directly inhibits HCV replication, which is based on mathematical modeling of HCV kinetics in vivo (Lam et al., 1997; Neumann et al., 1998), has been confirmed in various in vitro models. IFN-α was shown to inhibit replication of the HCV subgenomic replicon, a synthetic in vitro replication system using HCV non-structural proteins in HuH7 cell culture (Frese et al., 2001; Guo et al., 2001; Lanford et al., 2003). In addition, we recently demonstrated that IFN-α inhibits HCV replication in primary cultures of normal human hepatocytes, the model closest to the naturally infected human liver (Castet et al., 2002). Numerous IFN-induced proteins and enzymatic pathways are involved in establishing an antiviral state in infected cells, but only a few have so far been identified (Sen and Ransohoff, 1993), such as the 2′–5′ oligoadenylate synthetase (2′–5′ OAS) system, Mx proteins, and double-strand-RNA-dependent protein kinase (PKR). It is likely that a combination of several IFN-activated pathways ultimately inhibit HCV replication, but the exact mechanisms are unknown.

The second-phase fall in HCV RNA during IFN-α-based therapy has been suggested to result from gradual clearance of infected cells by the host immune system, which may be accelerated by IFN-α (Lam et al., 1997; Neumann et al., 1998). IFN-α binding to its receptors at the surface of immune cells triggers complex and intricate effects. In particular, IFN-α induces class I major histocompatibility complex antigen expression, activates effector cells such as macrophages, natural killer cells and cytotoxic T lymphocytes, and has complex interactions with the cytokine cascade (Peters, 1996; Tilg, 1997). It also stimulates the production of type 1 T-helper (Th1) cells and reduces the production of Th2 (suppressor) cells (Peters, 1996; Tilg, 1997). However, the role of the immunomodulatory properties of IFN-α in the clearance of infected cells has not yet been convincingly demonstrated in vivo, and it remains possible that infected cells are simply cleared by the normal immune response while IFN-α efficiently suppresses viral replication. The advent of antiviral molecules with negligible immunomodulatory properties should help to resolve this question.

2.3. Mechanisms of action of ribavirin

Ribavirin, a synthetic guanosine analog, was mainly used for the treatment of severe respiratory syncytial virus infection in infants. The mechanisms of ribavirin's antiviral action were recently reviewed (Lau et al., 2002). When added to standard IFN-α administered three times per week, ribavirin increases the initial response, i.e. the proportion of patients who clear HCV RNA while therapy lasts (McHutchison et al., 1998; Poynard et al., 1998). When combined with standard IFN-α administered three times per week or with

pegylated IFN-α administered once a week, ribavirin also markedly reduces the relapse rate after the end of treatment (Fried et al., 2002; Manns et al., 2001; McHutchison et al., 1998; Poynard et al., 1998). The mechanisms underlying these effects are not yet fully understood. Ribavirin, like other antiviral nucleoside analogs, undergoes intracellular phosphorylation, giving rise to ribavirin mono-, di-, and triphosphates. Ribavirin triphosphate is the major intracellular metabolite. It is believed that ribavirin has only a modest antiviral action on HCV in vivo, and that most of its effects are related to its capacity to modulate the immune response and possibly to enhance the action of IFN-α by accelerating the clearance of infected cells.

We recently found that ribavirin monotherapy had a significant but moderate (<0.5 $\log_{10}$ copies/ml reduction) and transient (days 2 and 3 of administration) inhibitory effect on HCV replication, observed in about 50% of patients (Pawlotsky et al., 2000). This effect appears to be additive with that of standard IFN-α administered three times per week, and could explain the more frequent virological responses observed in patients treated with the combination (Pawlotsky et al., 2000). These results are in keeping with the observation that ribavirin weakly inhibits the in vitro replication of bovine viral diarrhea virus (a pestivirus close to HCV), and synergizes the antiviral effect of IFN-α in this model (Lau et al., 2002). Ribavirin triphosphate is misincorporated into the HCV RNA chain by the viral RNA-dependent RNA polymerase, and weakly inhibits this enzyme in vitro (Lau et al., 2002). Finally, ribavirin weakly inhibits the replication of the subgenomic replicon in HuH7 cells, a non-productive in vitro cell culture model of HCV replication. However, as ribavirin can be cytotoxic even at relatively low concentrations, these data must be interpreted with care (Lau et al., 2002). In addition, the mechanisms by which ribavirin may inhibit HCV replication are unclear. Several hypotheses have been raised (Lau et al., 2002). First, ribavirin could act as a nucleotide analog and be misused by HCV RNA polymerase. Second, ribavirin could inhibit the cellular enzyme inosine monophosphate dehydrogenase (IMPDH) and exert its antiviral action by depleting GTP stores. However, other IMPDH inhibitors have been found to have no effect on HCV replication, either in vitro or in vivo. Finally, ribavirin was recently suggested to be an RNA mutagen, driving viral quasispecies to "error catastrophe", i.e. loss of fitness through a lethal acceleration of the accumulation of nucleotide mutations during replication (Crotty et al., 2000, 2001). No evidence that such a phenomenon occurs in HCV infection has yet been obtained.

The principal benefit of ribavirin is that it prevents relapse after IFN combination therapy (Fried et al., 2002; Manns et al., 2001; McHutchison et al., 1998; Poynard et al., 1998). This suggests that ribavirin accelerates the clearance of infected cells. Such an effect would probably be due to the immunomodulatory properties of ribavirin, which could be additive or synergistic with those of IFN-α. It is unclear how ribavirin might modulate the immune response, but there is evidence that it can tilt the Th1/Th2 balance towards Th1 responses, leading to more efficient elimination of infected cells by specific immune effectors (Fang et al., 2000; Hultgren et al., 1998).

3. Viral responses to IFN-α-based therapy

3.1. Viral kinetics

HCV RNA load values measured during and after IFN-α-based treatment categorizes patients into different virological response groups. The main goal of treatment is a "sustained virological response" (SVR), defined as HCV RNA undetectability in serum 24 weeks after the end of therapy. It was recently shown that nearly 98% of patients who achieve an SVR are cured of HCV infection (McHutchison et al., 2001). Patients who do not achieve an SVR are said to be in "treatment failure". Treatment failure can correspond to different virological patterns. "Non-responders" have no significant fall in HCV RNA load (>1 log) at any point of treatment. Partial responders have a significant fall (>1 log) in HCV RNA during therapy, but the viral genome remains detectable in serum. "Responder-relapsers" become serum HCV RNA-negative during treatment but relapse after treatment withdrawal (generally within 24 weeks). Finally, "responders with breakthrough" initially become HCV RNA-negative but relapse during treatment. This categorization lacks precision, because treatment dose reduction or discontinuation, as well as poor adherence to therapy, can play a role together with biological mechanisms (Fried et al., 2002; Manns et al., 2001).

A recent study of viral kinetics has thrown light on virological responses during the first weeks of IFN-α-based treatment. An international group of investigators (Neumann et al., unpublished) recently proposed a four-category patient classification based on viral kinetics during the first 4 weeks of treatment (Fig. 2) with any IFN-α-based schedule. "Rapid viral responders" (RVR) have a significant first-phase (day 1) fall in HCV RNA, followed by a significant second-phase fall, with a slope of at least −0.3 log HCV RNA international units (IU)/ml/week. "Slow partial responders" experience a significant first-phase fall followed by a significant second-phase fall but with a slope of less than −0.3 log IU/ml/week. "Flat partial responders" have a significant first-phase decrease but no second-phase decrease. Finally, "null responders/rebounders" either have no significant first-phase decline (>1 log), or rebound immediately after such a decline (Fig. 2). With the current treatment, most sustained virological responders (SVR) belong to the RVR group of the new classification, while the vast majority of patients in the three non-RVR categories fail to achieve an SVR; however, not all patients with an RVR have an SVR. These different early kinetic patterns of viral clearance in the group of patients who ultimately fail to eradicate the infection point to the existence of different

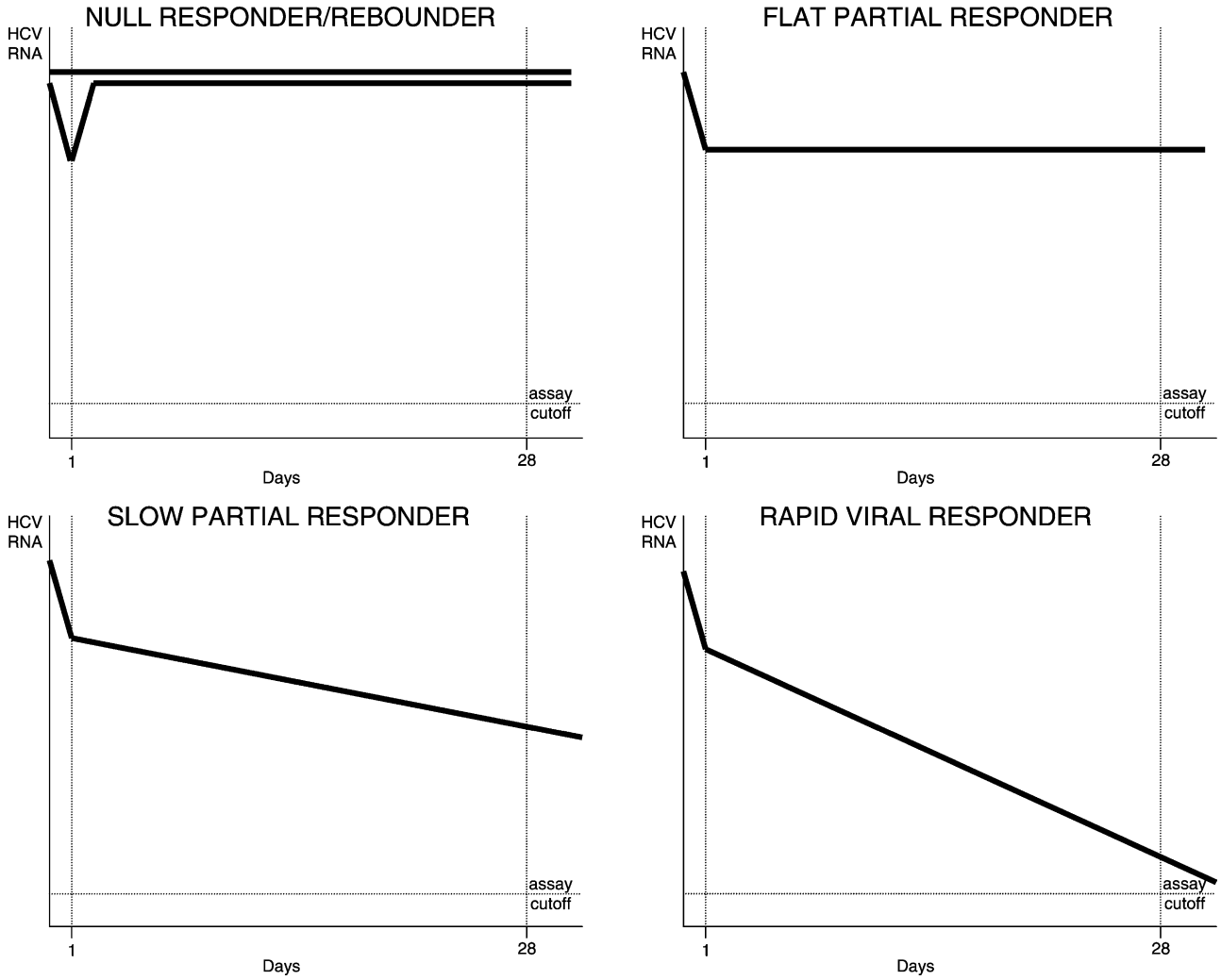

Fig. 2. Nomenclature of early viral kinetics at week 28 in patients with chronic hepatitis C receiving IFN-α-based antiviral therapy (Neumann et al., unpublished). Rapid viral responders are characterized by a second decline in viral load of at least −0.3 log IU HCV RNA/ml/week.

"failure mechanisms". Preliminary results suggest that early viral kinetics are predictive of the ultimate outcome of therapy and could be used to tailor treatment to the individual patient's virological response (Neumann et al., 2002a), but further studies are needed to establish reliable prognostic parameters, based on their positive and negative predictive values.

Simple monitoring of viral kinetics during therapy has successfully been used to predict treatment outcome. HCV RNA assay at baseline and at week 12 of pegylated IFN-α-ribavirin treatment was predictive of SVR in patients infected by HCV genotype 1 (Davis et al., unpublished data) (Fried et al., 2002). A fall in HCV RNA of 2 log or more (i.e. a >100-fold fall in the baseline HCV RNA load) had relatively poor individual positive predictive value for SVR (55–80%), but excellent negative predictive value (98–100%) (Davis et al., unpublished data) (Fried et al., 2002). This implies that patients who do not have at least a 2-log fall in HCV RNA at week 12 have virtually no chance of becoming sustained virological responders. As a result, the recent Consensus Conferences on Hepatitis C (2002a,b) recommended: (i) to continue treatment for a total of 48 weeks in patients with genotype 1 infection who experience at least a 2-log fall in viral load at week 12 and (ii) to withdraw treatment (if its antiinflammatory action is not needed to slow the progression of liver disease) in patients with genotype 1 infection who have a reduction of less than 2 log, as they have virtually no chance of achieving an SVR (Consensus Conference on Hepatitis C (2002a, 2002b). Patients who experience a 2-log reduction at week 12 but who are still HCV RNA-positive in a sensitive HCV RNA detection test at week 24 have a very low probability of SVR. A recent study suggested that such patients might benefit from longer treatment (72 weeks or more), but this needs to be confirmed prospectively (Buti et al., unpublished observations).

3.2. HCV quasispecies evolution

HCV behaves in the infected host as a quasispecies, i.e. a complex mixture of genetically distinct but closely related variants forming coexisting viral subpopulations (reviewed in Pawlotsky (2003)). The viral quasispecies is at a steady state at a given time point. This steady state is influenced by several opposing forces, including (1) continuous accumulation of nucleotide mutations during replication, leading to continuous generation of new variants; (2) selection pressures exerted by the environment in which the virus replicates (particularly the host immune response), which may fluctuate both spontaneously and under the influence of exogenous factors such as antiviral drugs; and (3) conservative constraints relating to essential viral genome and protein functions (Pawlotsky, 2003).

As IFN-α and ribavirin do not specifically target functional components of HCV, such as viral enzymes or the internal ribosome entry site (IRES), they do not exert specific selection pressures on functionally active sites of the corresponding proteins or RNA sequences (Soler et al., 2002a,b). Contrary to lamivudine in chronic hepatitis B or highly active antiretroviral therapy in AIDS, treatment of HCV infection does not select virus variants that are intrinsically IFN-α- or ribavirin-resistant. No particular pattern of viral mutations has been identified in patients failing to achieve SVR, despite extensive sequence analysis of HCV genes during and after therapy (Pawlotsky et al., 1998, 1999; Querenghi et al., 2001; Soler et al., 2002a,b). In addition, re-treatment of patients who have failed to achieve SVR, with the same schedule or a reinforced schedule, can sometimes induce an SVR (Chapman et al., 2001; Shiffman, 2002). This suggests that treatment failure is not due to a failure of IFN-α and ribavirin to clear the infection, but to a failure of the drug-stimulated host immune response to clear infected cells. Thus, viral quasispecies observed after treatment failure are composed of variants capable of escaping numerous treatment-stimulated host antiviral and immune responses.

Quasispecies changes after IFN-α-based treatment failure have been extensively characterized. Hypervariable region 1 (HVR1) is a 27-amino-acid stretch located at the N-terminus of the E2 envelope glycoprotein. HVR1 differs considerably among genotypes and also among strains within a given genotype, and varies substantially during the course of acute and chronic infections, both spontaneously and under the influence of external factors. Profound HVR1 changes are observed during and after IFN-α treatment in most patients with chronic hepatitis C (De Mitri et al., 2000; Farci et al., 2002; Gerotto et al., 1999; Gonzalez-Peralta et al., 1996; Odeberg et al., 1998; Pawlotsky et al., 1998, 1999; Thelu et al., 2001; Polyak et al., 1998). Using genetic and phylogenetic analyses, we showed that these changes are evolutionary and result from strong selection pressures on HVR1 (Pawlotsky et al., 1999). As HVR1 is one of the main neutralizing HCV epitopes, these changes are probably driven by treatment-enhanced humoral responses. However, the physicochemical characteristics of HVR1 residues are globally conserved despite amino acid changes, and fit into a consensus HVR1 sequence that we recently established on the basis of hydrophobic and basic characteristics of the residues (Penin et al., 2001). HVR1 evolution thus appears to be subject to strong conservatory constraints on the physicochemical properties of the residues, related to the need to maintain the conformation and functional role of HVR1 in the replicative cycle (Penin et al., 2001). Other genomic regions are subjected to even stronger conservatory constraints, for the same reasons; examples include the NS5A region, which has an unknown function in the replicative cycle; NS3 serine protease, which catalyzes the proteolysis of most HCV non-structural proteins; and IRES, which is located in the 5′ non-coding region and initializes HCV polyprotein translation (Pawlotsky et al., 1998; Soler et al., 2002a,b). IFN-α treatment does not induce significant evolutionary changes in these regions, but treatment failure has been linked to the emergence of variants bearing sporadic mutations that seldom involve the functional sites of the corresponding protein or IRES structures (Pawlotsky et al., 1998; Soler et al., 2002a,b). These variants appear to result from random accumulation of mutations during replication and to survive because the mutations do not compromise virus viability.

Thus, a sustained virological response would occur when no replication-capable quasispecies variants are able to survive. Post-treatment relapse (or breakthrough during therapy) is generally characterized by a peak of replication that corresponds to reinfection of the liver by the treatment-selected quasispecies (Pawlotsky et al., 1999). Subsequently, the new quasispecies replicates chronically and evolves genetically (Pawlotsky et al., 1999). This probably explains why IFN treatment can profoundly alter the outcome of HCV-associated liver disease—triggering rapid deterioration or significant improvement—even when SVR is not achieved.

4. Causes of treatment failure

Failure of IFN-α-based treatment to eradicate HCV infection is due to a combination of factors, such as a suboptimal treatment regimen, host factors, the type or severity of HCV-related disease, and “viral resistance”.

4.1. Treatment regimen

Since the first clinical trials of IFN-α in chronic hepatitis C in the mid-1980s (Hoofnagle et al., 1986), IFN-α dose regimens have been progressively refined (Fig. 3). Monotherapy with three megaunits of standard IFN-α administered three times a week for 24 weeks was associated with a 90% overall failure rate; this was reduced to 84% when treatment was extended to 48 weeks (McHutchison et al., 1998; Poynard et al., 1998). Addition of ribavirin reduced the overall fail-

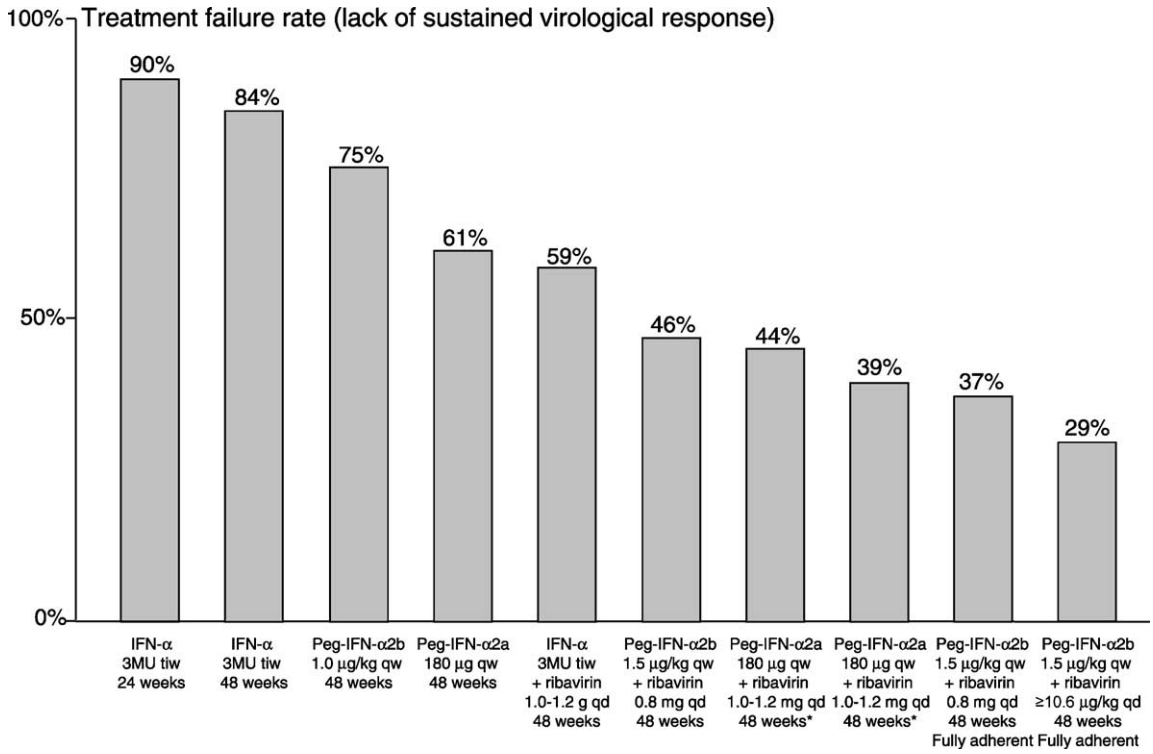

Fig. 3. The incidence of chronic hepatitis C treatment failure has fallen with the optimization of IFN-α administration schedules over the past 10 years (Fried et al., 2002; Hadziyannis et al., 2002; Manns et al., 2001; McHutchison et al., 1998; McHutchison et al., 2002; Poynard et al., 1998). The figure shows global results obtained in populations with different proportions of genotype 1 and genotype 2 or 3 infection. Note that the improvement in sustained virological response rates must be considered genotype by genotype: (∗), results from two different trials with the same drugs and administration schedule; (tiw), three times per week; (qw), once per week; (qd), once per day.

ure rate to approximately 60% (McHutchison et al., 1998; Poynard et al., 1998), and replacement of standard IFN-α three times per week by pegylated IFN-α once a week further reduced the global failure rate by 10% (Fried et al., 2002; Manns et al., 2001). Treatment can be further optimized by adjusting the dose of ribavirin and, in certain instances, that of pegylated IFN-α to body weight, and by full adherence to therapy over the entire treatment period (Hadziyannis et al., 2002; Manns et al., 2001; McHutchison et al., 2002). Overall failure rates of less than 30% have been obtained among patients receiving optimized therapy in the most recent trials; the corresponding failure rates in genotype 1 and genotype 2 or 3 infection were less than 40% and less than 10%, respectively (McHutchison et al., 2002). However, such low failure rates cannot be expected in routine practice, as the patients and treatment conditions differ considerably from those of large clinical trials (Falck-Ytter et al., 2002).

Studies of viral kinetics recently offered explanations for these gradually improving success rates. Standard IFN-α monotherapy was shown to induce a sharp fall in HCV RNA on day 1 (through inhibition of viral replication), followed by a significant rebound before the second injection, on day 2. Few of these patients showed a second phase of decline in viral load associated with subsequent viral eradication (Lam et al., 1997; Neumann et al., 1998; Pawlotsky et al., 2000). Longer treatment was associated with more efficient clearance of infected cells in the few patients who had a significant second decline in viral load, and slightly reduced the relapse rate after treatment withdrawal. The addition of ribavirin significantly increased early virological response rate (McHutchison et al., 1998; Poynard et al., 1998), possibly due, at least in part, to the additive effect of ribavirin's antiviral effect on days 2–3, attenuating the rebound before the second IFN-α injection (Pawlotsky et al., 2000). In addition, ribavirin significantly reduced the rate of post-treatment relapse, probably because it potentiated the clearance of infected cells by the immune system during treatment (Herrmann et al., 2003; McHutchison et al., 1998; Poynard et al., 1998). Finally, the sustained high concentrations obtained after a single weekly administration of pegylated IFN-α exert continuous antiviral and immunomodulatory pressure on the virus (effects apparently potentiated by ribavirin), leading to permanent eradication of infected cells in most patients (Buti et al., 2002; Fried et al., 2002; Herrmann et al., 2003; Manns et al., 2001; Zeuzem et al., 2001).

Can IFN-α-based treatment be further improved? It is possible that the current treatment schedules with pegylated IFN-α and ribavirin (Consensus Conference on Hepatitis C (2002a, 2002b)), when correctly followed, already offer the maximal eradicating rate achievable with these drugs. It has however been suggested that reinforced treatment schedules

during the first few weeks could further increase SVR rates by better inhibiting viral replication and triggering more efficient immune responses. The use of higher pegylated IFN-α doses, more frequent administration, longer treatment periods or other types of IFNs, as well as treatment tailoring to viral kinetics, are currently being tested in prospective clinical trials.

4.2. Host factors

Patient characteristics such as older age, male gender, and certain ethnic origins are associated with higher treatment failure rates (Buti et al., 2002; De Maria et al., 2002; Dev et al., 2002; Fried et al., 2002; Hadziyannis et al., 2002; Kimball et al., 2001; Kinzie et al., 2001; Manns et al., 2001; McHutchison et al., 1998, 2000; Poynard et al., 1998; Reddy et al., 1999). Failure rates show the following ethnic hierarchy: African-Americans > Caucasians > Asians (De Maria et al., 2002; Dev et al., 2002; Kimball et al., 2001; Kinzie et al., 2001; McHutchison et al., 2000; Reddy et al., 1999). Recent data suggest that IFN-α is significantly less effective at blocking viral replication in African-Americans than in Caucasians (Layden et al., unpublished data). Possible reasons include different genetic, hormonal and/or immunologic determinants of HCV sensitivity to IFN-α, and also socioeconomic confounding factors. Body weight can also influence the chances of successful IFN treatment by modifying the volume of distribution of the drug and its final concentration at the receptor level (Hadziyannis et al., 2002; Manns et al., 2001; Pawlotsky et al., 1996; Poynard et al., 2000). The ribavirin dose must be adjusted for body weight; both under-dosing and dose reductions for adverse effects increase the risk of treatment failure (Manns et al., 2001). As regards pegylated IFN-α, according to the manufacturers' recommendations, one of the two commercially available molecules (12 kDa-Peg-IFN-α2b) is administered on a weight-adjusted basis, while the other (40 kDa-Peg-IFN-α2a) is administered at a high fixed dose (Fried et al., 2002; Manns et al., 2001). These differences may be related to different volumes of distribution owing to the different sizes of the polyethylene glycol molecules used.

Various behaviors are also associated with lower response rates, such as active alcohol or intravenous drug use (Edlin, 2002; Peters and Terrault, 2002). The most important behavior is adherence to therapy: the risk of treatment failure is markedly increased by poor adherence (McHutchison et al., 2002).

4.3. Disease-related factors

Some types of HCV-related liver disease, such as advanced fibrosis and compensated cirrhosis, are associated with lower response rates (Fried et al., 2002; Manns et al., 2001; McHutchison et al., 1998; Poynard et al., 1998, 2000). Treatment is contraindicated in patients with decompensated cirrhosis (Consensus Conference on Hepatitis C (2002a, 2002b)). Patients dually infected by HCV and HIV also have lower response rates. This is partly due to unknown intrinsic mechanisms, and partly to poorer tolerance of pegylated IFN-α and ribavirin compared to patients infected by HCV alone (Sulkowski and Thomas, 2003). Large ongoing clinical trials will establish treatment failure rates in HCV/HIV-co-infected patients. Previous treatments were reported to fail more frequently in patients with extrahepatic manifestations of HCV infection such as mixed cryoglobulinemia and membranoproliferative glomerulonephritis than in patients without extrahepatic manifestations, but the published series could have been biased by cohort effects and the current standard of treatment has not been specifically studied in this setting.

4.4. Viral factors ("HCV resistance")

Strong arguments support a role of HCV characteristics in treatment failures, and suggest that true HCV "resistance" to IFN-α therapy can occur. The best argument is the difference between genotypes 1 and 4 on the one hand, and genotypes 2 and 3 on the other hand, in terms of the global results of therapy (Fig. 1) (Fried et al., 2002; Manns et al., 2001; McHutchison et al., 1998; Poynard et al., 1998).

Preliminary data on early HCV kinetics from an ongoing clinical trial of pegylated IFN-α and ribavirin provide clues to the determinants of HCV resistance to IFN-α-based therapy (Neumann et al., 2002b; Pawlotsky et al., 2002b). Viral kinetics were carefully monitored during the first 4 weeks of pegylated IFN-α2a-ribavirin treatment, and differences were observed between the different genotypes in three successive phases (Neumann et al., 2002b; Pawlotsky et al., 2002b). Distinct, complementary mechanisms could explain why genotypes 1 and 4 are less sensitive to treatment than are genotypes 2 and 3, especially if follow-up confirms another recent study suggesting that that early viral kinetics are predictive of final outcome (Neumann et al., 2002a). The principal findings were as follows: (i) The slope of the initial viral decline on day 1 was significantly steeper with genotypes 2 and 3 than with genotypes 1 and 4, suggesting that the latter genotypes are intrinsically more resistant to the direct, non-specific antiviral action of IFN-α. In addition, different IFN-α blocking effectiveness were observed on day 1 in different patients infected by a given genotype. (ii) The vast majority of patients infected by genotypes 2 and 3 had a biphasic decline in viral load, the second phase starting on day 2. In contrast, a significant number of responder patients with genotype 4 and, even more markedly, genotype 1 infection had a kinetic "shoulder" phase preceding the second-phase decline and lasting until the end of the first week of treatment. As the second phase of viral load decline is thought to reflect clearance of infected cells by immune effectors (Neumann et al., 1998), these findings suggest that the immune response may be delayed or otherwise altered in a viral-genotype-dependent manner. (iii) Finally, the slope

of the second phase of viral load decline was significantly steeper in responder patients infected by genotypes 2 and 3 than in those infected by genotypes 1 and 4. This suggests that the half-life of infected cells is longer in genotypes 1 and 4 infection than in genotypes 2 and 3 infection, and that HCV itself can modulate this half-life through direct interactions with cellular mechanisms and/or with immune responses (Neumann et al., 2002b; Pawlotsky et al., 2002b).

Thus, HCV resistance to IFN-α-based therapy appears to be mediated by a combination of HCV-induced alterations of the antiviral and immunomodulatory effects of IFN-α. HCV genotypes are defined on the basis of viral protein sequences and, possibly, their three-dimensional structure (Simmonds, 1999). Although these proteins theoretically support the same functions in the HCV replicative cycle, proteins from different genotypes (or from different patients infected by the same genotype) may display different functional efficiency or may have lost or acquired functions that are not mandatory for virus survival but that could play a role in resistance. The proteins and protein functions that mediate HCV resistance to IFN-α remain to be identified. Several hypotheses have been based on in vitro models testing the role of a given viral protein sequence in a given function. However, none of these interactions, nor their role in treatment failure, has been confirmed in vivo. For instance, a relationship has been reported between the sequence of the central region of the non-structural protein NS5A and the likelihood of sustained virological responses in genotype 1-infected patients, and a meta-analysis of conflicting reports confirmed that viral amino acid sequences differ more markedly from a prototype Japanese HCV 1b sequence in sustained virological responders than in patients who fail to eradicate infection (Witherell and Beineke, 2001). The corresponding 40-amino-acid region was termed the "interferon sensitivity-determining region" (ISDR) on the sole basis of this observed relationship (Enomoto et al., 1995, 1996). More recently, a relationship was found between a lack of sustained virological response and the sequence of a variable region called V3, located in the C-terminal part of NS5A (Nousbaum et al., 2000; Sarrazin et al., 2002). Interestingly, cell culture transfection with the HCV non-structural protein NS5A restores the replicative potential of a surrogate virus in the presence of IFN-α (Gale et al., 1999; Polyak et al., 1999). Results obtained in vitro suggested that this effect could be mediated by direct inhibition of PKR, a potent intracellular effector of the antiviral action of IFN-α (Gale et al., 1998, 1999). However, the role of the NS5A-PKR interaction was not confirmed in another in vitro model (Francois et al., 2000). In addition, we found no relationship between NS5A functional-site amino acid sequences (in particular the so-called ISDR, the PKR-binding site, and the V3 variable region) and IFN-α blocking efficiency on day 1 of treatment (due to the direct antiviral effect of IFN-α) in genotype 1-infected patients (Pawlotsky et al., 2002a). Thus, while an NS5A-PKR interaction may conceivably play a role in HCV resistance to naturally secreted IFN-α in the early stages of acute infection, it is very unlikely that this interaction plays a role in IFN-α-based treatment failure. HCV proteins (especially the core protein) were also suggested to inhibit the Jak-Stat pathway upstream of PKR (Heim et al., 1999) but, once more, no relationship between core sequences and IFN-α blocking effectiveness has been established. More recently, it was reported that NS5A expression in human cell lines induced interleukin 8 (IL-8) mRNA and protein expression, and that this effect correlated with inhibition of the antiviral effects of IFN-α in vitro (Polyak et al., 2001a). In addition, IL-8 serum levels were found to be significantly higher in IFN-non-responders than in responders (Polyak et al., 2001b). Whether or not this interaction exists in vivo and plays a role in treatment outcome remains to be determined. In addition, no information is available on mechanisms underlying potential HCV resistance to ribavirin or to ribavirin-induced immune responses.

In summary, the mechanisms underlying intrinsic HCV resistance to IFN-α-ribavirin treatment remain elusive. Studies of early viral kinetics during treatment indicate that specific viral interactions with both the antiviral and the immunomodulatory effectors of IFN-α are probably involved. The fact that IFN-α monotherapy cures the vast majority of patients with acute-phase HCV genotype 1 infection (Jaeckel et al., 2001), whereas it fails in approximately 50% of patients with chronic genotype 1 infection (Fried et al., 2002; Manns et al., 2001) further suggests that virus-induced modifications of the half-life of infected cells, possibly through direct intracellular interactions and/or interactions with the immune system, are probably key determinants of treatment failure.

5. Conclusion and future perspectives

The treatment of chronic hepatitis C is currently based on the pegylated IFN-α/ribavirin combination. Despite this treatment, approximately 20% of patients with genotype 2 or 3 infection, and 50% of patients with genotype 1 infection, fail to eradicate the virus. The risk of treatment failure is related to multiple factors, including the treatment schedule, adherence to therapy, host factors, and the severity of HCV-associated disease. Viral factors can also lead to true "HCV resistance". The mechanisms underlying this resistance are unknown, but indirect evidence suggests that chronic infection is associated with phenomena that protect HCV from the antiviral action of IFN-α and hinder the clearance of infected cells.

Several new drugs, including specific HCV inhibitors such as protease, helicase, polymerase and IRES inhibitors, are currently in the pipeline or are undergoing phases I and II clinical trials. Unfortunately, these drugs will probably select viral variants bearing resistance-conferring mutations and, eventually, other mutations restoring replication capacity and fitness. It is therefore unlikely that these drugs will rapidly replace IFN-α-based therapy as curative treatments.

They will more likely be used in combination with IFN-α and ribavirin (or ribavirin-like molecules). Efforts to understand the mechanisms underlying failures of IFN-α-based treatment must thus be pursued and amplified.

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