Digestives) trial in steroid-dependent patients¹² and the step-up versus top-down trial¹¹ also demonstrate that much higher rates of steroid-free remission may be obtained with infliximab, at least in the short term, in studies that do not include patients with Crohn disease who have had multiple treatment failures.

Against this evidence, favouring early introduction of anti-TNF treatment, is the observation that the durability of response to biological therapy is limited to a proportion of patients, and some advocate "saving" the drug for situations in which conventional therapies fail, sometimes ignoring that in this situation anti-TNF therapy is also more likely to fail. Proper pharmacoeconomic evaluation of therapeutic strategies is also urgently required. For years, we have had only the infliximab bullet, and when the effects of the shot waned, many patients faced complications or surgery. Now our gun is loaded with two more bullets that have been shown to regain the benefits of anti-TNF therapy in a proportion of patients after response to the first one has faded. In the GAIN (Gauging Adalimumab efficacy in Infliximab Nonresponders) induction study, 21% of patients who had lost previous response to or were intolerant of infliximab went into remission at week 4 after (2 weeks induction dose of adalimumab, then 160 mg followed by 80 mg).¹³ Admittedly, none of these bullets is quite magic, but these will be of great help in

modifying the disease course in a proportion of our patients, especially if introduced early in the disease course.

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Fatty liver in chronic hepatitis C infection

Fatty liver in chronic hepatitis C infection: unravelling the mechanisms

Jude A Oben, Emma Paulon

A step closer to understanding fatty liver due to HCV

Chronic hepatitis C virus (HCV) infection afflicts about 200 million people worldwide and is presently the most common cause of cirrhosis and hepatocellular carcinoma in Western countries. Over the last decade, an association between HCV infection, hepatic steatosis, insulin resistance (IR) and type II diabetes has been highlighted, although the cause–effect relationship underlying the co-existence of these phenomena has yet to be completely clarified.¹ These observations are important because the presence of steatosis and/or IR

in chronic HCV infection appears to modulate the progression of fibrosis and the response to antiviral therapy in chronic HCV infection.^{2–4}

Mechanisms underlying steatosis development in chronic HCV infection have been demonstrated to be genotype specific, with an apparent direct steatogenic effect of genotype 3, and an IR-associated steatosis effect exerted by genotype 1.^{5,6} The direct steatogenic effect of HCV genotype 3 is confirmed by findings such as those showing a correlation between HCV genotype 3 RNA level and steatosis

in chronically infected patients and the resolution of steatosis in genotype 3-infected patients after response to antiviral treatment.^{5–7}

Several mechanisms whereby HCV infection can directly cause steatosis have been described. HCV can affect de novo fatty acid biosynthesis, triglyceride (TG) assembly and secretion, and lipid peroxidation. HCV core protein has also been shown to localise in the periphery of TG-rich lipid droplets and on the cytosolic surface of the endoplasmic reticulum (ER) membrane, and it might physically interfere with lipids and other proteins involved in very low-density lipoprotein (VLDL) assembly.⁸ Lipid secretion can be altered by the effect of HCV on microsomal triglyceride transfer protein (MTP) and apolipoproteins. MTP is an enzyme that regulates VLDL assembly and its activity is impaired by HCV core protein in core-transgenic mice.⁹

In fact, in chronic HCV infection, hypo-betalipoproteinaemia is described, especially in patients infected with genotype 3.^{10,11} De novo fatty acid biosynthesis

is altered in chronic hepatitis C, and HCV appears to enhance the expression and activity of several enzymes involved in this process, such as fatty acid synthase (FAS) and acetyl-CoA carboxylase 1 (ACC1).¹² HCV genotype 3 in particular has been shown to induce transcription of stearoyl coenzyme A desaturase (SCD4), a rate-limiting enzyme in the synthesis of mono-unsaturated fats.¹³ In addition, core protein can cause mitochondrial injury and oxidative stress, with a consequent effect on lipid peroxidation.¹⁴

If on the one hand fat accumulation, steatosis, may have a role in the development of IR,¹⁵ and on the other hand IR is usually associated with genotype 1 (and not genotype 3), then the necessity arises to investigate a possible direct link between HCV genotype 1 infection and IR, ignoring for a moment what these two conditions may have as a common feature. HCV genotype 1 and the inflammatory response secondary to the infection can also play a role in the development of IR. HCV core protein has been observed to decrease the expression of the insulin receptor substrate (IRS)-1 and IRS-2, and to suppress insulin-induced phosphorylation of the p85 subunit of phosphatidylinositol 3-kinase (PI3K) and Akt, and activation of 6-phosphofructo-2-kinase, and to reduce glucose uptake.¹⁶ Tumour necrosis factor (TNF) α and the suppressor of cytokine signalling 3 (SOCS3), expressed as a consequence of the inflammatory response to HCV, suppress tyrosine phosphorylation of IRS-1 and IRS-2, to cause IR.^{17,18} Moreover, IRS-1 and IRS-2 down-regulation exerted by HCV core protein seems to be mediated by SOCS3.¹⁶ The effect of HCV on SOCS3 is particularly important in light of SOCS involvement in the regulation of expression of the sterol regulatory element-binding protein 1c (SREBP-1c). This transcription factor is a key regulator of lipogenesis in the liver and its activity is enhanced in the presence of IR and chronic hepatitis C infection.¹⁹

To investigate further the mechanisms through which HCV genotypes may differentially affect fat deposition in the liver, Jackel-Cram *et al*²⁰ studied de novo lipid synthesis and TG accumulation in hepatoma cells (Huh7) transfected with HCV 3a and 1b core genes, with a readout of the effect of these genes on FAS expression. Using immunofluorescence, they first confirmed that HCV-3a core protein, as previously demonstrated for HCV-1, localised on lipid droplets. Then they analysed FAS activity in the presence of HCV-3a and 1b core proteins. FAS plays a major role in fatty acid synthesis and its transcription is under the control of SREBP-1.²¹ To measure FAS expression they used a FAS promoter-luciferase

reporter, containing the SRE site, under the direct control of SREBP-1.

They showed that HCV core proteins significantly enhanced luciferase activity regulation by the FAS promoter, demonstrating an HCV effect on FAS expression. This increased FAS transcription was under SREBP-1 control, as FAS promoter-luciferase activity was undetectable when a FAS promoter-luciferase reporter with the SREBP-binding site deleted was used. In addition, they showed that the effect on FAS was greater for genotype 3a than for genotype 1b. These results are consistent with the already known strong association between genotype 3 and steatosis, as >70% of patients infected by this strain present with steatosis, compared with 40% of patients infected by HCV genotype 1.²

To understand better the relationship between core protein processing, lipid droplet accumulation and FAS up-regulation, the authors then altered the core protein structure. They demonstrated that unprocessed or fully processed forms of core protein can have different effects on FAS regulation and that reduced lipid droplet accumulation does not always correlate with reduced FAS up-regulation. On the other hand, nuclear localisation of fully processed core 3a protein prevented lipid droplet localisation as well as up-regulation of FAS, confirming further that these processes must somehow be related and that they take place in the cytoplasm.

Finally, experiments were performed to correlate genotype-specific sequences and genotype-related effects on lipid droplet localisation and FAS regulation. These studies demonstrated that certain sequences, namely 164YATG167/164FATG167 and 138PLVGAP143, are important for both genotypes in determining core protein lipid localisation, although deletion of these sequences does not always result in reduced FAS up-regulation. More importantly, they demonstrated that phenylalanine 164 of the genotype 3a core might be essential in determining the more potent effect of genotype 3a on FAS up-regulation, and hypothesised that this molecular mechanism could account for the direct steatogenic effect of HCV-3a.

Hourioux *et al*²² have continued this line of thought in this present issue (*see page 1302*), and further enhanced our understanding of the mechanisms of fatty liver in chronic HCV infection. It must of course be added that the above work by Jackel-Cram *et al* has only just been published in electronic form and was clearly unavailable to Hourieux and colleagues when their studies began. Moreover, the similar findings give us

confidence that we are indeed unravelling the mechanisms of hepatic steatosis in HCV infection.

The strategy adopted by Hourieux *et al* in their study²² again involved an in vitro cellular model, in this case using baby hamster kidney (BHK-21) cells. They investigated whether an HCV core protein with residues that were specific for genotype 3 increased the induction of lipid droplet accumulation in the BHK-21 cells. To begin with, Hourieux *et al* first compared the core protein sequences of 266 haplotypes, comprising the six HCV genotypes including their 25 subtypes. They found that at position 164 there was a phenylalanine (F) residue in the majority of genotype 3 sequences, whereas in all other genotypes, position 164 was occupied by a tyrosine (Y) residue. They then hypothesised that if this 164F residue was important for lipid accumulation, then replacing Y at position 164 with F in the known weakly steatogenic genotype 1a—that is, Y164F—should lead to a greater lipid accumulation induction by the mutant genotype 1a core protein.

The experimental design was then to transfect BHK-21 cells with the native (164Y) or mutant (164F) genotype 1a core proteins. The extent of lipid droplet accumulation induced by both types of constructs was then analysed by electron microscopy having first confirmed by confocal microscopy as above²⁰ that the HCV core proteins co-localised with the lipid droplets. Their results show that the extent of lipid droplet accumulation in cells expressing the mutant core protein was markedly greater than in cells expressing the wild-type core protein. They concluded that the F residue at position 164 is more steatogenic than Y at position 164. The explanation offered by the authors is biophysical, and plausible, and it is that the phenylalanine has a greater affinity for lipids than tyrosine and, as such, phenylalanine moieties act as a nucleus for lipid droplet accumulation.

Understanding the mechanisms of fatty liver in chronic HCV infection is of more than academic interest, in light of the recognised need to increase the response rate to antiviral treatment of patients affected by HCV and steatosis, who have been shown to be less responsive than patients without steatosis.²⁻⁴ If by reducing steatosis in genotype 3 we can enhance the response rates above the approximately 80–85% currently quoted for genotype 3 and above the approximately 50–55% for genotype 1, then our patients clearly stand to benefit. In addition, preventing steatosis and IR associated with chronic hepatitis C will allow us to reduce the inflammation and liver

damage that these conditions would cause in addition to that induced by HCV.

Finally, as steatosis is associated with IR in genotype 1-infected patients but not in those infected with genotype 3,^{5,6} sequence-specific effects on pathways responsible for IR need now to be investigated, in order to understand better the contribution of viral factors to IR in these patients. Given that in both genotypes the co-existence of steatosis correlates with a poor response outcome to antiviral treatment,^{23,24} understanding the molecular mechanisms that explain steatosis development in both genotypes would allow us to target antiviral treatment better, and perhaps the time is near when adjuvant anti-steatosis therapy will be a component of routine anti-HCV therapy. The present study is another welcome step in this worthy direction.

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