

Hepatitis C Virus Infection and Human Pancreatic β -Cell Dysfunction

MATILDE MASINI, MD¹
 DANIELA CAMPANI, MD²
 UGO BOGGI, MD³
 MICHELE MENICAGLI, MD²
 NICOLA FUNEL, MD¹
 MARIA POLLERA, MD¹
 ROBERTO LUPI, PHD¹

SILVIA DEL GUERRA, PHD¹
 MARCO BUGLIANI, PHD¹
 SCILLA TORRI, PHD¹
 STEFANO DEL PRATO, MD¹
 FRANCO MOSCA, MD³
 FRANCO FILIPPONI, MD⁴
 PIERO MARCHETTI, MD, PHD¹

Many patients with chronic hepatitis C virus (HCV) develop type 2 diabetes (1). This prevalence is much higher than that observed in the general population and in patients with other chronic liver diseases such as hepatitis B virus, alcoholic liver disease, and primary biliary cirrhosis. Furthermore, it has been shown that post-transplantation type 2 diabetes appears to be higher among patients with HCV (2). However, the pathogenetic basis for the association between HCV infection and diabetes has not been understood. A direct involvement of the virus in the development of insulin resistance has been proposed, and β -cell dysfunction in HCV-positive patients has been observed in some cases (1). Because HCV can infect many tissues other than the liver (3), we hypothesized that the virus might directly damage insulin-secreting cells. This article suggests that HCV may be present in human pancreatic β -cells and demonstrates that islet cells from HCV-positive patients have morphological and functional defects.

RESEARCH DESIGN AND METHODS

The pancreases of 5 HCV-positive (age 68 ± 9 years, 3 men

and 2 women, BMI 25.8 ± 1.6 kg/m²) and 10 HCV-negative (age 67 ± 9 years, 6 men and 4 women, BMI 26.8 ± 2.0 kg/m²) donors were harvested and studied with the approval of our local ethics committee. Histological studies were performed by immunohistochemistry (using the monoclonal mouse anti-HCV E2 protein, clone IGH222 [Innogenetics, Gent, Belgium]) and electron microscopy, as described elsewhere (4,5). Isolated islets were prepared by enzymatic digestion and density gradient purification, and islet functional and survival studies were accomplished as previously described (5,6).

RESULTS— Histology results are summarized in Fig. 1. No sign of islet cell staining was found in HCV-negative pancreases by immunohistochemistry (Fig. 1A); however, focal or diffuse HCV-positive islet cells were observed in HCV-positive pancreatic glands (Fig. 1B). Positive staining was found in $39 \pm 12\%$ of 140 examined islets, and the percentage of stained cells was $54 \pm 13\%$ per islet. The appearance of a control β -cell at electron microscopy is given in Fig. 1C,

showing the characteristic insulin granules and normally preserved mitochondria. In β -cells from HCV-positive pancreases, the presence of virus-like particles was observed, mainly close to the membranes of Golgi apparatus, which, in turn, appeared hyperplastic and dilated (Fig. 1D). The mitochondria appeared round-shaped with dispersed matrix and fragmented cristae (Fig. 1D). Additional β -cell changes were observed at the level of rough endoplasmic reticulum, which showed long and dilated tubular membranes, with numerous electron-dense ribosomes bound to the latter (not shown). These morphological changes were accompanied by reduced in vitro glucose-stimulated insulin release (Table 1); however, apoptosis was similar in control as in infected islet cells (Table 1).

CONCLUSIONS— Approximately 40% of patients with HCV infection will display symptoms of some extrahepatic manifestation during the illness (1). Most extraliver manifestations of chronic HCV infection are immunological; however, the virus may have a direct cytopathic action, because it can infect many tissues other than the liver (3). In the present article we have suggested the presence of HCV infection in pancreatic β -cells of human subjects, and we have provided evidence that this was associated with morphological cell changes and altered islet cell function. The immunohistochemical method we have used to show the presence of infection in islet cells has been previously validated (4), and the electron microscopy morphological alterations of the β -cell are similar to those reported in other cell types during HCV infection (7). The insulin secretion functional defects of islets from HCV-positive donors might contribute to the development of diabetes in predisposed subjects. On the other hand, the absence of increased apoptosis is in line with the observation that reducing viral load is associated with improvement of diabetes in HCV-positive patients (8). In conclusion, the present article proposes that HCV can infect human pancreatic β -cells and that this is accompanied

From the ¹Metabolic Unit, Department of Endocrinology and Metabolism, University of Pisa and Pisa University Hospital, Pisa, Italy; the ²Section of Transplantation Pathology, Division of Surgical, Molecular and Ultrastructural Pathology, Department of Oncology, University of Pisa and Pisa University Hospital, Pisa, Italy; the ³Referral Center for the Treatment of Pancreas Diseases, Department of Oncology, University of Pisa and Pisa University Hospital, Pisa, Italy; and the ⁴Liver Transplant Unit, University of Pisa and Pisa University Hospital, Pisa, Italy.

Address correspondence and reprint requests to Piero Marchetti, MD, Department of Endocrinology and Metabolism, Metabolic Unit, Ospedale Cisanello, Via Paradisa 2, 56124 Pisa, Italy. E-mail: marchant@immr.med.unipi.it.

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Abbreviations: HCV, hepatitis C virus.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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Table 1—Insulin secretion and apoptosis data of HCV-negative and HCV-positive pancreatic islets

	Insulin release (% insulin content)		Apoptosis	
	3.3 mmol/l glucose	16.7 mmol/l glucose	ELISA*	EM†
HCV ⁻	1.7 ± 0.3	3.8 ± 0.5‡	0.9 ± 0.1	2.0 ± 0.2
HCV ⁺	1.6 ± 0.3	2.9 ± 0.4‡§	1.0 ± 0.2	1.8 ± 0.2

Data are means ± SD. *Data are expressed as arbitrary units of optical density; †data are expressed as percentage of apoptotic β -cell over total number of counted β -cells; ‡ $P < 0.01$ vs. 3.3 mmol/l and § $P < 0.05$ vs. 16.7 mmol/l glucose, HCV⁻ by the two-tailed Student's *t* test. ELISA, enzyme-linked immunosorbent assay; EM, electron microscopy.

by β -cell dysfunction. A direct cytopathic effect of HCV at the islet cell level is there-

fore suggested to explain, at least in part, the association between HCV infection

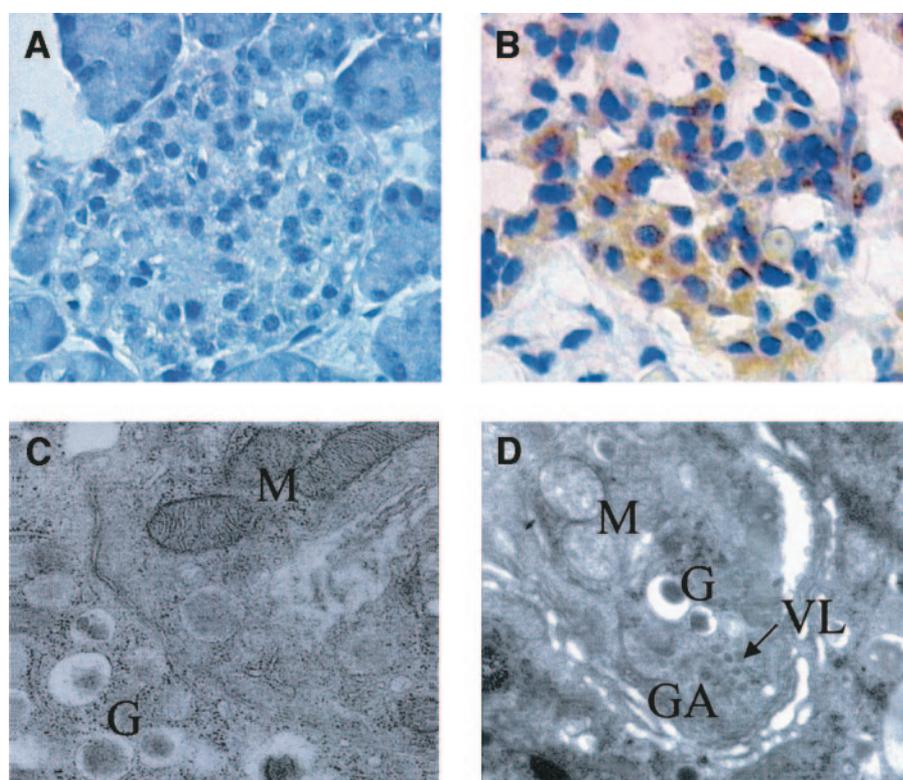


Figure 1—A and B show the results obtained by an immunoperoxidase technique for anti-HCV-E2 in pancreatic islets (original magnification $\times 400$). In A, the endocrine cells from a control pancreas are completely devoid of the viral antigen. In B, the endocrine cells from an HCV-positive pancreas show a brown, finely granular staining, indicating the presence of the HCV proteins. C and D show the results obtained by electron microscopy (original magnification $\times 46,000$). In C, a control β -cell is shown, with the characteristic insulin granules (G) and normal mitochondria (M). In D, a β -cell from an HCV-positive pancreas is represented, showing virus-like particles (VL) close to a dilated, hyperplastic Golgi apparatus (GA) and round-shaped mitochondria with dispersed matrix and fragmented cristae.

and diabetes, especially in predisposed subjects (1).

References

1. Lecube A, Hernandez C, Genesca J, Esteban JI, Jardi R, Simo R: High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. *Diabetes Care* 27:1171–1175, 2004
2. Bruchfeld A, Wilczek H, Elinder CG: Hepatitis C infection, time in renal-replacement therapy, and outcome after kidney transplantation. *Transplantation* 78:745–750, 2004
3. Mayo MJ: Extrahepatic manifestations of hepatitis C infection. *Am J Med Sci* 325:135–148, 2003
4. Verslype C, Nevens F, Sinelli N, Clarysse C, Pirenne J, Depla E, Maertens G, van Pelt J, Desmet V, Fevery J, Roskams T: Hepatic immunohistochemical staining with a monoclonal antibody against HCV-E2 to evaluate antiviral therapy and reinfection of liver grafts in hepatitis C viral infection. *J Hepatol* 38:208–214, 2003
5. Marchetti P, Del Guerra S, Marselli L, Lupi R, Masini M, Pollera M, Bugliani M, Boggi U, Vistoli F, Mosca F, Del Prato S: Pancreatic islets from type 2 diabetic patients have functional defects and increased apoptosis that are ameliorated by metformin. *J Clin Endocrinol Metab* 89:5535–5541, 2004
6. Marchetti P, Lupi R, Federici M, Marselli L, Masini M, Boggi U, Del Guerra S, Patanè G, Piro S, Anello M, Bergamini E, Purrello F, Lauro R, Mosca F, Sesti G, Del Prato S: Insulin secretory function is impaired in isolated human islets carrying the Gly(972)→Arg IRS-1 polymorphism. *Diabetes* 51:1419–1424, 2002
7. Falcon V, Acost-Rivero N, Chinae G, Gavilondo J, de la Rosa MC, Menendez I, Duenas Carrera S, Vina A, Garcia W, Gra B, Noa M, Reytor E, Barcelo MT, Alvarez F, Morale-Grillo J: Ultrastructural evidences of HCV infection in hepatocytes of chronically HCV-infected patients. *Biochem Biophys Res Commun* 305:1085–1090, 2003
8. Bahtiyar G, Shin JJ, Aytaman A, Sowers JR, McFarlane SI: Association of diabetes and hepatitis C infection: epidemiologic evidence and pathophysiologic insights. *Curr Diab Rep* 4:194–198, 2004