Therapeutic Vaccination of Chronic Hepatitis C Nonresponder Patients With the Peptide Vaccine IC41

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Background & Aims: **IC41 is a synthetic peptide vaccine containing 7 relevant hepatitis C virus (HCV) T-cell epitopes and the T helper cell (Th)1/Tc1 adjuvant poly-L-arginine. IC41 has been shown to be safe and to induce HCV-specific interferon (IFN)-γ-secreting CD4+ and CD8+ T cells in healthy volunteers. We aimed to investigate whether IC41 is able to induce HCV-specific T-cell responses also in chronic hepatitis C patients.** ***Methods:*** **Sixty HLA-A2-positive chronic HCV patients not responding to or relapsing from standard therapy were randomized in a double-blind phase II study into 5 groups to receive 6 vaccinations of IC41 (3 different dose groups), HCV peptides alone, or poly-L-arginine alone.** ***Results:*** **IC41 was well tolerated, and no drug-related serious adverse events or induction of hepatitis were observed. T-cell proliferation was recorded in up to 67% of patients in the 3 IC41 vaccine groups but only in 17% of patients treated with peptides alone. IFN-γ enzyme-linked immunospot assay responses were observed exclusively in the IC41 groups with response rates up to 42%. There were 3 RNA responders with transient >1-log declines of HCV serum RNA associated with the strongest IFN-γ enzyme-linked immunospot assay values within all 60 patients.** ***Conclusions:*** **This study showed that the HCV peptide vaccine IC41 can induce HCV-specific Th1/Tc1 responses in a subset of difficult to treat HCV nonresponder patients despite persisting viremia. However, changes in HCV RNA occurred only in single patients. Because strongest T-cell responses were associated with HCV RNA decline, further studies with optimized vaccine regimens and combination therapies have been initiated.**

Pegylated interferon (IFN)/ribavirin combination treatment elicits a sustained response, defined as lack of detectable viremia 6 months after end of treatment, in up to 80% of patients infected with genotypes 2 and 3 but only in 43% to 50% of patients infected with genotype 1, which is the most prevalent in Europe, the United States, and Canada.[1] In addition to limited efficacy, standard therapy is associated with substantial adverse effects, severely influencing the quality of life of the patients under treatment and considerable costs resulting from long-term pegylated IFN/ribavirin combination treatment.[1] Furthermore, a large number of HCV-infected patients are not suitable candidates for antiviral IFN-based therapy for reasons including nonadherence to evaluation procedures; medical or psychiatric contraindications; ongoing substance or alcohol abuse; or patient preference after considering possible adverse effects, duration of treatment, family planning issues, and route of administration.[2] Because of these limitations of standard therapy, new treatment options for chronic HCV infection are urgently required. This study was designed to evaluate the safety and immunogenicity of IC41, a novel vaccine designed to induce HCV-specific T-cell immunity.[3]

Primary HCV infection can cause broad and multispecific both CD4+ and CD8+ T-cell responses during acute infection. It has been reported that stronger, broader, and more sustained T helper cell (Th)1/Tc1 (IFN-γ) responses are associated with resolving infection.[4] Indeed T-cell responses can readily be detected in humans in the absence of viremia many years after clearing infection.[5–11] Although chronically infected patients also show some IFN-γ responses, these tend to be weaker and directed against less epitopes.[9,11] In addition, HCV-specific T cells appear functionally impaired in chronic infection.[12,13] The high mutation rate of an RNA virus and the existence of quasispecies in the same individual facilitate mutational epitope escape mechanisms that can undermine productive T-cell responses.[14] Additional potential immune deviations in chronic HCV include dysfunction of dendritic cells[15–17] and suppressor T cells.[18–21] Importantly, IFN-α-based therapy may

Abbreviations used in this paper: CTLs, cytotoxic T lymphocytes; IFN, interferon; PBMC, peripheral blood mononuclear cells; SI, stimulation index; Tc1, cytotoxic T cell; Th, T helper cell.

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Table 1. Patient Baseline Characteristics

	Vaccine groups n = 36		Control groups n = 24		Total n = 60	
Age (y), mean	46.1 (range, 23–63)		47.3 (range, 23–65)		46.5 (range, 23–65)	
Sex, n (%)						
Male	20	55.6	16	66.7	36	60.0
Female	16	44.4	8	33.3	24	40.0
Race, n (%)						
White	35	97.2	24	100.0	59	98.3
Oriental	1	2.8	0	0.0	1	1.7
Weight (*kg*), mean	74.6 (range, 50–99)		75.7 (range, 57–105)		75.0 (range, 50–105)	

not necessarily restore cellular immune responses in acute and chronic hepatitis C infection.[22–24]

For these reasons, eliciting anti-HCV immune response based on the induction of epitope-specific CD8+ cytotoxic T lymphocytes (CTLs) and CD4+ responses may be highly beneficial for an HCV-infected patient. It may be advantageous to concentrate on well-conserved regions within the HCV polyprotein. Choosing CTL epitopes restricted to HLA-A*0201 is mandated because most knowledge has accumulated for these epitopes, and the allele is frequently expressed in approximately 45% of white populations.[25] IC41 was designed to contain 5 synthetic peptides from core, NS3, and NS4. They harbor at least 4 HLA-A*0201 restricted CTL epitopes and 3 highly promiscuous CD4+ helper T-cell epitopes, all of which have been described in detail[26] and were independently confirmed to be targeted in patients responding to standard treatment or spontaneously recovering from HCV (own unpublished data, Klade, 2004). With one exception, peptide sequences are highly conserved in genotype 1. T helper epitopes for the peptides included in the IC41 vaccine have been shown to be active in the context of at least HLA-DRB1*0101, DRB1*0401, DRB1*0404, DRB1*0408, DRB1*0701, DRB1*0802, DRB1*0901, DRB1*1101, DRB1*1302, DRB1*1501, and DRB5*0101. Collectively, these account for at least 70% to 80% population coverage. IC41 contains poly-L-arginine as synthetic adjuvant, which has been shown to augment Th1/Tc1 (IFN-γ) responses in animal studies.[27–31] Data from a phase I study with IC41 indicated that administration of the vaccine is safe and well tolerated and that IC41 can induce HCV-specific Th1/Tc1 responses in healthy volunteers.[3] Here, we report a double-blind, randomized, multicenter, phase II study of immunization with IC41 HCV peptide vaccine together with poly-L-arginine, HCV peptide vaccine alone, or poly-L-arginine alone, in 60 patients with chronic HCV who did not respond to or relapsed from primary standard HCV therapy.

Materials and Methods

Study Design and Patients

This study was a double-blind, randomized, parallel group, multicenter comparison of immunization with IC41, HCV peptide vaccine alone, and poly-L-arginine alone, in HLA-A2-positive patients with chronic HCV who did not respond to or relapsed from primary standard HCV therapy. Inclusion criteria included a documented course of chronic hepatitis C (HCV RNA and antibody positive) for at least 6 months, nonresponse/relapse from primary standard HCV therapy of 6 to 12 months, and liver biopsy within 30 months before inclusion, demonstrating hepatic inflammation and/or fibrosis. Major exclusion criteria included cirrhosis or fibrosis with Ishak score ≥4 and any liver disease other than hepatitis C (Table 1). The IC41 HCV vaccine (Intercell AG, Vienna, Austria) contains 5 synthetic peptides (Ipep83, 84, 87, 89, 1426) derived from HCV genotype 1 core23–44 and 132–140, NS3 1073–1081 and 1248–1261, and NS4 1764–1786. These peptides contain 4 HLA-A*0201 CTL epitopes (core35–44 and 132–140, NS3 1073–1081, NS4 1764–1772) and 3 helper epitopes (core23–44, NS3 1248–1261, NS4 1767–1786). Sequences are conserved in the most prevalent HCV genotypes 1a (100%, 100%, 83%, 100%, 100% for the respective 5 peptides), 1b (98%, 90%, 15%, 94%, 88%), and 2 (91%, 96%, 13%, 91%, 87%). Three different formulations of IC41, peptide vaccine alone, and poly-L-arginine alone as controls were tested in different groups (Table 2). Six vaccinations were administered as a 0.5-mL subcutaneous injection in the upper arm every 4 weeks, and 3 follow-up visits 4, 12, and 24 weeks after the last vaccination were done. Peripheral blood mononuclear cells (PBMC) for T-cell analysis were obtained at baseline; at the time of the fourth, fifth, and sixth vaccination; and at the 3 follow-up visits. Objectives of the study were (1) to determine the immunologic profile (HCV peptide-spe-

Table 2. Dosing Groups

	Peptide dose/injection	Poly-L-arginine dose/injection
Vaccine groups (n)		
Group 1 (12)	2.5 mg	1.25 mg
Group 2 (12)	2.5 mg	2 mg
Group 3 (12)	5 mg	2 mg
Control groups (n)		
Poly-L-arginine (12)	0 mg	2 mg
Peptides (12)	5 mg	0 mg