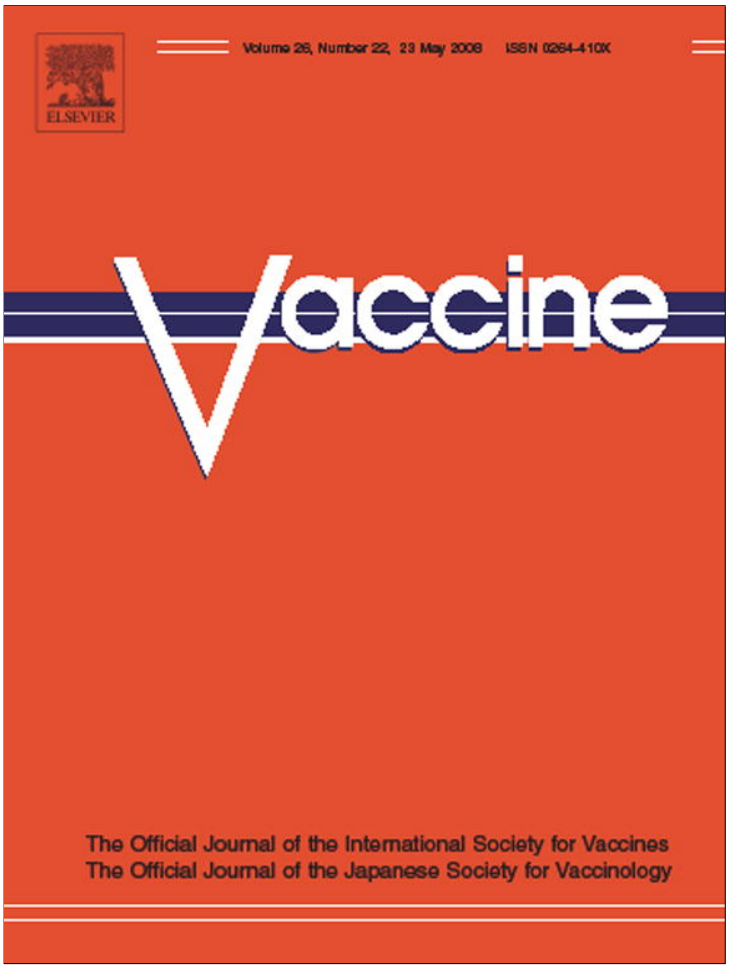

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Open-label trial of therapeutic immunization with oral V-5 Immunitor (V5) vaccine in patients with chronic hepatitis C

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Summary We evaluated whether V-5 Immunitor (V5) — tableted therapeutic bivalent vaccine comprising heat-inactivated HCV antigens from pooled blood of HBV- and HCV-infected donors — may produce clinical benefit through induction of oral tolerance and reduction of immune-mediated liver injury. Once daily dose of V5 was administered *per os* to 10 patients with chronic hepatitis C in an open-label study that lasted 1 month. Every patient who entered the study had elevated liver enzyme levels, which at the end of study have decreased in 100% of analyzed patients. The reduction was highly significant, from 157.7 ± 73.4 to 49.9 ± 43.8 U/L ($P = 0.0013$) and 147.0 ± 79.2 to 58.7 ± 56.6 U/L ($P = 0.0132$), for ALT and AST, respectively. The AST/ALT ratio has improved from 0.93 to 1.18 ($P = 0.00058$) indicating the reversion of progression to cirrhosis. None of intent-to-treat patients who were anti-HCV antibody positive at study entry, became negative after 1 month on V5 ($P = 0.998$). All patients, except one, reported complete recuperation from hepatitis C-associated clinical symptoms present at baseline ($P = 0.0016$) with Mantel Haenszel's odds ratio 9.4 ($P = 0.0021$) at 95% confidence interval: $2.7 < \text{OR} < 476.3$. No adverse events were observed at any time. The favorable biochemical and clinical responses have been observed in a small number of individuals for a limited time period. Larger scale and longer studies are needed to confirm our preliminary observations suggesting that V5 is safe and effective means for immunotherapy of chronic hepatitis C.

Introduction

Hepatitis C virus (HCV) is a global public health problem, infecting estimated 120–180 million people [1,2]. Mongolia has the highest reported rate of HCV infection in Asia; anti-HCV antibodies are found in 10–48% of adult popula-

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tion [3—5]. Approximately 85% of acute infections progress to chronic persistence of HCV and about 25% of these chronically infected individuals develop fatal liver diseases. Currently, there is no prophylactic vaccine to prevent the disease and no specific antiviral drug controlling HCV replication [1,2].

The current standard of care are interferon alfa-2a (Roferon-A) and interferon alfa-2b (Intron-A) or pegylated interferons (PEG-Intron, Pegasys) alone or in combination with ribavirin (Copegus, Rebetol). However, they have shown limited success and are associated with undesirable side effects [1,2]. The high cost of medications is another barrier that prevents wider use of anti-HCV therapy. For example, in Mongolia, only about 100 patients were treated with interferon in 2001 [6]. Thus, the treatment of the chronic HCV infection represents an unmet medical need. New strategies are being developed including therapeutic vaccines. The development of a therapeutic vaccine has considerable potential to aid chronic HCV carriers. They might help to clear the infection or prevent and delay cirrhosis and primary liver cancer.

Natural immune responses, both cellular and humoral, are not capable of terminating HCV infection in most patients. Over the last several years, studies have revealed some of the causes for the failure of the immune system to eliminate HCV infection [2]. It is now believed that if a therapeutic vaccine is administered to already-infected individuals it may help to induce a proper immune response that can be clinically beneficial. Nevertheless, therapeutic vaccines tested so far had shown modest clinical benefit [1]. In previous study we have shown that oral therapeutic hepatitis vaccine V-5 Immunitor (V5) developed by us, is beneficial to patients with hepatitis B [7]. In the present study we describe results from a pilot, open-label trial of V5 in patients with chronic hepatitis C.

Materials and methods

Subjects

Four female and six male patients with chronic hepatitis C infection were enrolled into an open-label, 1-month study. Patients were recruited among NRCID outpatients who gave their consent. Individuals who had higher than normal baseline liver enzyme transaminase ALT and AST levels were enrolled into the trial. All patients were positive for hepatitis C antibodies at study entry. The median age of patients was 31.5 years, range 17—64 years, mean $\pm$ S.D. equal to 35.7 ± 17.6 years. The average and median duration of infection prior to study initiation was between 10 and 11 years. None of patients were treated with interferon and/or anti-hepatitis drugs before or during the trial.

Vaccine

The vaccine is made from pooled blood of hepatitis B and C carriers by employing proprietary technology developed by us. The hepatitis viruses were killed by heat- and chemical inactivation and then formulated into a tablet. The process of manufacturing is described in detail earlier for a similar vaccine, V1, which is derived from the blood of HIV-positive patients many of whom had concomitant hepatitis virus infections [8]. The principle for production of V5 is not much different from established principles with old-fashioned killed vaccines, e.g., Hepatitis B vaccine made from pooled plasma. V-5 Immunitor is presented as 850 mg coated pill, 10 of which are sealed in a ''blister'' pallet, with 30 pills per one package. The recommended dose is 1—2 pills per day. The preparation is stable at ambient temperature for 5 years.

Administration schedule and monitoring

Patients were instructed to self-administer one oral tablet of V5 once per day at least half-an-hour before or after the morning meal. Each patient received 30 pills of V5 and was asked to come back 30 days later. The baseline and outcome parameters were established at study entry and at the return visit. The ALT and AST values were measured by LiquiUV test (Human GmbH., Germany). ELISA test kit for anti-HCV antibodies was from the same manufacturer.

Statistical analysis

Primary endpoints for this study were changes in serum ALT and AST liver aminotransferases, effect on anti-HCV antibodies, and clinical response. Parametric paired values were assessed by Student t-test and qualitative changes were analyzed by Chi-square (χ^2) 2×2 contingency table using GraphPad software. The odds ratio (OR) was estimated by Mantel Haenszel test with Wald's 95% confidence interval (CI). The significance level was set at $P \leq 0.05$.

Results

The results of V5 immunotherapy which lasted 1 month are shown in Table 1. The statistical values are provided at the bottom of the Table. One male patient (#10) failed to test for ALT and AST and have not completed his HCV-antibody tests. The patient #6 instead of taking one daily pill, took 2 pills per day for 15 days. Nevertheless, the statistical analysis of available, per intent-to-treat data shows that V5 produced significant positive changes, including three-fold decrease in ALT and AST levels and remarkable amelioration of clinical symptoms. Every endpoint has shown highly significant statistical value compared to baseline, indicating that these parameters were correlated with each other. The status of anti-HCV antibodies has not changed in any of tested patients.

Serum alanine aminotransferase rather than AST, is considered to be more reliable parameter in assessment of hepatic damage. Table 1 provides results from unpaired t-test since one patient had not ALT measured at follow-up. The reduction was highly significant, from 157.7 ± 73.4 to 49.9 ± 43.8 U/L ($P = 0.0013$) and from 147.0 ± 79.2 to 58.7 ± 56.6 U/L ($P = 0.0132$), for ALT and AST respectively. In order to prevent the bias we have excluded this patient and conducted more robust paired Student t-test on remaining nine patients. However, the overall outcome was not affected, as baseline mean ALT level 148.6 ± 71.5 U/L still shows the decrease to 49.9 ± 43.8 U/L ($P = 0.0009$). When

Table 1 Outcome of V5 vaccine therapy in 10 chronic hepatitis C virus patients

No.	Age	Sex	Diagnosis	Date of infection	ALT		AST		Serostatus		Clinical symptoms		Accompanying symptoms
					Before	After	Before	After	Before	After	Before	After	
1	61	F	HCV	2000	63.5	40.1	79.0	32.0	Anti-HCV+	Anti-HCV+	Fatigue, abdominal distension, malaise	Symptoms disappeared	Hypertension
2	18	M	HCV	2002	165.0	20.9	95.7	25.3	Anti-HCV+	Anti-HCV+	Fatigue, abdominal distension, malaise	Symptoms disappeared	
3	40	F	HCV	1989	156.0	20.4	124.0	41.4	Anti-HCV+	Anti-HCV+	Fatigue, abdominal distension, weight loss	Symptoms disappeared	Nephritis
4	23	M	HCV	2005	124.0	85.0	165.0	96.4	Anti-HCV+	Anti-HCV+	Fatigue, abdominal distension, weight loss, nausea	Symptoms disappeared	Nephritis
5	63	M	HCV	1981	296.0	154.0	324.0	196.9	Anti-HCV+	Anti-HCV+	Fatigue, abdominal distension, weight loss, diarrhea	Symptoms not improved	Hypertension
6	29	M	HCV	2003	84.3	28.4	97.4	54.2	Anti-HCV+	Anti-HCV+	No symptoms	No symptoms	
7	52	F	HCV	1989	165.5	36.8	108.0	29.4	Anti-HCV+	Anti-HCV+	Fatigue, abdominal distension, weight loss, diarrhea	Symptoms disappeared	Kidney, gall bladder, and pancreatic complications
8	17	F	HCV	2000	84.3	41.4	102.9	30.6	Anti-HCV+	Anti-HCV+	Fatigue, weight loss	Symptoms disappeared	
9	20	M	HCV	1992	198.4	22.5	124.0	21.9	Anti-HCV+	Anti-HCV+	Abdominal pain when walking	Symptoms disappeared	
10	34	M	HCV	1993	240	ND	250	ND	Anti-HCV+	ND	No symptoms	No symptoms	
	35.7 ± 17.6	4/6		1995.4 ± 7.8	157.7 ± 73.4	49.9 ± 43.8	147.0 ± 79.2	58.7 ± 56.6	10/0	9/0	8/2	1/9	
	Mean ± S.D.			Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.					
	31.5 Median			1996.5 Median	$P = 0.0013$ Student t-test		$P = 0.0132$ Student t-test		$P = 0.998$ (χ^2) Chi-square		$P = 0.0016$ (χ^2) Chi-square		

ND: not done.

ALT response was stratified by gender, 4 women with analyzable data had baseline 117.3 ± 51 U/L which decreased to 34.7 ± 9.7 ($P = 0.019$); while 5 men had ALT decreased from 173.5 ± 80.8 to 62.2.6 ± 57.8 ($P = 0.037$). After 1 month two of nine patients had higher than 45 U/L cut-off ALT levels, i.e., while remaining 77.8% patients had been able to achieve normal ALT (30.1 ± 9.2). Regardless, all patients with complete sets of data (100%) had favorable biochemical response as measured by both ALT and AST markers. When AST data were analyzed the same way as we did with ALT the results were also highly significant. The ratio of AST to ALT had improved from 0.93 to 1.18 ($P = 0.00058$).

Out of ten patients one has not tested for anti-HCV antibody at the end of trial. Even if we assume that his status has not changed we still have nine out of nine (100%) patients who remained seropositive at study conclusion ($P = 0.998$).

The most remarkable finding from patients' perspective is the complete disappearance of baseline clinical symptoms after 1 month on V5 immunotherapy. Only one patient (#5), who had been infected for 25 years, continued to complain of fatigue and abdominal distension at the end of study. Two patients who had no symptoms at baseline, remained symptomless after 1 month. While such clinical parameters are subjective by nature, the statistical analysis supports the patients' impression of the clinical benefit of V5. The categorical analysis of symptoms reduced into 2×2 χ^2 table of dichotomous variables, i.e., baseline symptoms versus outcome symptoms for ''yes'' or ''no'' binary categories, reveals that the difference is highly significant ($P = 0.0016$). This translates into Mantel Haenszel's odds ratio 9.4, ($P = 0.0021$) at 95% confidence interval: $2.7 < OR < 476.3$.

Discussion

Pilot, open-label, 1-month trial of V5 vaccine administered *per os* once per day revealed approximately three-fold decrease in ALT and AST levels, increase in AST/ALT ratio, and remarkable improvement in clinical symptoms among 10 patients with chronic hepatitis C. No changes were observed in HCV serostatus. Despite limited number of enrolled subjects and short duration of the study the changes at every clinical endpoint are characterized by highly significant statistical probability values. All observed *P* values were under 0.05 cut-off level. The time to response appears to be only 1 month and, perhaps, even less as suggested by example of patient #6 who took V5 for 2 weeks only. Considering that conventional hepatitis C therapies require protracted treatment periods, which can be as long as 1 year, the observed rate is noteworthy.

These findings support our previous open-label, study with V5 vaccine which was made from the blood of donors co-infected with hepatitis B and C viruses [7]. V5 vaccine is different from V1 since it is derived from the blood of hepatitis B and C donors and does not contain HIV antigens [8]. Nevertheless, the results are strikingly similar. Favorable biochemical responses in this study were observed in 100% of patients. In earlier published study 95% of 19 hepatitis patients with abnormal baseline liver enzymes had their ALT and AST levels back to normal levels at the end of the first month of follow-up. The *P* values seen in

that study were comparable to those seen in the present study, i.e., $P = 0.00000064$ and $P = 0.0000049$ for AST and ALT, respectively.

Sheath et al., and other investigators indicated that AST to ALT ratio ≥ 1 had a 100% specificity and positive predictive value in distinguishing cirrhotic from non-cirrhotic patients [9,10]. Although the prognostic value of AST/ALT ratio as a reliable marker of cirrhosis is debated [11] our data indicates that there is a clear distinction between baseline and outcome ratios ($P = 0.00058$). Statistically significant increase in AST/ALT ratio from under 1 to more than 1, seen in our patients, suggests that the progression to cirrhosis can be reversed by V5 therapy. Although we observed improvement in AST/ALT ratio in 100% of patients, our study population is clearly small and this impression needs to be verified in larger studies.

From prior experience with V1 we know that HCV viral burden can be reduced significantly as a result of therapeutic vaccination. However, due to technical and financial constraints we were not able to measure HCV RNA and thus we do not know the effect of V5 on viral load. This possibility is not excluded since statistically significant correlation between HCV RNA and aminotransferase levels, especially AST, were noted for example by Zechini et al. [12]. We need to do conduct additional studies to find this out.

Several vaccination strategies including those for therapeutic indication are now being explored for hepatitis C [1]. These include DNA immunization, peptide-based vaccines, administration of plant-expressed vaccines, virus-like particles, and presentation of HCV antigens via dendritic cells [1,13]. In addition to academic and non-profit institutions many biotech companies are involved in development of therapeutic HCV vaccines. These include Berna/Pevion (Switzerland); Chiron/Novartis (USA); Chlorogen (USA); CSL (Australia); Curocom (Korea); Dynavax (USA); Enzo (USA); Epimmune (now IDM, USA); GeneCure (USA); GenPhar (USA); GlobeImmune (USA); Inovio (USA); Intercell (Austria); Innogenetics (Belgium); Lipoxen (UK); Merix (USA); Monserum (Mongolia); Oxxon (UK); Sinovac (China); Transgene (France); Tripep (Sweden); VGX (USA); and ViRexx (Canada). Most vaccines from these companies are in the preclinical stage. A few vaccines like TG4040 (Transgene); PeviPRO (Pevion); GI-5005 (GlobeImmune); and HCV/MF59 (Chiron/Novartis) have advanced into safety Phase I stage.

Only three vaccines have been tested in advanced efficacy trials. The Phase II trial of attenuated MTH-68/B avian bursal virus vaccine has shown that significantly more patients progressed into active chronic hepatitis C on conventional therapy (26%) than in the vaccine-treated group (9%) [14].

Intercell's IC41 therapeutic vaccine comprises five T-cell antigens and poly-arginine IC30 adjuvant [15]. According to analysis of earlier study no statistical improvements were seen in patients since it appeared that the doses were suboptimal. An ongoing study to assess the effectiveness of IC41 at an optimal dose is currently underway. The preliminary data from 25 out of 50 patients revealed statistically significant viral load reduction and very good safety profile.

Despite earlier promising results showing fibrosis and inflammatory reduction, the recent data from the Phase IIb trial of INNO0101 (HCV E1) vaccine from Innogenetics revealed that the difference between vaccine-treated patients and the placebo group did not reach statistical significance [16]. For this reason the study was extended for an additional 15 months. Based on outcome of this extension, as announced in September 2007, the company decided to abandon E1 development.

The therapeutic vaccine most relevant to this study is an oral preparation made from protein extract of human hepatocytes mixed with NS3 protein of HCV. This vaccine, which was developed by Yaron Ilan and his team at Hadassah-Hebrew University with support of ENZO company, has shown good safety profile in Phase I trial [17]. An improvement in the histological necroinflammatory score was observed in 2/12 (17%) of the chronic HCV patients. However, no significant decrease in HCV RNA was noted in any of the patients. The postulated mechanism of Ilan et al., vaccine is similar to that of V5 or V1 [18]. The oral administration of viral antigens entails a complex immune tolerance effect, characterized by simultaneous enhancement and suppression of different elements of the immune response in a manner that benefits the host [19,20]. Such a manipulation of the oral immune response toward viruses may achieve a combination of specific antiviral immunity and inhibition of immune-mediated liver damage.

Very little is known as to how the liver becomes damaged as a result of HCV infection [2]. One of theories is that progressive hepatic injury is associated with hepatocyte-directed immune reaction of certain T-cell subsets [21]. Although the autoimmune nature of liver injury has been advocated by some investigators [22], others maintained that virus-induced autoimmunity in hepatitis C virus infection is a rare event [23]. So far no specific T cells were implicated in this process but it is clear that elevated serum ALT and AST aminotransferases and increase in levels of other biochemical markers, e.g., bilirubin and alkaline phosphatase, are signs of ongoing liver damage associated with HCV [24]. As this concept is relatively new there are not too many studies that have addressed specifically the immunological basis of action of orally delivered vaccines [25]. For this reason we can only speculate what the exact mechanism of V5 action is.

In conclusion, our results indicate that one tablet of V5 administered daily for as little as 2 weeks can produce an outcome that has seldom been seen in prior hepatitis C therapeutic vaccine trials. In a separate trial we have shown that V5 produces similar beneficial results in patients with chronic hepatitis B, which was not surprising since V5 is produced from pooled blood from hepatitis C as well as B donors. The production of multivalent vaccines against unrelated pathogens is a common practice in vaccine industry. For example, two hexavalent vaccines including recombinant hepatitis B vaccine in combination with diphtheria, tetanus, pertussis, poliomyelitis, and Haemophilus influenzae type B were licensed and introduced in Europe in 2000 by GSK and Aventis Pasteur [26,27]. The bivalent edible vaccine against HBV and HIV was recently expressed in a tomato plant [28]. Candidate bivalent DNA vaccines encoding for HCV and HBV antigens were tested in mice [29,30].

Hepatitis treatment options in developing countries such as Mongolia are extremely limited due to high cost of medications. A bivalent therapeutic vaccine derived from readily available source certainly represents an affordable and safe means to control chronic HCV and HBV infections.

Further studies of V5 are required to confirm our preliminary findings.

References

[1] Leroux-Roels G. Development of prophylactic and therapeutic vaccines against hepatitis C virus. Expert Rev Vaccine 2005;4:351—71.

[2] Dustin LB, Rice CM. Flying under the radar: the immunobiology of hepatitis C. Annu Rev Immunol 2007;25:71—99.

[3] Lvov DK, Samokhvalov EI, Tsuda F, Selivanov NA, Okamoto H, Stakhanova VM, et al. Prevalence of hepatitis C virus and distribution of its genotypes in Northern Eurasia. Arch Virol 1996;141:1613—22.

[4] Fujioka S, Shimomura H, Ishii Y, Kondo J, Fujio K, Ikeda F, et al. Prevalence of hepatitis B and C virus markers in outpatients of Mongolian general hospitals. Kansenshogaku Zasshi 1998;72:5—11.

[5] Tsatsralt-Od B, Takahashi M, Nishizawa T, Inoue J, Ulaankhuu D, Okamoto H. High prevalence of hepatitis B C and delta virus infections among blood donors in Mongolia. Arch Virol 2005;150(12):2513—28.

[6] Ebright JR, Altantsetseg T, Oyungerel R. Emerging infectious diseases in Mongolia. Emerg Infect Dis 2003;9:1509—15.

[7] Batdelger D, Dandii D, Jirathitikal V, Bourinbaiar AS. Open label trial of therapeutic hepatitis B vaccine V-5 Immunitor (V5) delivered by oral route. Lett Drug Des Deliv 2007;4:540—4.

[8] Jirathitikal V, Metadilogkul O, Bourinbaiar AS. Normalization of elevated liver enzymes due to V-1 Immunitor therapy. Electr J Biotechnol 2003;3:76—9.

[9] Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1998;93:44—8.

[10] Park GJ, Lin BP, Ngu MC, Jones DB, Katelaris PH. Aspartate aminotransferase: alanine aminotransferase ratio in chronic hepatitis C infection: is it a useful predictor of cirrhosis? J Gastroenterol Hepatol 2000;15:386—90.

[11] Reedy DW, Loo AT, Levine RA. AST/ALT ratio > or = 1 is not diagnostic of cirrhosis in patients with chronic hepatitis C. Dig Dis Sci 1998;43:2156—9.

[12] Zechini B, Pasquazzi C, Aceti A. Correlation of serum aminotransferases with HCV RNA levels and histological findings in patients with chronic hepatitis C: the role of serum aspartate transaminase in the evaluation of disease progression. Eur J Gastroenterol Hepatol 2004;16:891—6.

[13] Houghton M, Abrignani S. Prospects for a vaccine against the hepatitis C virus. Nature 2005;436:961—6.

[14] Csatary LK, Telegdy L, Gergely P, Bodey B, Bakacs T. Preliminary report of a controlled trial of MTH-68/B virus vaccine treatment in acute B and C hepatitis: a phase II study. Anticancer Res 1998;18:1279—82.

[15] Firbas C, Jilma B, Tauber E, Buerger V, Jelovcan S, Lingnau K, et al. Immunogenicity and safety of a novel therapeutic hepatitis C virus (HCV) peptide vaccine: a randomized, placebo controlled trial for dose optimization in 128 healthy subjects. Vaccine 2006;24:4343—53.

[16] Leroux-Roels G, Batens AH, Desombere I, Van Den Steen B, Vander Stichele C, Maertens G, et al. Immunogenicity and tolerability of intradermal administration of an HCV E1-based vaccine candidate in healthy volunteers and patients with resolved or ongoing chronic HCV infection. Hum Vaccine 2005;1:61—5.

[17] Israeli E, Safadi R, Melhem A, Pappo O, Shibolet O, Klein A, et al. Induction of oral immune regulation towards liver-extracted proteins for treatment of chronic HBV and HCV hepatitis: results of a phase I clinical trial. Liver Int 2004;24:295—307.

[18] Bourinbaiar AS, Abulafia-Lapid R. Clinical experience with therapeutic AIDS vaccines. Expert Rev Vaccine 2005;4:289—304.

[19] Margalit M, Ilan Y. Oral immune regulation: a novel method for modulation of anti-viral immunity. J Clin Virol 2004;31(Suppl 1):S63—8.

[20] Bourinbaiar AS, Abulafia-Lapid R. Autoimmunity, alloimmunization and immunotherapy of AIDS. Autoimmun Rev 2005;4:403—9.

[21] Khakoo SI, Soni PN, Savage K, Brown D, Dhillon AP, Poulter LW, et al. Lymphocyte and macrophage phenotypes in chronic hepatitis C infection correlation with disease activity. Am J Pathol 1997;150:963—70.

[22] Ichiki Y, He XS, Shimoda S, Ishibashi H, Keeffe EB, Rossaro L, et al. T cell immunity in hepatitis B and hepatitis C virus infection: implications for autoimmunity. Autoimmun Rev 2005;4:82—95.

[23] McFarlane BM, Bridger C, Tibbs CJ, Saleh MG, Fuzio A, Verucchi G, et al. Virus-induced autoimmunity in hepatitis C virus infections: a rare event. J Med Virol 1994;42:66—72.

[24] Mangoud AM, Sanad SM, Hendawy A, el-Hady G, el-Sherbiny GT. Non-histological assessment of liver fibrosis in HCV infection. J Egypt Soc Parasitol 2004;34:383—95.

[25] Ilan Y, Chowdhury JR. Induction of tolerance to hepatitis B virus: can we 'eat the disease' and live with the virus? Med Hypotheses 1999;52:505—9.

[26] Zepp F, Knuf M, Heininger U, Jahn K, Collard A, Habermehl P, et al. Safety, reactogenicity and immunogenicity of a combined hexavalent tetanus, diphtheria, acellular pertussis, hepatitis B, inactivated poliovirus vaccine and Haemophilus influenzae type b conjugate vaccine, for primary immunization of infants. Vaccine 2004;22:2226—33.

[27] Mallet E, Belohradsky BH, Lagos R, Gothefors L, Camier P, Carriere JP, et al., Hexavalent Vaccine Trial Study Group. A liquid hexavalent combined vaccine against diphtheria, tetanus, pertussis, poliomyelitis Haemophilus influenzae type B and hepatitis B: review of immunogenicity and safety. Vaccine 2004;22:1343—57.

[28] Shchelkunov SN, Salyaev RK, Pozdnyakov SG, Rekoslavskaya NI, Nesterov AE, Ryzhova TS, et al. Immunogenicity of a novel, bivalent, plant-based oral vaccine against hepatitis B and human immunodeficiency viruses. Biotechnol Lett 2006;28:959—67.

[29] Jin J, Yang JY, Liu J, Kong YY, Wang Y, Li GD. DNA immunization with fusion genes encoding different regions of hepatitis C virus E2 fused to the gene for hepatitis B surface antigen elicits immune responses to both HCV and HBV. World J Gastroenterol 2002;8:505—10.

[30] Nam SH, Park JH, Kang JH, Kang SY, Kim JH, Kim SY, et al. Modulation of immune response induced by co-administration of DNA vaccine encoding HBV surface antigen and HCV envelope antigen in BALB/c mice. Arch Pharm Res 2006;29:1042—8.