Clinical Experience with Therapeutic Vaccines Designed for Patients with Hepatitis

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Abstract: Franciscan missionary Giovanni Di Plano Carpini traveled in 1245 to a country named Yeke Tartar, to visit a certain man called Genghis Khan. His journey's report narrated peculiar dietary habits of the locals: "they eat anything, even lice". Little that Carpini knew, he had actually documented the earliest known to us record of oral vaccination against blood-borne infections – an approach that is still used occasionally in the present-day Mongolia for therapy of hepatitis. Currently, efforts aimed at developing therapeutic hepatitis vaccines have switched to more palatable path, but we may still benefit from the insight of medieval Mongols. This review provides an update on development of hepatitis B and C vaccines as related to immunotherapy of hepatitis. Immune therapy is a fast-moving field but the results so far failed to pitch woo. Current trends in research on therapeutic vaccine candidates and liver immunology are discussed. We subscribe to the idea that viral hepatitis is essentially an autoimmune disease generating immune-mediated liver damage. Therapeutic vaccines need to be designed in such a way that self-destructive immunity of the host is targeted not the virus, which is not cytopathic.

HEPATITIS B AND C

Hepatitis B virus (HBV), DNA virus of large hepadnaviridae family, affects estimated 350 million individuals and is the leading cause of chronic liver disease and hepatocellular carcinoma worldwide [1]. Approximately 500,000 deaths per year are attributed to HBV. A plasma-derived hepatitis-B vaccine has become widely available since 1981. In 1987 the FDA licensed the first genetically engineered hepatitis B vaccine - Recombivax HB manufactured by Merck Sharp & Dohme. While vaccination programs have reduced HBV incidence in many countries, it still remains a major public health problem, especially in Asia and Africa.

Hepatitis C virus (HCV) is an enveloped RNA virus in the flaviviridae family which appears to have a narrow host range. Humans and chimpanzees are the only known species susceptible to infection, with both species developing a similar disease. HCV is another global public health problem, infecting estimated 170 million people. The WHO estimates that 3 to 4 million persons are newly infected with HCV each year. 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 [2].

CURRENT THERAPIES FOR HEPATITIS

The current standard of care for hepatitis C 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). Treatment response is dependent on viral subtype, with 76 to 80% of those with genotypes 2 and 3, but only approximately 40% with genotype 1 or 4 achieving a sustained virologic response [2]. The most prescribed treatment for hepatitis B are interferon alpha and its pegylated version Pegasys. Glaxo-Wellcome's product lamivudine (Epivir) was launched in 1998. A second hepatitis drug, adefovir dipivoxil (Hepsera, Gilead Sciences), was approved by the FDA in September 2002. Entecavir (Baraclude) from Bristol-Myers Squibb was approved in March 2005. Recently approved telbivudine (Tyzeka) is another nucleoside analogue and was developed by Idenix [1]. These drugs act as virostatic agents by reducing the amount of replicating virus, however the virus generally returns once the treatment is discontinued [3]. In addition, most of these drugs have significant adverse or toxic effects. Many patients drop out or decline the treatment because of side effects. Viral resistance is another drawback of long-term antiviral chemotherapy. Lamivudine monotherapy is associated with higher resistance (year 1, 10-27%; year 2, 37-48%; year 4, 60-65%) than adefovir (year 1, 0%; year 2, 3%; year 5, 29%) or telbivudine (year 1, 3-4%; year 2, 9-22%). Entecavir resistance is rare in naive individuals (year 4, <1%), but increases over time to 43% in lamivudine-resistant patients [3].

It is thus clear that better treatment modalities are needed to improve clinical outcome in hepatitis patients. One such approach is so-called therapeutic vaccination – a subject that will be reviewed in detail in the following chapters.

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RATIONALE FOR THERAPEUTIC VACCINES FOR HEPATITIS

In contrast to preventive vaccines, a therapeutic vaccine is administered to already-infected individuals aimed at enticing the immune system to suppress or clear the infection. Several studies have shown that priming with hepatitis antigens can stimulate cell-mediated and humoral immune responses suggesting that therapeutic vaccination may work as means for controlling the disease. The efforts aimed at directing immunity towards viral elimination have been on the research agenda of academic institutions and biopharma industry since early 1980's.

Dienstag *et al.*, were first to describe this approach in 1982 when they administered preventive vaccine with modified surface antigen (HBsAg) to 16 patients with chronic hepatitis B [4]. While, in retrospect, their results appeared to be promising, there was a long period of inactivity in this area, due perhaps to the pessimistic interpretation by the authors of their own data. However, today the field became very active with many vaccine candidates for both hepatitis B and C.

EXPERIENCE WITH PROPHYLACTIC HEPATITIS B VACCINES TESTED AS A THERAPY

Eleven years after original Dienstag study, Pol *et al.*, reported that GenHevacB (Aventis Pasteur) could decrease or abolish viral replication in 50% of treated patients [5]. However, the same vaccine tested by Turkish investigators in pediatric population produced very limited response with no statistical difference in the mean alanine aminotransferase (ALT) values and viral load compared to controls [6]. Another Turkish group led by Yalcin *et al.*, also concluded that GenHevacB does not offer any therapeutic benefit [7]. In contrast, Pata *et al*., found negative HBV DNA in 7 out of 19 patients (36.8%). Furthermore, four patients (36.35%) had HBeAg seroconversion and one patient (5.2%) became negative for HBsAg at the end of 12 months' study [8]. When GenHevacB was compared to Recombivax (Merck) in a multicenter 118 patients' trial the proportion of HBV DNA negative individuals in the vaccine groups (16.3%) was higher ($p=0.033$) than in the control group (2.7%) but clearance of serum HBsAg was not observed in any of the patients [9]. The triple antigen Hepacare vaccine (Medeva) administered to 11 chronic HBV carriers induced immune responses detectable *in vitro* but no clinical benefits were reported [10]. In Japanese study of Meinyu vaccine (Meiji Dairies) there was a significant decrease in the serum HBV DNA level in the vaccinated group compared to the control group ($p=0.03$). However, the rate of HBsAg clearance and anti-HBs seroconversion data were not available [11]. In another Japanese study, which also employed commercially available HBsAg vaccine, the sustained normalization of DNA-polymerase activity and ALT were seen in 6 of 11 (55%) patients [12]. Positive, although not definitive effect on viral replication in Japanese patients who received in addition the antiviral drug lamivudine was also reported recently [13]. Hepatitis B plasma vaccine developed by Public Health Institute in Mongolia was tested in 30 symptomless HBsAg carriers. The vaccination produced complete clearance of HBV DNA in 43.3% patients and undetectable HBsAg in 10 cases, while 14 others had shown significant decrease in HBsAg titers [14].

Nevertheless, the efficacy of prophylactic HBV vaccines as a therapy was found to be unpredictable, often leading to contradicting outcomes. In essence, experimental or commercial prophylactic vaccines have failed to meet initial expectations, which suggest that different vaccine preparations and optimal immunization protocols need be considered. For example, nasal formulation of Cuban prophylactic vaccine Heberiovac consisting of HBV surface and core antigens is now being tested as a therapeutic modality that could elicit mucosal instead of systemic immune response [15].

DISCONTINUED OR ON HOLD THERAPEUTIC HEPATITIS B VACCINES

There were several studies that have attempted to use vaccine formulations other than commercially available preparations. Most of these studies have been either abandoned at preclinical and clinical stage, or put on hold due to insufficient funding, or discontinued due to lack of promise or interest from investors (Table **1**). In many cases clinical data from such vaccines were never published and available information is usually found in companies' press releases only.

In a pilot study of Chiron the anti-HBs seroconversion was achieved in 11 of 13 patients when MF59 was used as an adjuvant to HBV surface and preS2 antigen vaccine. Administration of this vaccine resulted in a biochemical flare but with a subsequent reduction in viral levels [16]. However, the development of this vaccine was discontinued at Phase II stage.

Theradigm-HBV or CY-1899 (Cytel/Epimmune) was another experimental vaccine that has been discontinued by its developer. The vaccine consisted of HBV core antigen peptide, T-helper peptide, and two palmitic acid molecules that were postulated to enhance immunogenicity. This lipopeptide vaccine was well tolerated; subsequent studies revealed vaccine-induced specific cytotoxic T lymphocytes, but its antiviral effect was marginal. No significant changes in liver biochemistry or viral serology were observed during follow-up of 90 vaccinated patients in efficacy trial [17].

HepaVaxx B from ViRexx (Canada) consisted of a recombinant chimeric molecule containing hepatitis B viral antigen and Fc fragment of mouse antibody. The vaccine was expressed in insect cells. Although safety Phase I trial has been completed the results were not published and the company has been recently delisted from Toronto Stock Exchange indicating that this vaccine is likely to be abandoned.

Therapeutic Coval vaccine platform based on heat shock proteins covalently fused with hepatitis antigen was shown by Stressgen (USA) to demonstrate the ability to induce antigen-specific cytotoxic T lymphocytes, Type 1 cytokines and anti-tumor immunity [18]. Company has apparently not pursued hepatitis application and concentrated on other targets such as cervical dysplasia.

CDX-2101 (Celldex) virus-like particle comprising 240 modified HBcAg protein subunits with or without RC-529 adjuvant developed by Edgar Ribi's company was an-

nounced to be IND-ready treatment of hepatitis B infection. However, this vaccine was apparently discontinued as no new information has been published in last few years.

Lipoxen's HepaXen liposomal vaccine was though to become therapeutic product against hepatitis A, C and E, as well as candidate prophylactic vaccine. In preclinical studies of HepaXen as a prophylactic vaccine against hepatitis B, the antibody immune response generated was 20 times greater than that of a leading prophylactic vaccine [19]. While liposome-based delivery of vaccine is worth being investigated it is unclear, at this stage, whether Lipoxen will ever come up with clinically valid candidate.

The candidate vaccine from Microscience (acquired by Emergent BioSolutions) is so-called "drinkable vaccine" based on attenuated Salmonella that was planned to deliver hepatitis B core antigen [20]. According to company it passed into Phase II, but no published information is available and it appears there was a switch to other disease targets such as development of typhoid vaccine.

Oxxon Therapeutics HI-8 HBV therapeutic vaccine completed Phase IIa in 2006 involving 54 patients either on vaccine alone, vaccine plus lamivudine or lamivudine alone. The vaccine comprised DNA plasmid as prime and modified vaccinia virus Ankara as boost, both vectors expressing the same HBV surface antigen. By 52 weeks, HBeAg clearance in 24% and HBeAg seroconversion in 19% of patients receiving the Hi-8 alone was seen. In patients that seroconverted a viral load decrease of up to 3 logs was observed. No synergy in terms of increased efficacy was seen in the group that received lamivudine. The trial was conducted at 11 sites in Poland, Serbia and Montenegro. The results were never published and there is no mention of this vaccine being further pursued by Oxford Biomedica, a company which acquired Oxxon.

Another candidate vaccine INNO102 - HBV polyepitope vaccine candidate from Innogenetics (Belgium) - was said to be safe and well tolerated in Phase I study but again its efficacy, except some data from animal studies, has not been reported in scientific publications [21]. This vaccine has apparently been abandoned after Innogenetics transferred vaccine programs into now defunct Genimmune.

Dynavax had intentions to develop HBV-ISS (Heplisav), a vaccine based on hepatitis B virus (HB V) antigens and immunostimulatory DNA sequence ISS-1018, an adjuvant that acts as a TLR-9 agonist, for treatment of HBV infection [22]. However, several years had passed since that announcement and until today no news appeared of advancement in this direction. As the FDA had recently advised Dynavax against continued clinical evaluation of prophylactic Heplisav vaccine in healthy subjects it is not clear when and whether the therapeutic version will be produced.

Large randomized, controlled study of GlaxoSmithKline Biologicals which involved co-administration of HBsAg/AS02 vaccine and lamivudine has been carried out in 195 patients for 52 weeks. Although vaccine was safe and well tolerated, but did not improve the HBe seroconversion rate (18.8%) compared to lamivudine alone (16.1%) (p=0.6824). The authors concluded that despite induction of a vigorous HBsAg-specific lymphoproliferative response, cytokine production and anti-HBs antibodies, therapeutic vaccination did not demonstrate superior clinical efficacy than lamivudine therapy alone [23]. It is thus unlikely that this vaccine, in its present form, will be pursued further and perhaps radically new formulation needs to be designed to optimize the therapeutic effect. Similar opinion can be advanced in regard to other vaccines described above

CURRENTLY PURSUED HEPATITIS B VACCINES

Despite setbacks described above there is a sufficient current activity in hepatitis B area with few candidates that may deserve further investigations. Many of these, however, are in preclinical stage and thus no sufficient information is available at this time (Table **1**).

In 2003 Phase II study reported by Safadi *et al*., HBV envelope proteins, HBsAg+preS1+preS2, were administered *per os* to 42 chronic HBV patients [24]. Favorable response in one of the primary endpoints was achieved in 28/42 patients (66.6%). A significant decrease in viral load was observed in 15 patients (35.7%). HBsAg/HBcAg biopsy scores improved in 41% and 57.1% of patients, respectively. Histological improvement in liver necroinflammatory score was noted in 12/40 patients (30%). In all, 80% showed biochemical response. Five of 19 HBeAg positive patients (26.3%) became negative for HBeAg. Favorable anti-HBV specific T cell responses were seen: increased HbsAg specific T cell proliferation (78%); cytotoxicity (75%); IFN gamma positive T cell clones (62.9%); and decrease in the IL10 gamma T cell clones (48.1%). Natural killer T (NKT) lymphocytes increased in 100% treated patients. In a study reported one year later 14 patients with chronic HBV were treated with oral formulation that contained hepatocyte-extracted allogeneic proteins in addition to HBV antigens. A significant decrease in viral load was observed in 5/14 (35.7%) of patients. HB surface antigen/HB nucleocapsid antigen scores on liver biopsy improved in 46.1% and 50%, respectively, and the necroinflammatory score improved in 4/13 (30.7%). Forty percent of the patients with elevated liver enzymes showed a favorable biochemical response. These results indicate that administration of HBV envelope proteins, especially within allogeneic context, can alleviate the immune-mediated liver injury *via* induction of oral tolerance, while enhancing the effective antiviral immunity.

The large Phase II study in China by Xu *et al*., describes 242 HBeAg+ patients immunized with yeast-derived HBsAg-hepatitis-Antibody immune complex (YIC). The primary endpoints were loss of HBeAg, or presence of anti-HBe antibody or suppression of HBV DNA, while the secondary endpoint was both HBeAg seroconversion and suppression of HBV DNA [26]. Statistical significance was not reached in neither of primary endpoints, however, at the end of follow-up, HBeAg seroconversion rate was 21.8% and 9% (p=0.03) in highest dose of YIC and placebo groups respectively. The earlier published data with the same vaccine had shown that after 24 weeks 5 of 10 patients (50%) responded to YIC immunization showing over 2 logs decrease of serum HBV DNA with loss or marked reduction of HBeAg and appearance of anti-HBe or anti-HBs antibodies. However the flares of ALT were observed in 4 of 5 responders and in 2 out of 10 control patients.

Table 1. Summary of Therapeutic Hepatitis Vaccines Tested or Under Development by Companies and Academic Institutions

Company	Country	Name of Vaccine	Description of Vaccine	Status
Apovia acquired by Lorantis (now Celldex)	Germany USA	CorVax	HBV vaccine	Preclinical (abandoned)
Bionor Immuno	Norway		HCV vaccine	Preclinical
Bioven CIGB	Malaysia Cuba		Nasal spray HBV vaccine developed by CIGB	Phase I
Boyce Thompson Institute and Health Research, Inc.	USA		HBsAg expressed in potatoes	Phase I
Celldex (now Avant)	USA	CDX-2101	Virus-like particle comprising 240 modified HBcAg subunits	Preclinical (abandoned)
Centro de Ingeniería Genética y Biotecnología	Cuba	Heberiovac CIGB-230	Nasal formulation of HBsAg and HBcAg (core antigen) pIDKE2 plasmid expressing HCV structural antigens with recom-binant core Co.120	Phase I Phase I
Chiron	USA	Hepatitis B Hepatitis C	HBV surface and preS2 antigens with MF59 adjuvant ChronVac-C DNA vaccine	Phase II (discontinued) Phase I
Chiron and CSL	US/ Australia		Recombinant HCV with CSL's ISCOMATRIX adjuvant - saponin from Quillaja saponaria tree	Preclinical
Chongqing University	China		HBsAg-loaded dendritic cells	Phase II
Corixa acquired by GlaxoSmithKline	USA		Ribi 529 or RC-529 adjuvant (synthetic LPS mimetic aminoalkyl glucosaminide 4-phosphate) and Lorantis' CV-1831 hepatitis B core antigen	Preclinical (discontinued)
Cytel/Epimmune	USA	Theradigm-HBV	HBV core peptide 18-27 as CTL epitope, t etanus toxoid peptide 830-843 as T helper peptide, and 2 palmitic acids as lipid adjuvants	Phase I (abandoned)
Dynavax	USA		HBV surface and core antigen vaccine with TLR9 agonist Immunostimulatory DNA sequence (ISS) with type C TLR9 agonist	Phase I (on hold) Phase I (on hold)
Emergent Biosolutions Microscience	USA	Spi-VEC	Drinkable vaccine based on attenuated Salmonella to deliver hepatitis B core antigen	Phase II
Endorex (Dor Biopharma)	USA		pre-S HBV peptides with Endorex Orasome(TM) polymerized liposome technology for oral delivery	Preclinical (discontinued)
Ehime University	Japan		HBsAg-pulsed dendritic cells	Phase I
Enzo	USA	EHT899	Orally delivered vaccine	Phase II (discontinued)
Genencor/Epimmune acquired by Innogenetics	USA	Epigene	DNA-based HBV vaccine licensed from Epimmune	Phase I (passed to Innogenetics)
Genexine with Dong-A Pharmaceutical	Korea	GX-110	HBV DNA vaccine of 3 pGX10-based plasmids with HBV Env, Core/Pol and mutant IL-12 gene	Phase I/IIa
GlaxoSmithKline Biologicals	Belgium		HBsAg with AS02B adjuvant	Phase II (discontinued)
Globeimmune	USA	GI5005	Inactivated recombinant Saccharomyces cerevisiae expressing HCV NS3-core fusion protein	Phase II
Green Peptide with Kurume University	Japan		Polypeptide HCV vaccine with HLA-binding motif	Phase I
IDM Pharma (Epimmune)	USA		Therapeutic HBV vaccine together with Innogenetics based on PADRE	Sold to Pharmexa after Phase I
Immunitor (Monserum)	USA Thailand Mongolia	V5	Tableted vaccine containing heat-inactivated HBV and HCV from pooled blood	Phase II

(Table 1) contd….

Company	Country	Name of Vaccine	Description of Vaccine	Status
Immunotope (ImmunoVaccine Technologies)	USA		HBV and HCV	Preclinical (abandoned)
Innogenetics (span-off to GenImmune)	Belgium	E1 INNO102	Therapeutic HCV vaccine based on envelope E1 protein INNO102 polyepitope HBV vaccine	Phase II (abandoned) Phase I (abandoned)
Intercell with Novartis	Austria	IC41	Eight or five T-cell antigens and poly-arginine adjuvant (IC31).	Phase II
Lipoxen	UK	Hepaxen	Liposome-based HBV antigens and DNA plasmid co-delivery	Preclinical (discontinued)
Lorantis (Celldex)	UK	Hepvax CDX	HBV core antigen in VLP with RC-529 adjuvant from Corixa	Preclinical (discontinued)
Microscience (acquired by Emergent)	UK	Spi-Vec	Salmonella bacterium with HBV core antigen	Phase II (discontinued)
Okairòs (spin-out from Merck Sharpe & Dohme)	Italy Switzerland	TerCvax	Adenovirus vectors encoding NS of HCV containing large number of T-cell epitopes as prime and plasmid DNA with same antigens as boost	Preclinical
Oxxon acquired by Oxford Biomedica	UK	HI-8	DNA plasmid prime and modified vaccinia virus Ankara (MVA) boost, expressing the same HBV surface antigen.	Phase IIa (abandoned)
Pevion Biotech	Switzerland	PEV2A PEV2B	Virosome vaccine with peptide antigens from HCV to induce CTL (PeviTER) and helper cells (PeviPRO)	Phase I
Pharmexa with Genimmune	Denmark Norway USA	EP2210 EP2220	HBV and HCV PADRE peptide vaccine platform acquired from IDM	Phase I (abandoned)
Powdermed acquired by Pfizer	USA	pdpSC18 pPWRG7128	Dual antigen DNA-based HBV vaccine (inhaled) Single antigen DNA-based HBV vaccine (inhaled)	Phase I (unknown) Phase I (abandoned)
Public Health Institute	Mongolia		Plasma-derived HBV vaccine	Phase II
SciGen OctoPlus	Singapore Netherlands	Sci-B-Vac	PreS1 and PreS2-HBV antigen with Intercell's adjuvant IC 31™	Preclinical
Shanghai Fudan-Yueda Bio-tech	China	YIC	Yeast-derived HBs Ag-and Immunoglobulin complex	Phase II
Shenzhen Thyx Biopharmaceutical	China		Adenovirus-based vaccine with HBV core and IL-2 insert	Preclinical
Stressgen (now Nventa)	US/Canada	HspBcor	CoVal fusion protein engineered from HBV core antigen (HBc) and Hsp65 from Mycobacterium bovis BCG	Preclinical (abandoned)
Theravax (Viropro)	Canada		HBV vaccine	Preclinical (unknown)
Transgene	France	TG4040	Recombinant MVA vaccinia virus encoding non-structural NS3, NS4 and NS5B proteins of HCV	Phase I
Transgene Biotek	India		Oral liquid HBV vaccine	Preclinical
Tripep	Sweden	ChronVac-C	HCV NS3/4A-based DNA vaccine delivered by electroporation device developed by Inovio (USA)	Phase I
United Cancer Research Institute	USA	MTH-68/B	Chicken Newcastle disease virus HBV and HCV vaccine	Phase II
Vaxin	USA		Therapeutic hepatitis C based on PER.C6 cell line from Crucell	Preclinical (abandoned)
Vical/Merck	USA		Therapeutic "naked" DNA vaccine HBV and HCV	Preclinical (abandoned)
Virax	Australia	VIR401	HBV vaccine based on Co-X-GeneTM technology and fowlpox virus vector	Preclinical (on hold)
ViRexx	Canada	Chimigen CHB-111 HepaVaxx B	Comprises pre-S1/S2 antigen bound to a fragment of murine monoclonal antibody	Phase I (abandoned)

DNA vaccine developed by Korean company Genexine comprises most HBV genes together with genetically engineered interleukin-12 (IL-12N222L). Twelve chronic hepatitis carriers in Ukraine and Lithuania were treated with this vaccine along with lamivudine [27]. Vaccination appeared to be well-tolerated and showed 50% of virological response rate in CHB carriers. However, ALT levels have risen significantly in 5 patients (41.7%). The authors concluded that combination of DNA vaccine with chemotherapy may be valid immunotherapeutic approach.

V-5 Immunitor (Immunitor, USA) is a tableted vaccine comprising heat-inactivated HBV antigens from pooled blood of HBV- and HCV-infected donors, which is postulated to produce clinical benefit through induction of oral tolerance instead of activation and consequently reduction of immune-mediated liver injury and viral clearance [28]. In pilot Phase II trial one tablet of V5 was administered daily one month to 10 patients with chronic HBV. Aminotransferase levels have decreased in all analyzed patients from 112.4 to 44.4 U/L (p=0.00009) and 118.8 to 46.1 U/L (p=0.0032), for ALT and AST respectively. In addition, 50% of patients who were HBsAg positive at study entry, became negative after one month (p=0.0098). All patients, except one, reported complete recuperation from hepatitis-associated clinical symptoms present at baseline (p=0.0016). No adverse events were observed at any time. While favorable biochemical, virological and clinical responses indicate that V5 is safe and effective, placebo-controlled, randomized study is required to confirm these findings.

CURRENTLY PURSUED AND ABANDONED THERAPEUTIC HEPATITIS C VACCINES

Considerably fewer HCV vaccines have been tested in advanced clinical trials [1,2,4]. One of reasons is that this virus was discovered relatively recently and there is still no prophylactic vaccine against it. Several vaccination strategies including those for therapeutic indication are now being explored for hepatitis C. These include DNA immunization, peptide-based vaccines, virus- or microorganism-based delivery, administration of plant-expressed vaccines, and presentation of HCV antigens *via* dendritic cells. In addition to academic and non-profit institutions many biotech companies are involved in development of therapeutic HCV vaccines (Table **1**). Most vaccines from these companies are in the preclinical stage. A few vaccines have advanced into clinical stages. In this chapter abandoned and actively pursued clinical trials are described.

The earliest known to us advanced clinical trial was performed by Hungarian researchers Csatary *et al*., in 1998. The Phase II trial of attenuated MTH-68/B avian Newcastle disease virus vaccine involving, in addition to 43 HBV cases, 41 patients with HCV has shown that significantly more patients progressed into active hepatitis on conventional therapy (26%) than in the vaccine treated group (9%) [29]. Relapses occurred less frequently in the vaccine group (32%) than in the control group (79%), with remissions within one month observed more often in treated patients (50%) than in the control (21%). While this study was the first successful proof-of-concept that immunotherapy of HCV is feasible it has been unfortunately ignored and even derided despite the fact that Laszlo Csatary has pioneered currently popular viral vector approach back in 1968 [30].

HCV vaccine IC41 from Intercell (Austria) which includes variable number of MHC I and II-restricted epitopes with poly-L-arginine adjuvant has been tested in randomized, double-blind Phase II study in 60 patients. T-cell proliferation was recorded in up to 67% of patients in the 3 different vaccine doses recipients but only in 17% of patients treated with peptides alone. IFN-gamma ELISPOT assay responses were observed in the IC41 groups with response rates up to 42%. There were 3 RNA responders with transient >1-log declines of HCV RNA associated with the strongest IFN-gamma values within all 60 patients. According to outside analysts the study failed to find statistical improvement in patients given IC41 since it appeared that the doses were sub-optimal. Such results, according to Klade *et al*., suggest that further studies with optimized vaccine regimens and combination therapies need to be initiated [31].

Another peptide candidate designed to induce effector cells was recently tested in Phase I by Yutani *et al*., in 12 HCV-positive patients unresponsive to interferon therapy [32]. Vaccine was administered bi-weekly at three different doses. Decrease of ALT and HCV-RNA levels after the 14th vaccination was observed in 5 and 3 patients, respectively. Given that study had limited number of patients per each dosing it is not clear whether statistical difference is reachable at this size. However, results appear to be more favorable compared to IC41 results. This technology, which apparently has been assigned to Green Peptide company (Japan) needs to be tested in larger trial.

The HCV structural E1 protein-based vaccine (INNO-0101) with aluminum hydroxide adjuvant (Innogenetics) has been shown to be safe in Phase I and had passed through Phase II trial [33]. However, no changes in HCV RNA were observed and histological ameliorations in liver were not different from placebo thus confirming open label trial results reported earlier [34].

Globeimmune's GI-5005 (Tarmogen) is a whole, heat-killed recombinant yeast genetically modified to express HCV NS3-core fusion protein [35]. A randomized Phase II study evaluating GI-5005 plus pegylated interferon and ribavirin has been conducted recently. It was found out that it was well tolerated; HCV specific cellular immune responses were observed in 23% of treated subjects; ALT normalization was seen in up to 50%; and higher viral load reductions were generally seen in GI-5005 group 6/54 (11%) than in placebo but the difference was not statistically significant (p=0.23). While encouraging, these results may have been masked by interferon and ribavirin adjunct treatment and it is clear that another study will be needed to reveal the clinical benefit.

Hepatitis vaccine ChronVac-C from Tripep (Sweden) has been in development since 1999. This vaccine is a preparation of codon-optimized HCV non-structural (NS) 3/4A DNA-gene expressed under the control of the cytomegalovirus immediate-early promoter and delivered by electroporation by a device from Inovio (USA) company. In Phase I study safety, immunogenicity, and effects on the viral load were evaluated by Sällberg *et al*., [36]. Twelve patients with

HCV genotype 1 and a viral load <800,000 IU/mL were divided into four groups of 167µg, 500µg, and 1,500µg given as four monthly doses. In the 167µg group no severe side effects appeared, two mounted transient T cell responses, and none had reduced viral load. In the 500µg group no severe side effects appeared and two developed HCV-specific T cell responses. These two patients had reductions in the viral load of up to 0.89 log10 and 1.5 log10. In the 1500 µg dose no severe side effects nor antiviral effects have been noted. It is not clear whether such results can be considered positive and it is likely that Tripep will go back to a drawing board to come up with better version of its candidate.

French Transgene company designed hepatitis C virus (HCV) T cell-based MVA vectored vaccine (TG4040) expressing three viral antigens known to be targets of potent CD8 and CD4-mediated responses [37]. This candidate vaccine has now moved into Phase I clinical trial. According to the company press release the preliminary results apparently indicated its safety.

The oral liquid preparation made from protein extract of human hepatocytes mixed with NS3 protein of HCV has been developed by Yaron Ilan and his team at Hadassah-Hebrew University with support of ENZO company (USA). It has shown good safety profile in Phase I trial [16]. 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 [25].

V-5 Immunitor (V5), is another oral vaccine, but was formulated as a tablet. Once-daily dose of V5 was administered *per os* to 10 patients with chronic HCV in an open-label Phase II study that lasted one month [38]. Every patient who entered the study had elevated liver enzyme levels, which have decreased 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 one 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<OR<476.3. No adverse events were observed at any time. Similar results were seen in another 20 HCV patients study which was just completed (Table **2**). Mean decrease in bilirubin levels was from 22.1 to 10.9 µmol/L (p=0.0000000006) and ALT from 172.1 to 18.2 IU/L (p=0.000000000005). The positive response was seen in 19 of 20 patients (95%). Thus favorable biochemical and clinical responses were observed indicating placebo-controlled, randomized study is required to confirm these findings

CIGB-230 is a vaccine developed by Cuban Centro de Ingeniería Genética y Biotecnología, it is based on the mixture of pIDKE2, plasmids expressing HCV structural antigens, with recombinant HCV core protein Co.120 [39]. In Phase I study CIGB-230 was administered by intramuscular injection on weeks 0, 4, 8, 12, 16 and 20 to 15 HCV-chronically infected individuals. Neutralizing antibody responses against heterologous viral pseudoparticles were modified in eight individuals, including six de novo responders. In addition, 73% of vaccinees exhibited specific T cell proliferative and IFN-gamma responses 24 weeks after primary immunization. Despite persistent detection of HCV RNA, more than 40% percent of vaccinated individuals improved or stabilized liver histology, particularly reducing fibrosis, which correlated with cellular immune response against more than one HCV antigen (p=0.0053). These results appear to be more promising than outcomes with other DNA based vaccines.

Chiron (USA) is working on several variations of hepatitis C vaccine. Company filed an investigational new drug (IND) application with the FDA for a phase I trial of the E1-E2 vaccine in combination with their adjuvant MF59. Preliminary results indicated a good safety profile and induction of antibodies and T-cell proliferative responses at all tested doses [40]. Another candidate vaccine consisting of recombinant HCV polyprotein, with non-structural NS3, NS4, NS5a, NS5b, and core formulated in ISCOM (or MF59) adjuvants, was immunogenic in rodents, but showed no efficacy against heterologous challenge in chimpanzees. Chiron is also pursuing a T-cell based subunit vaccine using HCV core protein produced in yeast and formulated in ISCOM, which had shown induction of T-cell responses in rhesus macaques.

Table 2. Results from Open-Label Phase II Trial in 20 HCV Patients Co-Infected with HIV and TB, Treated with Daily One Tablet of V5 for One Month

No.	Sex F/M	Age	TB and HIV Co-Infections	Liver Size in cm Over Normal		Erythrocyte Sedimentation Rate (ESR) mm/Hour		Leukocyte $\times 10^9$ L		Hb g/L		Weight Change kg		Total Bilirubin µmol/L		ALT IU/L	
				Before	After	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
20	5/15	36.1 ±10.6	20	3.5 ±1.4	0.95± 1.1	32.3 ±11.4	9.9 ±6.4	14.3± 3.9	4.7± .4	114 ±7.1	123.4 ±6.6	65.3 ±8.1	73 ±9.6	22.1 ±3.4	10.9 ±2.5	172.1 ±34	18.2 ±28.2
Statistical significance				Mean decrease 2.55cm P=2.893E-009		Mean decrease 22.4 P=3.713E-008		Mean decrease =9.6x10^9L P=7.162E-010		Mean gain=9.3g/L P=1.419E-007		Mean gain=7.7 kg P=4.604E-007		Mean decrease =11.2 µmol/L P=5.679E-009		Mean decrease =153.9 IU/L P=5.027E-012	

DENDRITIC VACCINES

The vaccinologists embraced these particular antigen-presenting cells as convenient vehicles for inducing effector cells [41]. Ag presentation by cytokine-activated dendritic cells (DCs) is argued to break tolerance and trigger an antiviral CTL response and it was suggested that this strategy is more efficient than DNA or other form of immunization. The consensus is that selective functional deficit in DC/T cell interaction is a crucial mechanism in chronic hepatitis virus infection. Whether this approach is worthy of merit is unknown since until today no commercial product with proven efficacy has emerged. These vaccines are set apart in this chapter since the rationale for their use differs from postulated principles of classical vaccines although, in reality, the underlying mechanism of action is likely to be the same. It is likely that an antigen from any vaccine will end up in APCs in order to produce an immune response.

There are several published works exploring these cells for immunotherapy of hepatitis. Most studies are in preclinical stage investigating their potential in *in vitro* or animal models, some of which have shown interesting results [42-48]. The first human trial known to us was published in 2004 by Akbar *et al*., who injected into 5 healthy human volunteers autologous DCs pulsed with commercially available HBV vaccine [49]. No evidence of physical, biochemical, and immunological abnormalities were documented during the next 28 days. The administration of HBsAg-pulsed DCs resulted in upregulation of HBs antibodies in 2 anti-HBs+ and *2* HBs-negative volunteers.

Soon after Chinese investigators did the first efficacy trial in hepatitis B patients [50]. Based on their data 11 of 19 (57.9%) patients had a clinical response to DC-treatment. HBeAg of 10 (52.6%) patients became negative. The authors concluded that autologous dendritic vaccine can effectively suppress HBV replication, reduce the virus load in sera, eliminate HBeAg and promote HBeAg/anti-HBe transformation. Not only the patients with high serum ALT levels but also those with normal ALT levels were capable to respond to DC vaccine treatment. However, the effect of vaccine appeared to be more pronounced in two patients who combined lamivudine with the vaccine.

In clinical trial published by Duan *et al*., twelve patients with chronic HBV and 10 normal control subjects were enrolled [51]. However the goal of their study was to evaluate whether impaired function of DC in patients with chronic HBV may be restored by supplementation *in vitro* with a cocktail of cytokines. Another attempt to investigate the role of dendritic cells in humans was published by Verkade *et al*. [52]. In this trial granulocyte-monocyte-colony stimulating factor (GM-CSF) was given to hemodialysis patients who received conventional prophylactic B vaccine. The hypothesis was that that this growth factor may stimulate dendritic cells and thus enhance immunogenicity of the vaccine. Paradoxically, contrary to *in vitro* situation, patients who were injected with GM-CSF had lower number of circulating dendritic cells and decreased antigen presenting capacity. Shi *et al*., approached this problem from another angle - they argued that DCs are functionally impaired in hepatitis patients [53]. In order to overcome this problem they injected autologous cytokine-induced killer (CIK) cells obtained from 14 CHB patients back to patients expecting that this will restore function of DCs. Half of patients exhibited a sustained decrease in HBV load. The rate of HBVe antigen loss or seroconversion was also higher in virological responders. The decrease in HBV DNA load was however not correlated with ALT levels. The frequency and cytokine-producing capacity of DCs increased significantly in virological responders, but not in non-responders.

The use of dendritic cells as a vaccine strategy for hepatitis was proposed ten years ago by Chisari *et al*. [54]. But so far it appears that more creative immunization strategies with APCs are needed to effectively induce an immune response of sufficient quality and magnitude to achieve sustainable therapeutic effect.

EDIBLE OR ORAL VACCINES

Another fast-growing direction in hepatitis vaccine research is in so-called edible vaccines. This field was pioneered in 1992 by team of Charles J. Arntzen who purposefully expressed hepatitis B antigens in transgenic tobacco plants [55]. Many papers were published since, perhaps too many to cite them all. So far the stability and immunogenicity of orally delivered antigens from plants appears to vary greatly, often producing disappointing results. Only one hepatitis vaccine candidate has been tested in humans [55]. In Thanavala *et al*., study HBsAg expressed in potatoes and delivered orally to 42 previously vaccinated individuals had increased serum anti-HBsAg titers in 10 of 16 volunteers (62.5%) who ate three doses of potatoes; in 9 of 17 volunteers (52.9%) who ate two doses of transgenic potatoes; and in none of the volunteers who ate non-transgenic potatoes. These remarkable results were achieved without the co-administration of a mucosal adjuvant or the need for buffering stomach pH. Edible vaccines are certainly attractive as a concept but the fact that they are genetically modified plants and uniformity of dosing has yet to be solved makes them less appealing than traditional oral approaches. Furthermore, ecological and human risks from field-grown genetically modified plants expressing vaccine components are not fully known. Despite intense academic research activity during last 15 years no commercial product is yet emerged and it is unlikely that this type of vaccines will be available soon unless radical regulatory policy changes will be implemented.

Besides plants, higher forms of carrier organisms are studied as means of oral delivery. The earliest known instance of this approach - has been described by Franciscan missionary Giovanni Di Plano Carpini (c.1180-1252) - in the account of his spy mission to Mongolia in 1245-1247 [57]. In his report delivered to Pope Innocent IV he affirms that Mongols "...eat lice." (Fig. **1**). Obviously, the diet on lice has little value in terms of caloric intake and Carpini have failed to understand why Mongols would engage in insecticide in a manner that was disagreeable and offensive even to medieval senses. Had Carpini investigated this phenomenon in depth this could have saved the world from the pandemic of Black Plague that Mongol armies, which were immune to the disease, introduced during siege of Genoese city of Caffa (now Feodosiya, Ukraine) in 1347 [58]. In Mongolia, even today, patients with hepatitis or other blood-borne diseases refractory to all tried treatment would routinely seek lice to swal-

low. Based on our own experience and testimony of doctors familiar with the outcome of this procedure the results are often quite dramatic. In retrospect, chitin covered ectoparasites, filled with ingested blood containing viruses and other pathogens, are an ideal delivery vehicle for an oral vaccine [59]. This unusual method of therapy is also known in other ethnic medical practices, e.g., in Russia and Ukraine, but understandably it does sit well with modern medical notions.

Fig. (1). Magnified picture of a head/body louse (*Pediculus humanus*) that has recently fed and the blood is still visible in its alimentary canal (courtesy of Dr. Vincent S. Smith, The Natural History Museum, London, UK).

There are nevertheless published studies which indicate that this approach has valid grounds. In report published by Ali *et al*., microwave irradiated larvae of Trichinella spiralis given orally to mice had significantly reduced the incidence of trichinellosis among animals challenged with parasitic worm [60]. Mice which were given drinking water spiked with Biomphalaria snails infected with Schistosoma mansoni were subsequently found to be immune to schistosomiasis [61]. Scientists from Paravax company (now Heska) had immunized dogs with soluble antigens from the midguts of fleas. They found out that significantly fewer live fleas were recovered from vaccinated dogs and recovered live female fleas produced significantly fewer eggs [62]. In 16th century China pills made from ground-up fleas collected from sick cows were given as a prophylactic/therapeutic remedy against smallpox [59]. Chitin, 1-4 linked polymer of 2-acetamido-2-deoxy-b-D-glucose, is the third most abundant natural polymer in nature and has been quite popular as a delivery means for oral vaccines, which need to survive digestive degradation in the stomach, in order to confer protective immunity [59]. Our experience with V5 Immunitor in both hepatitis B and C suggests that oral delivery of antigens merits serious consideration. In any case, oral vaccine delivery is certainly attractive as an approach and the vaccine field is now abuzz with new reports that appear on a daily basis. Whether Mongols were right in their empirical vaccine approach or they will be dismissed as unsophisticated licemongers will be judged by further studies in edible/oral vaccines area.

IMMUNOLOGY OF HEPATITIS AND RELATION TO THERAPEUTIC VACCINES

Two completely different viruses, HBV and HCV, infect human hepatocytes and cause very similar clinical manifestations. HBV since its discovery in 1967 and HCV in 1989 were subjects of intensive research [63,64]. The literature on immunology of HBV and HCV is vast and often contradictory. It is generally accepted that the cellular but not humoral response plays the most important role in determining the outcome of hepatitis virus infections. Often there is an inverse relationship between lymphocyte responses and antibody levels in infected patients. Vigorous and polyclonal CD4 and CD8 T-cell responses in early stages of the infection are associated with viral clearance. Depletion studies in chimpanzees, the only other host of HCV besides humans, have shown that both CD4 and CD8 lymphocytes are required for virus elimination [65]. Similar studies conducted in HBV animal models such as ducks also indicate the predominant role of cellular immunity [66]. However, the cellular immune response is also strongly associated with hepatitis, i.e., liver inflammation [67].

The principal argument in designing therapeutic vaccines is as follows. Control of primary infection with hepatitis viruses is associated with robust and broad T cell immunity. In contrast, chronic infection is characterized by weak T cell responses (often termed "tolerance") suggesting that one needs to boost immune response and thus clear the virus. In most reported studies therapeutic hepatitis vaccines did enhance the immune response as evidenced by various immune assays. But immunogenicity did not correlate with clinical outcome, forcing us to conclude that, either these assays are inadequate or there is a deep inherent problem with what immunologists assume and how virus and liver play their own game. Careful analysis of available data suggests that immune activation triggered by therapeutic vaccines in addition to purported immune elimination of infected hepatic cells also brings upon an excessive collateral liver damage. Synchronous induction of antiviral and liver-damaging effects is the key as to why so far, most if not all, therapeutics vaccines have failed. It is crystal clear that both, HBV and HCV, are not cytopathic. Hepatitis is a result of self-directed immune damage. Therapeutic vaccines ought to target autoimmunity, not the virus. The question is, how we do that?

ALLOIMMUNIZATION

Few hepatologists subscribe to the wholesale idea that viral hepatitis is an autoimmune disease. However, this is perhaps the only way how we should view both hepatitis B and hepatitis C [68]. The medical literature abounds with description of autoimmune symptoms that are commonly associated with hepatitis. There is a consensus that cell-mediated immune response is the primary culprit of liver damage [69]. Yet, there is a little conscious effort to bring together these observations, which is illogical. This inconsistency harms the progress in development of effective hepati-

tis vaccines. In this chapter we try to assemble our arguments in a cohesive manner in a hope that it may create fresh outlook on the problem.

If hepatitis is an autoimmune disease then there should be indications that suppression of the immune response can alleviate symptoms of the disease. Indeed, numerous studies have been published which support this premise. Cyclosporine, a potent immunosuppressor, was found to increase the chance of a sustained virological response after liver transplantation [70]. Corticosteroids – another class of immunosuppressants – are also used in treating hepatitis although the results are inconsistent [71]. This indicates that non-specific, broad scope immunosuppression is not the answer, especially considering that this may re-activate viral replication [72]. Therefore more subtle immune modulation is needed to.

Ten years ago Ilan and Chowdhury proposed to exploit the phenomenon of immune tolerance as a way of treating hepatitis [73]. Tolerance, for example, can be induced by oral feeding of antigens – a phenomenon that was first observed more than 100 years ago [59]. Nevertheless, oral tolerance has not been studied well but what is clear is that it does not result in simple immune suppression. This type of tolerance needs to be distinguished from another "tolerance" – a term commonly used by hepatologists to describe weak T cell responses in chronic hepatitis stage. The oral administration of viral antigens produces very complex effect, characterized by simultaneous enhancement and suppression of different elements of the immunity in a manner that produces antiviral activity and benefits the host [74]. Indeed, in Ilan *et al*., oral vaccination studies [24,25] and in our V5 studies [28,38] this approach has been shown to be safe and clinically effective. However besides viral antigens there is a critical second component required for the induction of host tolerance. This component is called alloantigen – a term better known to transplantation immunologists. However this is the hidden component that is present in all successful classical vaccines.

In 1796 Edward B. Jenner – the official founder of modern vaccinology – has inoculated James Phipps, son of his gardener, with lymph from the cowpox blisters of the hand of Sarah Nelmes, a milkmaid who had caught cowpox from a cow called Blossom. The subsequent generations of vaccinologists made emphasis on the fact that Jenner's vaccine contained poxvirus but completely ignored that the preparation also contained alloantigens and later on even xenoantigens - when vaccine was harvested from the skin of calves. Nevertheless, Jenner's vaccine is the only vaccine in existence today that had completely eradicated viral disease – fact that was announced by the World Health Organization in 1980. We will probably never find out whether this remarkable achievement owed its success to alloantigens, but we believe, it did.

Is there any evidence that would support the benefit of alloimmune approach in hepatitis vaccine research? The first generation prophylactic hepatitis B vaccine was made from the pooled plasma of hepatitis carriers. But published information concerning the amount of allogeneic material in the final preparation is in short supply except admission that a vaccine had traces of serum proteins [75-78]. Thus we cannot determine whether this vaccine was *bona fide* alloimmune vaccine although no one seems to have purposefully investigated the contribution of contaminant alloantigens. However, data from unrelated studies suggest that even recombinant hepatitis B vaccine may possess allogeneic properties [79].

Nevertheless, there are published studies showing that vaccination with virus and accompanying infected cells – a common practice in preparation of veterinary vaccines - produced very interesting outcomes. Miller *et al*., examined the ability of a whole-cell vaccine, expressing the duck hepatitis B virus (DHBV) core antigen (DHBcAg) to prevent *de novo* DHBV infection [80]. Liver biopsies at day 4 after challenge demonstrated that vaccination did not prevent the infection. But analysis of liver tissue obtained at 9 or more days post-challenge revealed that 9 out of 11 vaccinated ducks and 8 out of 11 ducks vaccinated with cells+DHBcAg plus anti-DHBc antibodies had rapidly resolved DHBV infection. In contrast, 10 out of 11 of unvaccinated controls developed chronic DHBV infection. These results demonstrate that even if viral infection is not prevented, the vaccine-mediated immune response can successfully clear the virus – which in essence is the goal of therapeutic vaccination. In another study, reminiscent of famous alloimmunization experiment by Stott with HIV vaccine preparation [81], Chinese investigators investigated whether vaccination with spleen lymphocytes or peripheral blood lymphocytes could prevent mice from developing experimental hepatitis [82]. The immunized mice developed much milder disease, exhibited lower levels of ALT and had less inflammatory lesions in their livers. The outcome was associated with inhibition of major inflammatory mediators, pro-inflammatory cytokines and chemokines. All this happened without any virus present at any time during vaccination experiments.

Obviously, in humans such experimentations will be not permissible but there are plenty of indirect data pointing out to optimal design of next generation of hepatitis vaccines. There are other ways and means to induce tolerance and overcome problems surrounding the inclusion of an allocomponent. For this, one needs to go back to basics of immunology, especially transplantation immunology that had dealt with allograft and MHC histocompatibility issues for ages. There are many extremely curious cases in the literature that describe various situations involving transplant-receiving patients with or without hepatitis and who were either vaccinated or were already infected with hepatitis viruses [see for example Refs.79,83].

Vaccines based on dendritic cells can also contribute significant insight since cells which were pulsed with viral antigens will be certainly seen by the host not only as merely antigen-presenting autologous cells but as "foreign" cells as well due to *ex-vivo* manipulation of their phenotypic make-up. The information on the role of these cells in hepatitis is often contradictory. Most indicate that they are defective while others could not find any abnormality [84,85].

Same stands true with so-called adoptive transfer of immunity whereby host cytotoxic cells primed *in vitro* against virus are injected back to the donor [53]. Guidotti and Chisari have shown that HBV-specific cytotoxic T cells can abolish virus gene expression and replication in the liver

without killing the hepatocytes [69]. Even though these cells are autologous, they will be not viewed by the host as self, since they have changed their immune phenotype due to extracorporeal passaging. The phenomenon of immune phenotype switching of cells after passage *in vitro* is well known [86]. Thus, it is not clear whether the observed beneficial effect in adoptive immunity transfer experiments was due to CTL activity or to introduction of allograft-like situation by delivering autologous cells that have changed their make-up after *in vitro* passage.

Obviously, these arguments may sound simplistic and against current consensus that chronic hepatitis results from lack of functional virus-specific T cells which are supposed to clear the infection. However, since neither HBV nor HCV are cytopathic, what then causes immune-mediated liver injury? Exhausted T lymphocytes? Over-expression of the programmed death 1 (PD-1) molecule is considered the hallmark of exhausted T-cells, which affect negatively T-cell activation and function in both HBV and HCV [87,88]. If PD-1 over-expressing cells are dysfunctional allowing hepatitis to persist then which cells create the havoc? Perhaps we do not need to look for culprit further. In all likelihood these cells are PD-1 expressing autoreactive cells since they have been identified as havoc-triggers in acute hepatitis [89]. Unless the cause-and-effect relationship is straightened we will not know whether these two views are conciliatory. An interesting report in a mouse model of fatal acute hepatitis provides a clue to this seemingly contradictory situation even though the authors did not appear to have interpreted their results from the alloimmune viewpoint [90].

CONCLUSION

In this review we have attempted to chronicle the research on therapeutic hepatitis vaccines. Notwithstanding the medieval experience of Mongols, very little was known about immunology of hepatitis forty years ago [63]. Now, plentiful data are available regarding immune regulation of chronic hepatitis and hopefully they will accelerate the development of vaccines in the right direction. The next few years will be decisive in showing whether therapeutic hepatitis vaccines will become a reality. Well-known phenomenon of spontaneous clearance from hepatitis B and C viruses indicates that the Nature has a solution [91,92]. We just need to find it.

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