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Brief report

Chimeric hepatitis B virus (HBV) / hepatitis C virus (HCV) subviral envelope particles induce efficient anti-HCV antibody production in animals pre-immunized with HBV vaccine

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ABSTRACT

The development of an effective, affordable prophylactic vaccine against hepatitis C virus (HCV) remains a medical priority. The recently described chimeric HBV-HCV subviral envelope particles could potentially be used for this purpose, as they could be produced by industrial procedures adapted from those established for the hepatitis B virus (HBV) vaccine. We show here, in an animal model, that pre-existing immunity acquired through HBV vaccination does not influence the immunogenicity of the HCV E2 protein presented by these chimeric particles. Thus, these chimeric HBV-HCV subviral envelope particles could potentially be used as a booster in individuals previously vaccinated against HBV, to induce protective immunity to HCV.

Keywords:

Viral hepatitis

Preventive vaccination

Subviral envelope particles

Immune response

Liver diseases

1. Introduction

Hepatitis C virus (HCV) infection, one of the leading causes of severe chronic liver disease, affects more than 2% of the world population [1]. In recent years, considerable advances have been made in the treatment of HCV, with the development of direct-acting antiviral agents (DAA) [2]. However, DAA therapies are very expensive and are therefore unlikely to be adopted universally, even in high-income nations. DAA costs could be sharply decreased for lower-income countries, as was done for antiretroviral drugs for the treatment of HIV infection, if similar public pressure, licensing policies, and private/public funding could be applied [3]. However, most HCV-infected subjects are not aware of their infection, and the cost of large-scale HCV screening and DAA treatment would remain very high, even if the cost of this treatment could be reduced in low-income nations. Moreover, it is estimated that the world reservoir of HCV-infected individuals increases by three to four million newly infected subjects each year. In areas with limited resources, iatrogenic transmission, through unsafe blood transfusion and medical procedures, is the major mode of HCV transmission. By contrast, in industrialized countries, the main route of HCV transmission is the use of intravenous drugs. Reports from the Centers for Disease Control and Prevention (CDC) in the USA have documented an increase in the frequency of HCV infection diagnosis in adolescents and young adults over the last decade, due to escalating epidemics of intravenous drug use and needle sharing in both urban and non-urban areas [4]. Healthcare-associated transmission is also observed, with one to two outbreaks reported each month in the USA [5]. Moreover, although HCV is rarely transmitted by sexual activity in heterosexual couples, sexual transmission is increasingly recognized in men who have sex with men (MSM), whose

traumatic sexual practices and HIV infection are associated with a higher risk of HCV infection [6]. For all these reasons, the development of a safe, effective and affordable prophylactic vaccine against HCV is a major medical priority, providing the best long-term hope for controlling the global epidemic and decreasing the burden on healthcare systems. Such a vaccine would be of interest for at-risk populations in high-income countries, and probably for the entire population in many low-income countries.

The recently described chimeric HBV-HCV subviral envelope particles, which could be produced by industrial procedures adapted from those established for the hepatitis B virus (HBV) vaccine, constitute an affordable potential prophylactic vaccine against HCV [7, 8]. These particles belong to the family of modular virus-like particles (VLPs), for which good process knowledge and capability is emerging in vaccine design [9]. The highly arrayed structure of VLPs allows ordered repetitive presentation of heterologous epitopes on the particle surface, offering a favorable platform that is extensively exploited for generation of epitope-based VLPs to target various diseases [9]. Although the process for this new vaccine will require revalidation, the generic HBV vaccine process backbone might be an appropriate starting point for this process of revalidation, saving considerable time and cost. Antibodies produced in small-animal models in response to immunization with chimeric HBV-HCV subviral particles bearing the full-length HCV genotype 1a envelope E2 protein have been shown to neutralize *in vitro* infection with HCV pseudoparticles and cell-cultured viruses derived from different heterologous 1a, 1b, 2a and 3a strains [7]. Moreover, the humoral anti-hepatitis B surface (anti-HBs) response induced by these chimeric particles has been shown to be equivalent to that induced by a commercial HBV vaccine, suggesting that this vaccine could replace existing vaccines against HBV, while providing the additional benefit of protection

against HCV. However, as mother-to-child transmission is a leading cause of high rates of chronic HBV infection worldwide, more than 180 countries have added the HBV vaccine into routine childhood immunization programs [10]. Given the high percentage of the human population already immunized against HBV, this study aimed to investigate, in an animal model, whether prior HBV vaccination interfered with the production of antibodies against the HCV E2 full-length protein fused to the HBV S protein.

2. Materials and methods

2.1. Immunization of New Zealand rabbits

Chimeric HBV (adw genotype)-HCV (1a genotype) envelope subviral particles bearing the full-length HCV E2 protein (S+E2-S particles) were produced and purified, as previously described [7]. HBs antigen (HBsAg) levels were determined in a quantitative microparticle chemiluminescence immunoassay (ARCHITECT system; Abbott Laboratories, Abbott Park, IL), and immunogens were mixed, immediately before immunization, with a squalene-based oil-in-water nanoemulsion (Addavax; Cayla-InvoGen, San Diego, CA). Five rabbits were first immunized subcutaneously with Engerix, a commercially available HBV vaccine, by four injections of 15 µg of HBsAg on days 0, 14, 28 and 42. These animals were then further immunized by four injections of 15 µg of the HBV-HCV particle-adjuvant mixture on days 84, 98, 112 and 126. Serum samples were collected from animals at various time points (days 0, 14, 28, 42, 56, 70, 84, 98, 112, 126, 140, and 154), for the characterization of antibody responses. Serum samples collected from five rabbits previously immunized by three

injections of 15 μg of the immunogen-adjuvant mixture and naive for HBV vaccine immunization were used as controls. All animal experiments were conducted by an accredited company (Agro-Bio, La Ferté Saint-Aubin, France), in accordance with current guidelines for animal experimentation.

2.2. Analysis of anti-S and anti-E2 antibody responses

Anti-HBs antibodies were quantified with the ARCHITECT system (Abbott Laboratories). Anti-E2 responses were evaluated with an "in-house" ELISA, based on the use of solid phases consisting of lysates of BHK-21 cells expressing HCV E1E2 proteins, which were compared with control cells expressing β-galactosidase, as previously described in detail elsewhere [7]. For each serum sample tested, the optical density (OD) value obtained for wells with capture by β-galactosidase protein was subtracted from that obtained for wells with capture by E1E2 proteins. The final data are expressed as the difference in OD (E1E1-β-gal).

3. Results

Antibodies directed against the HCV E2 protein were detected in the serum samples of all rabbits pre-immunized by four injections of a commercial HBV vaccine (Engerix) that had developed high levels of anti-HBs antibodies (Figure 1A). More than 150 days after the first HBV vaccine injection and 70 days after the first S+E2-S chimeric particle injection, we detected high levels of anti-E2 antibodies in these five animals (Figure 1A). Anti-E2 antibody levels were not strictly comparable to those of animals naive for HBV vaccination, due to the use of a slightly different immunization

protocol and variations in the individual responses of the animals, but the kinetics of anti-E2 antibody production appeared to be more homogeneous in animal pre-immunized with the HBV vaccine (Figure 1A and 1B). In both groups, all rabbits displayed a strong humoral anti-HBsAg response, induced early by the commercial HBV vaccine in the first group (Figure 1A), or concomitantly with the anti-E2 response in the animals from the second group, which were immunized only with the S+E2-S chimeric particles (Figure 1B).

4. Discussion

Our results show that pre-existing immunity to HBsAg did not influence the immunogenicity of the HCV E2 protein fused to the HBV S protein. A previous study conducted on mice immunized with HBV subviral envelope particles bearing hypervariable region 1 (HVR1) of the HCV E2 protein also showed that pre-existing anti-HBs responses did not hamper the production of anti-HVR1 antibodies [11]. However, in these particles, the HBV S immunodominant “a” domain was disrupted by the insertion of the HVR1 encoding sequence. The situation is different with our chimeric HBV-HCV subviral envelope particles, in which the N-terminal transmembrane domain of S is replaced by the transmembrane domain of E2 to incorporate the full-length E2 protein while preserving the immunogenicity of the HBV S protein in the chimeric subviral particle [7]. Nevertheless, all animals recently immunized with the HBV vaccine and still presenting high levels of anti-HBs antibodies were able to produce anti-E2 antibodies following immunization with the chimeric HBV-HCV subviral envelope particles. Interestingly, other experimental vaccines based on carrier VLPs such as murine polyomavirus capsids demonstrated

that these VLPs were able to raise antibodies against the displayed antigenic element despite a strong pre-existing anti-carrier immune response [12].

Our findings for small animals require confirmation in immunization studies on humans. However, it has been shown that the malaria vaccine candidate RTS,S, consisting of the C-terminal region of the circumsporozoite protein (CS) fused to the N-terminus of the HBV S protein efficiently induces anti-CS antibodies in children previously vaccinated with the HBV vaccine [13]. This, and our data suggest that chimeric HBV-HCV subviral particles could be used to induce protective immunity to HCV in subjects previously vaccinated against HBV. In countries in which the disease is highly endemic and HBV is spread principally from mother to infant at birth or from child to child during early childhood, the first dose of HBV vaccine is administered as soon as possible after birth, preferably within 24 h. This birth dose is then followed by two or three booster doses of monovalent or combined vaccines [14]. The WHO currently considers young adults immunized in this way during the neonatal period to have memory immunity, and a booster dose therefore considered not strictly necessary in routine immunization programs [14]. Nevertheless, for populations at risk of exposure to HBV infection, such as healthcare workers, a three-dose booster is recommended, as memory immunity has been shown to decline in a substantial proportion of individuals [15]. Our results suggest that the chimeric HBV-HCV vaccine could be used in such young health workers who previously received neonatal HBV immunization, to give the added benefit of protection against HCV. This recommendation could be extended to other young adults or adolescents at risk of contact with infected blood, such as intravenous drug users, MSM, fire-fighters, policemen, military personnel or household contacts of one or more HCV-infected individuals.

Although further assays, including immunization in humans, will be required before definitive conclusions can be drawn, our results suggest that the chimeric HBV-HCV envelope subviral particles could be used in two different strategies: (i) in primary vaccination, to induce protective immunity to both HBV and HCV, in areas of the world in which the population is exposed to the risk of iatrogenic transmission of these blood-borne pathogens; or (ii) as booster doses in individuals previously vaccinated against HBV and remaining at risk of exposure to hepatitis viruses, to ensure full protection against HBV and to induce protective immunity to HCV.

Authors' contributions

EB and PR conceived, designed and performed the experiments, analyzed the data, and wrote the paper. The two authors read and approved the final manuscript.

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Conflict of interest

The authors have no conflict of interest to declare or financial disclosures to make.

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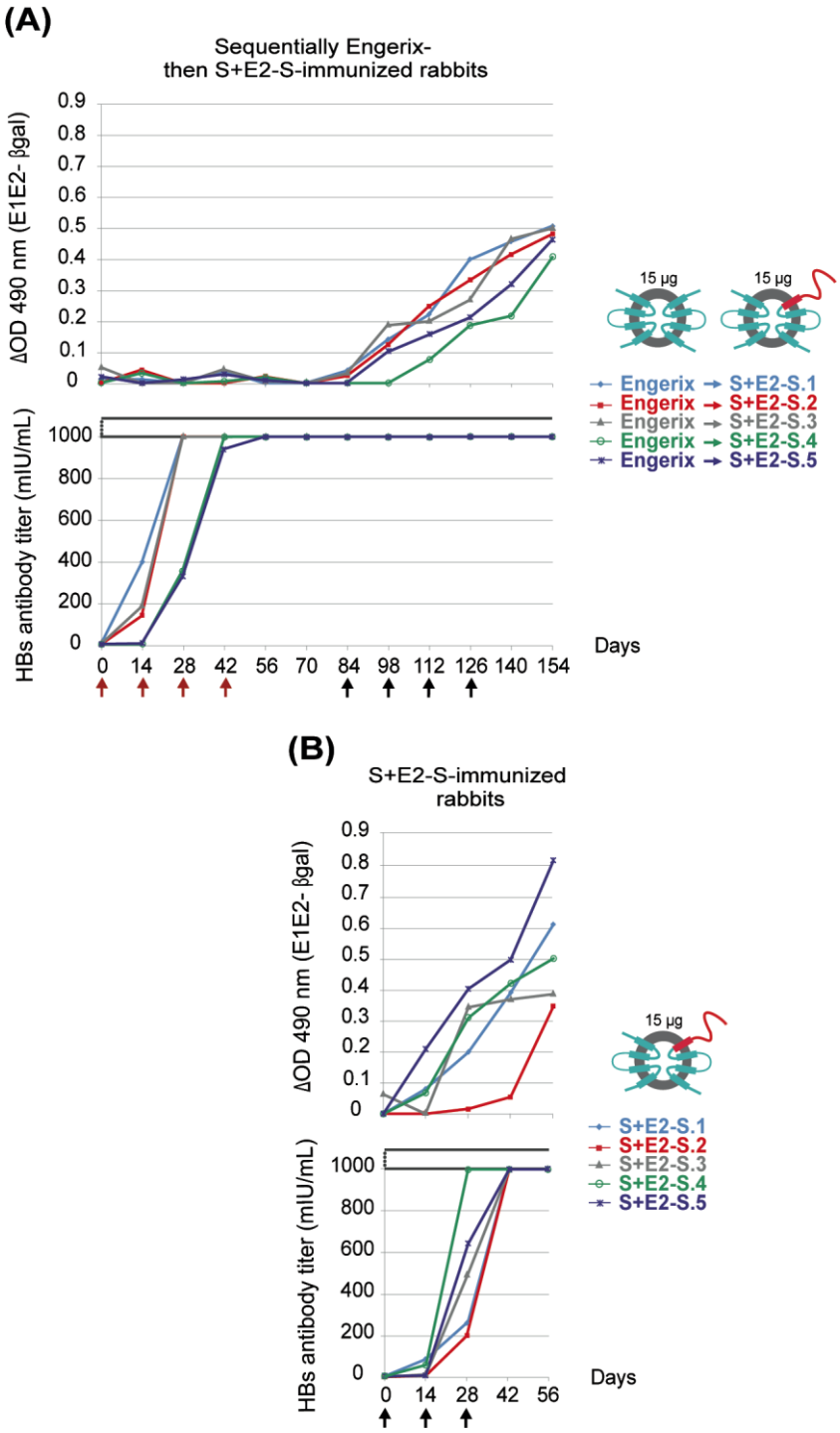

Fig. 1. Humoral immune responses induced in rabbits immunized with the chimeric HBV-HCV subviral envelope particles carrying the HCV E2 protein in the presence (A) or absence (B) of pre-immunization with a commercial HBV vaccine (Engerix). Anti-E2 and anti-HBs responses were evaluated with rabbit serum samples collected at various time points, with an "in-house" ELISA and a routine immunoassay (Abbott), respectively. Red and black arrows indicate the time at which immunization occurred with the HBV vaccine and the chimeric HBV-HCV subviral envelope particles, respectively. Results are expressed as the difference in OD (E1E1-β-gal) and anti-HBs titer (mIU/ml), respectively.