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VIRAL HEPATITIS

Cell-culture-derived HCV —a promising vaccine antigen

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With millions of humans infected yearly with HCV, leading to cirrhosis and cancer, a vaccine is urgently needed. Cultured virus particles constitute the antigen in most antiviral vaccines. A study in mice demonstrated induction of neutralizing antibodies by immunization with cell-culture-derived HCV, providing new possibilities for vaccine development.

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Approximately 3–4 million new HCV infections occur yearly, with >150 million chronic infections worldwide.^{1,2} Efficient therapeutics that target specific HCV proteins, defined steps of the HCV life cycle or essential host factors are under development.³ However, owing to the asymptomatic nature of most infections, economic constraints, as well as drug adverse effects and contraindications, only a minority of HCV-infected individuals will be diagnosed and treated. A vaccine is urgently needed to prevent HCV infection globally. Akazawa *et al.*⁴ have now provided proof-of-concept for immunogenicity of cell-culture-derived HCV particles (HCVcc). HCVcc of genotype 2a were concentrated, purified, UV-inactivated and formulated with an experimental oil–water adjuvant. Four consecutive immunizations of BALB/c mice elicited antibodies with neutralizing capacity against HCV genotypes 1a, 1b and 2a *in vitro*, and with an apparent protective effect against genotype 2a HCVcc infection in a human liver chimeric mouse model. Thus, HCVcc seems to be a promising antigen for a whole-virus, inactivated HCV vaccine.

Protective immunity against HCV exists—~25% of acutely infected individuals clear HCV—and, in some cases, protection from reinfection was observed.^{1,2,5} From early studies in humans and chimpanzees (considered the only true animal model for

studies of HCV infection⁶), activated HCV-specific CD4⁺ and CD8⁺ T cells are required for control of HCV infection. The development of culture systems, specifically HCV pseudo-particles (HCVpp) and HCVcc,³ enabled *in vitro* studies of HCV neutralizing antibodies; evidence was subsequently obtained that neutralizing antibodies are of importance for HCV clearance.^{1,2,5} Indeed, most antiviral vaccines use inactivated and/or attenuated whole viral particles as antigen and depend, at least in part, on the induction of neutralizing antibodies. HCV vaccine research has focused on recombinant protein-based, peptide-based, DNA-based, vector-based, or virus-like-particle vaccine candidates, with the most advanced candidates in phase II clinical trials.^{1,2}

Vaccine candidates containing adjuvant-formulated HCV envelope glycoproteins (E1 and E2), or virus-like-particles containing HCV envelope proteins, most closely resemble a whole-HCV particle vaccine. Immunization with virus-like-particles induced humoral and cellular immune responses in several animal models; however, no HCV-specific antibodies were induced in chimpanzees.^{1,2} Although immunizations with heterodimeric E1–E2 induced strong humoral immune responses in chimpanzees and humans (phase I trial), neutralizing antibody responses were detected in <50% of immunized humans.^{1,2}

Of note, production of these heterodimers seems to be technically difficult. DNA-based or vector-based vaccines primarily induce cellular immunity, which might limit their use as a prophylactic vaccine. The efficacy of viral-vector-based vaccines might also be compromised by pre-existing antibodies neutralizing the viral vector.^{1,2} These limitations of prophylactic HCV vaccine candidates might restrict their use, and their protective effect in humans is yet to be established.

In 2005, the first cell-culture system to produce infectious HCV was developed, which enabled the same research group to study HCVcc immunogenicity in mice (Figure 1).⁷ Immunization with HCVcc of genotype 2a with an adjuvant elicited anti-E1 and anti-E2 antibodies, as well as HCV neutralizing antibodies.⁴ Serum from immunized mice had 50% neutralization titres of ~1:100 against HCVpp of genotype 1a, 1b and 2a and ~1:500 against HCVcc of genotype 1b and 2a; in the latter experiment, however, pre-immunization control sera were not included. The serum 50% HCVpp neutralization titres were comparable to those found in chimpanzees and humans after immunization with the E1–E2 vaccine candidate.² Polyclonal IgG purified from immunized mice neutralized HCVpp of genotype 1b and 2a and HCVcc of genotype 2a, with EC₅₀ values in the upper range of those observed for human monoclonal antibodies with therapeutic potential.^{4,5} Of note, pre-absorption of anti-E1 and anti-E2 antibodies in purified polyclonal IgG with recombinant E1 and E2 resulted only in a ~threefold increase in EC₅₀.⁴ Neutralizing antibodies, therefore, seem to target not only the HCV envelope proteins but also other components of the vaccine preparation. Although whole-virus vaccines mainly elicit humoral immune responses, any T-cell responses triggered by HCVcc-based vaccines should also be studied (Figure 1).

Experimental use of chimpanzees has been restricted, and it is unclear if this animal model will be available for HCV immunization and challenge studies in the future (Figure 1).⁶ The only small animal model to support robust HCV infection are SCID-uPA mice, whose auto-degenerating liver can be repopulated with human hepatocytes (Figure 1).⁶ Polyclonal IgG from BALB/c mice immunized with HCVcc of genotype 2a apparently had a protective effect against challenge with HCVcc of genotype 2a in this mouse model.⁴ When challenged with the minimal infectious

Figure 1 | Development of a whole-virus, inactivated HCV vaccine candidate.

dose (equivalent to 10^3 HCV RNA copies), but not with higher doses, all of the IgG-loaded mice ($n=6$) were HCV negative post-challenge, whereas four of the control mice ($n=6$) became infected. Whether the observed protective effect was conveyed by IgG in serum of challenged mice remains to be determined, as mixing of challenge virus with IgG might lead to *in vitro* viral neutralization.⁸ The polyclonal IgG dose of 100 μ g given in this study compares favourably to previous *in vivo* passive immunization studies, in which polyclonal IgG from a patient with chronic HCV achieved protection in human liver chimeric mice at a dose of 0.2–1 mg per g body weight.⁸

It will be interesting to investigate if HCVcc-vaccine-induced IgG protects against challenge with native HCV particles derived from *in vivo* infections, which might differ from HCVcc. Indeed, HCVcc are believed to resemble the native HCV particle.² Thus, it was encouraging that, when tested head-to-head with recombinant E1 and E2 protein using the same adjuvant, HCVcc proved to be a more potent antigen regarding induction of neutralizing antibodies.⁴ However, the recombinant E1 and E2 used by Akazawa *et al.*⁴ differed from extracted and purified

intracellular heterodimeric E1–E2 previously used as vaccine antigen.^{1,2} Different antigens might show optimal efficacy in different adjuvants.

Although HCV serotypes have not been defined, different genotypes and subtypes show differential sensitivity to neutralizing antibodies *in vitro*.⁹ Single amino acid changes can also alter susceptibility to neutralizing antibodies, facilitating HCV immune escape.⁵ HCVcc of genotype 2a showed resistance to neutralization by patient sera, which could be overcome by human monoclonal antibodies and also by polyclonal IgG from mice immunized with HCVcc of genotype 2a.^{4,10} Whether induced antibodies target conserved neutralizing epitopes is currently unknown.⁵

Given the high regional prevalence of HCV genotypes 3–6, it should be determined if antibodies induced by HCVcc of genotype 2a, capable of neutralizing HCV genotype 1 and 2, are also able to neutralize these genotypes. The immunogenic potential of HCVcc of other major genotypes should also be tested,⁹ as a multivalent vaccine might be required to efficiently protect against all major HCV genotypes.¹ Lastly, HCV particles engineered to unshield neutralizing epitopes should be developed and tested. For example, HCV particles without the E2 motif HVR1 (which apparently shields susceptible neutralizing epitopes) showed increased sensitivity to neutralizing antibodies, and so might be useful in vaccine development.⁵

Akazawa *et al.*⁴ used the experimental oil–water based adjuvant MPL-TDM, which is not licensed for human vaccinations. To develop a whole-virus, inactivated vaccine, adjuvants suitable for humans, able to elicit strong humoral and possibly also cellular immune responses, need to be found. Furthermore, this experimental vaccine was administered intraperitoneally; for human use other routes of immunization need to be explored. As the use of components of animal origin should be limited in human vaccine development, it is of importance to establish cell-culture conditions that enable production of large amounts of HCVcc without the use of bovine serum. Further optimization of HCVcc purification procedures might also be required before this antigen can be used in humans.

This study provides evidence that immunization of mice with HCVcc results in induction of neutralizing antibodies, able to protect against HCVcc infection in cell culture and in human liver chimeric mice. These promising findings warrant exploration of this novel

HCV vaccine approach, including immunogenicity studies in mice to determine the effect of different HCVcc antigens and adjuvants and an analysis of elicited neutralizing antibody and T-cell responses (Figure 1). The protective effect of promising vaccine candidates needs to be tested in high incidence populations in clinical trials. Eventually, a whole-viral-particle vaccine might benefit from combination with technologies that primarily elicit T-cell responses, such as viral-vector-based vaccines.¹

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Competing interests

The authors declare no competing interests.

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