Immunization of Human Volunteers With Hepatitis C Virus Envelope Glycoproteins Elicits Antibodies That Cross-Neutralize Heterologous Virus Strains

To the Editor—Ray and colleagues recently demonstrated hepatitis C virus (HCV) glycoprotein-specific neutralizing responses against homologous HCV-1 and the closely related genotype 1a HCV strain H77 [1]. Using well-characterized pseudoparticle (HCVpp) and cell culture replicating virus systems (HCVcc) [2], we set out to further investigate the breadth of the neutralizing responses elicited by vaccination. We tested serum samples from 8 of the volunteers reported in the study by Ray et al [1], selecting representative samples that had low, intermediate, and high anti-E1E2 antibody titers as measured by enzyme immunoassay (Novartis Vaccines and Diagnostics, Emeryville, CA).

Generation of cross-reactive neutralizing antibodies (nAb) in response to vaccination has been a major hurdle for RNA viruses such as human immunodeficiency virus (reviewed in [3]). We reported that immunizing rodents with HCV E1E2 heterodimer or truncated soluble E2 derived from the genotype 1a HCV-1 strain elicited high titer cross-reactive nAb [2]. Here we report that immunization of healthy human volunteers with the same recombinant HCV-1 E1E2 glycoproteins can induce a cross-reactive neutralizing antibody response. Serum samples from 8 healthy immunized volunteers were assessed for their ability to neutralize a panel of HCVpp strains. Briefly, pre- and postimmune serum samples at a final dilution of 1/100 were preincubated with HCVpp encoding a luciferase reporter for 1 hour at 37°C prior to infecting Huh-7.5 cells for 6 hours at 37°C. Infection was quantified after 72 hours by monitoring luciferase activity (Figure 1). All immune serum samples neutralized HCVpp expressing the closely related genotype 1a H77 glycoproteins, the heterologous genotype 1b glycoproteins CON1 and OH8, and the more distantly related genotype 2a strain J6, albeit with reduced efficiency. Preimmune and postimmune serum samples had no effect on murine leukemia virus pseudoparticle infection (Figure 1).

To ascertain the ability of immune serum samples to neutralize HCVcc, we tested the sensitivity of chimeric JFH-1 viruses expressing H77 and J6 structural proteins to inhibition by immune serum samples. All serum samples were clearly capable of neutralizing both heterologous HCVcc viruses, although less efficiently in the case of the 2a virus (Figure 1). Our experiments demonstrate that immunization of human volunteers with recombinant E1E2 glycoproteins derived from the genotype 1a strain elicits antibodies that can cross-neutralize the in vitro infectivity of heterologous strains derived from genotypes 1a, 1b, and 2a.

Despite indications that HCV can transmit in vitro in the presence of antibodies targeting the viral encoded glycoproteins via direct transfer between adjacent contacting cells [4], recent studies with chimeric SCID-uPA mice have yielded encouraging results for a protective role of nAb to prevent or ameliorate virus infection in vivo [5, 6]. Our studies using HCVpp and matching HCVcc strains expand upon the work of Ray et al [1] and demonstrate that vaccination of human volunteers elicits antibody responses with significant cross-neutralizing activity against heterologous 1a, 1b, and 2a HCV genotypes, warranting the continued clinical development of...
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References

Figure 1. Recombinant hepatitis C virus type 1 (HCV-1) genotype 1a E1E2 glycoproteins elicit cross-neutralizing activity in vaccinated humans. Eight healthy adult volunteers were immunized with 4–100 μg of E1E2 with adjuvant MF59 at 0, 1, and 6 months. A, Serum samples obtained at month 7 were tested for their ability to neutralize HCV pseudoparticles (HCVpp) expressing diverse glycoproteins at a final dilution of 1/100. Data are presented as the percentage of neutralization of HCVpp-H77 (black bars), Con1 (diagonal striped bars), OH8 (white bars), and J6 (horizontal striped bars) relative to infection in the presence of each volunteer’s preimmune serum at the same dilution. B, Pseudotype viral particles expressing murine leukemia virus (MLV) glycoproteins were used to confirm serum neutralization specificity. Luciferase measurements (in relative light units [RLUs]) are given for MLV pseudoparticles (MLVpp) incubated with preimmune serum samples (white bars), postimmune serum samples (black bars), or no serum samples (diagonal striped bar). No ENV, luciferase signal given by empty vector. C, Immune serum samples were tested at a final dilution of 1/100 for their ability to inhibit cell culture replicating HCV containing heterologous 1a H77/JFH (black bars) or 2a J6/JFH (horizontal striped bars). The bars represent the mean of 4 replicate wells, and the error bars represent standard deviation (SD) values. Percentage neutralization values were measured using each volunteer’s own preimmune serum as a base-line signal.
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