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MAJOR ARTICLE

Immunization With a Subunit Hepatitis C Virus Vaccine Elicits Pan-Genotypic Neutralizing Antibodies and Intrahepatic T-Cell Responses in Nonhuman Primates

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Background. The global control of hepatitis C virus (HCV) infection remains a great burden, owing to the high prices and potential drug resistance of the new direct-acting antivirals (DAAs), as well as the risk of reinfection in DAA-cured patients. Thus, a prophylactic vaccine for HCV is of great importance. We previously reported that a single recombinant soluble E2 (sE2) vaccine produced in insect cells was able to induce broadly neutralizing antibodies (NAbs) and prevent HCV infection in mice. Here the sE2 vaccine was evaluated in non-human primates.

Methods. Rhesus macaques were immunized with sE2 vaccine in combination with different adjuvants. Vaccine-induced NAbs in antisera were tested for neutralization activities against a panel of cell culture–derived HCV (HCVcc), while T-cell responses were evaluated in splenocytes, peripheral blood mononuclear cells, and hepatic lymphocytes.

Results. sE2 is able to elicit NAbs against HCVcc harboring structural proteins from multiple HCV genotypes in rhesus macaques. Moreover, sE2-immunized macaques developed systemic and intrahepatic memory T cells specific for E2. A significant correlation between the sE2-specific immunoglobulin G titers and neutralization spectrum was observed, highlighting the essential role of sE2 immunogenicity on achieving broad NAbs.

Conclusions. sE2 is a promising HCV vaccine candidate that warrants further preclinical and clinical development.

Keywords. Hepatitis C virus; vaccine; neutralizing antibodies; nonhuman primates.

Despite the substantially improved sustained virologic response rates realized by the new direct-acting antiviral (DAA) drugs [1], DAA-based therapies against HCV are unaffordable for most patients, and DAA-resistant HCV has the potential to emerge. Additionally, DAA-cured patients do not develop sufficient antiviral immunity and thus remain susceptible to reinfection. Therefore, to control HCV prevalence and eradicate HCV, a prophylactic HCV vaccine—the most effective and far more economical approach to prevent infectious disease—is urgently needed as a complement to drug treatments. Unfortunately, such a vaccine is still unavailable, mainly because of the highly genetic diversity of HCV and because the relatively weak immunogenicity of HCV envelope proteins leads to difficulties in inducing both broadly neutralizing antibodies (bNAbs) and T-cell responses [2, 3]

Previous studies suggest that neutralizing antibodies (NAbs) may play an important role in protecting against HCV infection [4–11]. Indeed, multiple trials have focused on inducing bNAbs [12–19] targeting HCV envelope proteins E2 and/or E1, because E1/E2 interact with numerous host molecules during HCV entry [20, 21]. Although significant progress has been made toward developing a prophylactic HCV vaccine based on eliciting bNAbs, the complexity of immunization regimens (such as heterologous prime-boost regimens) and unsatisfactory yield of vaccine strains (such as inactivated cell culture-derived HCV [HCVcc]) still render vaccine production and vaccination difficult. Besides NAbs, there are numerous studies showing that T cells play an important role in shaping the outcome of HCV infection [22]. Proof-of-principle studies have shown that several vaccine approaches could induce potent, cross-reactive T-cell responses in chimpanzees, resulting in accelerated viral clearance after challenge [23–25]. Notably, polyfunctional T cells and long-time T-cell memories were also elicited by viral-vectored vaccines expressing conserved HCV nonstructural proteins in clinical trials [26, 27]. Therefore, a vaccine capable of eliciting not only

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broadly NAbs but also long-time T-cell immunity would be highly preferable.

In a previous study, we reported on a high-yield subunit sE2 vaccine produced in insect cells, in which the glycosylation patterns are altered, resulting in enhanced immunogenicity. sE2 vaccine induced bNAbs and protected genetically humanized mice from HCVcc challenge [28]. Herein, we aimed to extend our characterization of sE2 immunogenicity to nonhuman primates. Specifically, we tested the insect cell–derived sE2 in rhesus macaques and examined the humoral and cellular immune responses following sE2 vaccination.

MATERIALS AND METHODS

Cells, Antibodies, and Viruses

Drosophila S2 cells and Huh-7.5.1 cells were cultured as described previously [28–30].

HCV E2 monoclonal antibody (mAb) AR3A [7] was kindly provided by Dr Dennis Burton and Dr Mansun Law (The Scripps Research Institute). E2 mAb AP33 [31] was kindly provided by Dr Arvind Patel (University of Glasgow). Horseradish peroxidase (HRP)–conjugated AP33 and AR3A antibodies were generated as previously described [28]. NS5A mAb was customized from Abmart. HRP-conjugated anti–monkey immunoglobulin G (IgG) antibody was purchased from Santa Cruz (catalog no. sc-2458). For the enzyme-linked immunospot (ELISPOT) assay, an interferon γ (IFN-γ) antibody pair (anti-human IFN-γ capture antibody [catalog no. 51-2555KZ] and biotinylated anti-human IFN-γ detection antibody [catalog no. 51-1890KZ]) and an interleukin 4 (IL-4) antibody pair (anti–human IL-4 capture antibody [catalog no. 51-1865KZ] and biotinylated anti–human IL-4 detection antibody [catalog no. 51-1850KZ]) were all purchased from BD Biosciences.

A panel of HCVcc strains covering genotypes 1–7 were used for the neutralization assay. All of these HCVcc strains were produced as previously described [28].

Animal Immunization

The expression, purification, and characterization of sE2 (amino acids 384–661; Con1 strain; genotype 1b; accession no. Q9WMX2) from stable *Drosophila* S2 cell clones were performed as previously described [28]. Rhesus macaques were purchased from XiShan ZhongKe Laboratory Animal Company (Suzhou, China). Ten male and 10 female macaques (age range, 3–6 years old; weight ranges, 5–9 kg for males and 3–6 kg for females; all were free of known primate pathogens) were randomly assigned (with 5 animals per group) to receive one of the following 4 vaccines intramuscularly: (1) 500 μg of alum (Alhydrogel 2%; Invivogen) as the control, (2) 200 μg of sE2 containing 500 μg of alum, (3) 200 μg of sE2 containing 500 μg of alum and 500 μg of CpG 7909, and (4) 200 μg of sE2 containing 500 μg of alum and 50 μg of MPL (Invivogen, San Diego, CA). Macaques were injected at months 0, 1, and 2. Blood samples were collected at months −1, 1, 2, 3, 4, and 5. To test sE2-induced immune memory, we boosted the macaques at month 5, when their serum titers had decreased, and collected blood samples again at months 6, 7, and 8. At month 8, all macaques were anesthetized by intravenous injection of propofol and euthanized. Macaque livers were perfused with sterile phosphate-buffered saline (PBS) from the inferior cava vein to the portal vein, and the spleens and livers were harvested for lymphocyte isolation and cellular immune response tests.

All the animal studies were performed at the Institut Pasteur of Shanghai and were approved by the Institutional Animal Care and Use Committee at the Institut Pasteur of Shanghai (protocol no. A2013007). The animals were cared for in accordance with institutional guidelines.

Antibody Measurement

To measure E2-specific antibody responses in serum samples by enzyme-linked immunosorbent assay (ELISA), 96-well enzyme immunoassay/radioimmunoassay flat-bottomed plates were coated overnight with 100 ng/well of sE2. After blocking, serially diluted sera from monkeys were added as primary antibodies, followed by addition of secondary antibodies diluted 1:5000. After color development, colorimetric analysis was performed at 450 nm in a 96-well plate reader. The end point titers of serum were defined as the last diluted specimens that gave positive results (defined as a titer of >0.1 above the preimmune titer).

Competitive ELISA

For the competitive ELISA, 96-well enzyme immunoassay/radioimmunoassay flat-bottomed plates were coated overnight with 100 ng/well of sE2. After blocking, serially diluted monkey antisera were added to the wells and incubated at 37°C for 1 hour, followed by 3 washes with PBS. Then, HRP-conjugated AP33 (2 μg/mL) or HRP-conjugated AR3A (2 μg/mL) was added to the wells and incubated at 37°C for 1 hour. After color development, colorimetric analysis was performed at 450 nm in a 96-well plate reader.

Measurement of Neutralization Activity of the Antisera

Neutralization assays were performed as previously described [28]. Briefly, serum samples were heat inactivated, diluted, and mixed with HCVcc (200 foci-forming units [FFU]/well) and incubated at 37°C for 2 hours. The virus-serum mixture was transferred to Huh-7.5.1 cells seeded 12 hours previously in 96-well plates (1×10^4 cells/well) and replaced with complete Dulbecco's modified Eagle's medium after 4–6 hours of incubation at 37°C. The cells were incubated at 37°C for 72 hours, followed by fixation and NS5A immunostaining. The neutralization percentage was calculated by comparing the focus numbers of immune serum to that of a preimmune serum control at the same dilutions.

Measurement of Cellular Immune Responses by ELISPOT Assay

Spleen, liver, and blood specimens were collected from each macaque when the animals were euthanized. Peripheral blood mononuclear cells (PBMCs) were isolated from whole blood samples by using Ficoll density gradient centrifugation, while hepatic lymphocytes were isolated from livers by using 40% Percoll density gradient centrifugation, as previously described [32, 33]. Splenocytes were also isolated from spleens of mice and monkeys. The isolated cells were stimulated by sE2 and analyzed by ELISPOT assay.

For macaque cells, 96-well polyvinylidene difluoride plates (Millipore) were precoated with human IFN-γ capture antibody (1:200 dilution) or human IL-4 capture antibody (1:200 dilution) at 4°C overnight. Plates were blocked with 200 μL/well of complete Roswell Park Memorial Institute 1640 medium (Gibco) supplemented with 10% fetal bovine serum, 10 mM HEPES buffer, 100 mg/L of L-glutamine, 1% NEAA, 100 U/mL penicillin/streptomycin, and 50 μM 2-mercaptoethanol (Gibco) for 2 hours at 37°C. Then, the plates were decanted, and freshly isolated splenocytes, PBMCs, or hepatic lymphocytes were added to the plates. sE2, medium (negative control), or concanavalin A (positive control) was diluted in complete Roswell Park Memorial Institute 1640 medium, added to each well at a final concentration of 10 μg/mL, and incubated for 48 hours at 37°C and 5% CO_2. Subsequently, the plates were incubated with human IFN-γ detection antibody (1:250 dilution) or human IL-4 detection antibody (1:250 dilution) for 2 hours and then with alkaline phosphatase–conjugated streptavidin (Mabtech) diluted 1:1000 in PBS for 1 hour. After washing the plate 6 times with PBS, 100 μL/well of NBT/BCIP substrate (Promega) was added to the plates, and the plate were incubated for 15–30 minutes for color development. The cytokine-secreting cell spots were imaged and counted on an immunospot reader (Cellular Technology).

Statistics

Significance comparisons were calculated with the 2-tailed Student *t* test or Kruskal-Wallis 1-way analysis of variance. For correlation analysis, a nonparametric Spearman test was used. All statistical analyses were performed on GraphPad Prism, version 5.0c (GraphPad Software, San Diego, CA).

RESULTS

sE2 Immunization Induces Both Humoral Response and Long-lasting T-Cell Memory Responses in Rhesus Macaques

To determine the immunogenicity of sE2 in a nonhuman primate model, 3 groups of 5 rhesus macaques were immunized intramuscularly with sE2 formulated with alum (macaque identifiers: B1–B5), alum plus CpG (C1–C5), or alum plus MPL (D1–D5). As a control, a fourth group of macaques (A1–A5) was injected with alum alone. The kinetic profile of sE2-specific antibody titers following immunization is shown in Figure 1A. In general, for the 3 vaccine groups, sE2-specific antibody was detectable after the first injection; the antibody titers increased after the second and third injections, peaked at month 3, and then gradually declined but still remained at high levels until month 5. Following a homologous boosting at month 5, levels of sE2-specific antibody titers rapidly rose, with an approximately 2-log increase observed at month 6, indicating the establishment of B-cell memory. In contrast, no significant anti-sE2 response was detected in the control macaques throughout the observation period.

The T-cell response to sE2 in the macaques was examined by ELISPOT analysis. IFN-γ– and IL-4–producing cells were detected in splenocytes from most of the sE2-immunized macaques but not from the control animals (Figure 1B). In pools of peripheral blood mononuclear cells (PBMCs) collected at month 4, a significant increase in IFN-γ–secreting cells upon sE2 stimulation was observed in the sE2/alum/CpG and sE2/alum/MPL groups, whereas only the sE2/alum and sE2/alum/MPL groups significantly demonstrated an IL-4 secretory response (Figure 1C). At month 8, none of the groups exhibited increases in IFN-γ secretion upon sE2 stimulation; however, IL-4–secreting cells were still present at high frequencies in animals of the sE2/alum and sE2/alum/MPL groups (Figure 1C). The change in the profiles of IFN-γ– and IL-4–producing cells over time suggests a shift from a T-helper type 1 (Th1) response to a Th2 response. Strikingly, intrahepatic lymphocytes collected at month 8 from all 3 sE2 immunization groups strongly produced IFN-γ and IL-4 following antigen stimulation (Figure 1D), indicating the successful establishment of resident T-cell memory.

Sera From sE2 Immunized Rhesus Macaques Efficiently Neutralize Multiple HCV Genotypes

Antisera from the immunized macaques were analyzed for their neutralization ability against the HCVcc panel described above. As shown in Figure 2A, at a 1:20 dilution none of the control antisera had significant neutralizing activity; in contrast, individual antisera from the 3 vaccine groups exhibited different efficiencies in neutralizing distinct HCV genotypes. In particular, Con1, PR52B6mt, PR79L9, JFH1, J8, ED43, and HK6a were effectively neutralized (average neutralization, ≥50%), with H77, J6, S52, SA13, and QC69 neutralized to a lesser extent (average neutralization, <50%). Among the 3 groups, sE2/alum/MPL antisera had the broadest neutralization spectrum, with average neutralization efficiency of ≥50% against all tested HCV strains except H77. By competitive ELISA, we found that, compared with the sE2/alum group, the sE2/alum/CpG group and sE2/alum/MPL group possessed more AP33-like and AR3A-like bNAbs (Figure 2B), suggesting that both CpG and MPL adjuvants may augment the induction of bNAbs.

As a quantitative assessment, we summarized the neutralization breadth—the number of HCVcc strains that can be

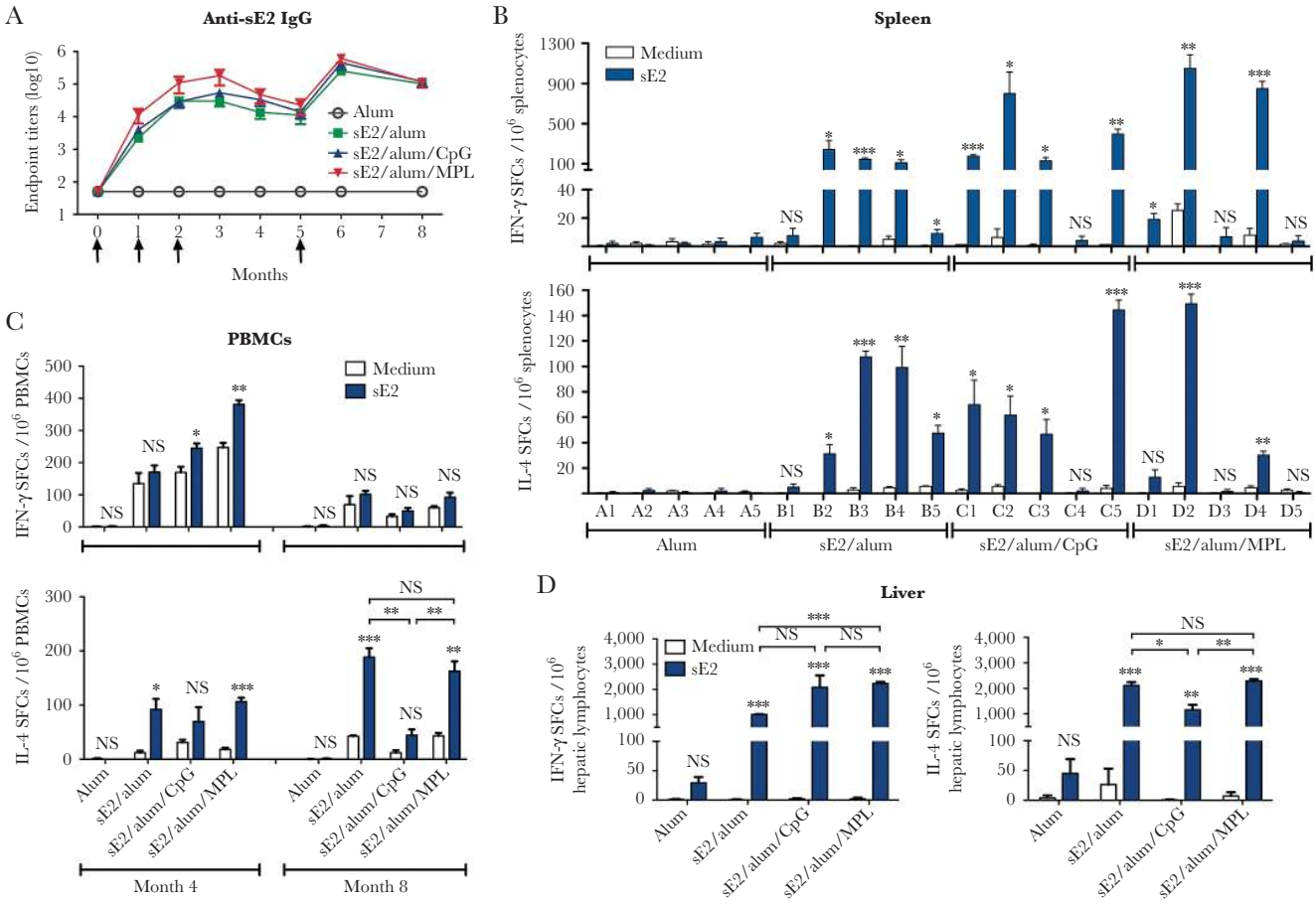

Figure 1. Induction of both humoral and cellular immune responses in rhesus macaques. *A*, Kinetics of sE2-specific antibody response. Rhesus macaques (5 per group) were immunized intramuscularly at months 0, 1, 2, and 5, as indicated by the black arrows. Serum samples were collected monthly and titrated by enzyme-linked immunosorbent assay. Mean titers ± standard errors of the mean (SEMs) for each group are shown. *B–D*, The interferon γ (IFN-γ)– and interleukin 4 (IL-4)–secreting cell responses in spleens (*B*), peripheral blood mononuclear cells (PBMCs; *C*), and livers (*D*). Splenocytes from each macaque (collected at month 8), pooled PBMCs from each group (collected at months 4 and 8), and pooled intrahepatic lymphocytes from each group (collected at month 8) were stimulated with sE2 protein and then analyzed by IFN-γ and IL-4 enzyme-linked immunospot assays. Data are means ± SEMs from 2 independent experiments performed in triplicate. Asterisks represent significant differences between medium (white bars) and sE2 stimulation (blue bars) in each group or between groups. NS, not significant ($P \geq .05$); SFC, spot-forming cell. *$P < .05$, **$P < .01$, and ***$P < .001$.

neutralized by a given sera—of the antisera (Table 1). Sera from 5 of 15 sE2-immunized macaques (B1, C4, D1, D3, and D5) had a highly broad neutralization spectrum (≥50% neutralization against no less than 10 strains); sera from 8 were moderately broad (≥50% neutralization against 6–9 strains); and only 2 sera (from macaques B3 and C2) had narrow neutralization spectra (≥50% neutralization against 3 and 4 strains, respectively). Notably, antisera from macaques D3 and D5 neutralized all 12 strains at a 1:20 dilution (Table 1). Further titration showed that all individual antisera from animals in group D (the sE2/alum/MPL group) had 50% neutralization titers of ≥5 against each individual HCVcc strain (Table 2).

Neutralization Breadth Correlated With sE2-Specific IgG Titer

We observed that all macaque antisera with very broad neutralization breadths also had high titers of sE2-specific IgG, whereas the 2 sera with narrow neutralization breadth (from macaques B3 and C2) had the lowest sE2-specific IgG titers. Spearman correlation analysis of all the 15 sE2-immunized macaque sera revealed a significant correlation (Figure 3A) between the sE2-specific IgG titers and the neutralization breadths. Based on this result, total IgG purified from the sera from macaques B3, C2, and D5 was tested for its ability to neutralize antigenically diverse HCV strains of different genotypes (H77, S52, SA13, and QC69). In accordance with previously published work demonstrating that intergenotypic HCVcc chimeras harboring envelopes from these isolates are harder to neutralize [28], 1:20 diluted antisera from most of the macaques neutralized only weakly these isolates (Table 1). IgG from macaque A1 served as a control in this assay. As shown in Figure 3B, a dose-dependent trend of neutralization against all 4 of these HCV strains was observed for IgGs from macaques B3, C2, and D5 but not A1. This indicates that, like sera from macaque D5, sera from

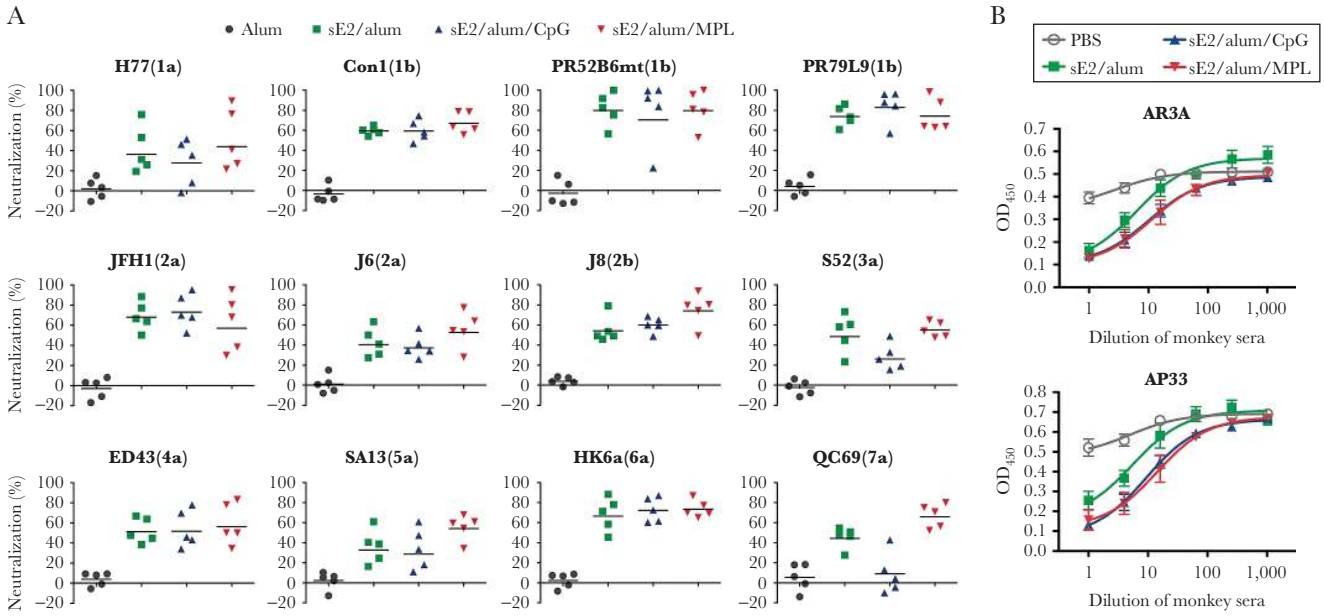

Figure 2. Neutralization of the sE2-immunized macaque sera. *A*, The macaque sera collected at month 6 were diluted 1:20 and then tested for neutralization of various cell culture–derived HCV (HCVcc) of genotypes 1–7 as indicated. Each symbol represents 1 animal, and the horizontal lines indicate the geometric means for each group. The data are representative of 3 independent neutralization experiments. *B*, Competitive enzyme-linked immunosorbent assay. Macaque antisera were serially diluted and tested for inhibition of AR3A and AP33 binding sE2. Means ± standard errors of the mean of the OD_{450} readings for all animals in each group are shown. PBS, phosphate-buffered saline.

macaques B3 and C2 sera also possess bNAbs, albeit at low levels (Figure 3C).

DISCUSSION

Prophylactic HCV vaccines capable of eliciting both NAbs and HCV-specific T-cell responses are highly desirable [3, 18]. While a number of vaccine candidates have been shown to be able to induce cross-NAbs and cellular immune response in small-animal models, only a fraction of them were advanced to immunogenicity studies in nonhuman primates [13, 14, 23–25]. The present study aimed to evaluate the immunogenicity of our recently developed sE2 vaccine [28] with Food and Drug Administration (FDA)–approved adjuvants in nonhuman primates.

In our previous study, NAbs were induced to different extents with different adjuvants (eg, alum, Freund adjuvant, and Toll-like receptor ligand CpG) [28]. Herein, 3 adjuvants or combinations were compared in our macaque immunization studies,

Table 1. Summary of the Neutralization Potency of Individual Macaque Antiserum Against 12 Cell Culture–Derived Hepatitis C Virus (HCVcc) Strains

	Alum					sE2/Alum					sE2/Alum/CpG					sE2/Alum/MPL				
HCVcc Strain	A1	A2	A3	A4	A5	B1	B2	B3	B4	B5	C1	C2	C3	C4	C5	D1	D2	D3	D4	D5
H77(1a)	7.7	−5.3	−10.5	3.3	15.3	75.9	25.8	19.3	53.1	31.4	−1.8	8.2	46.2	51.3	35.4	27.0	21.7	76.6	41.2	89.5
Con1(1b)	10.4	−9.7	−8.6	−8.5	−0.7	65.1	57.6	60.2	60.6	54.2	54.3	46.9	58.2	74.6	66.9	55.7	61.7	78.8	64.0	78.6
PR52B6mt(1b)	15.0	−11.6	−10.3	6.2	−12.9	75.4	91.7	56.6	100.0	82.7	83.8	22.6	99.4	100.0	92.1	52.9	78.3	100.0	81.0	95.6
PR79L9(1b)	5.2	15.6	−2.3	7.4	−5.8	81.6	60.7	73.3	86.1	69.7	84.4	56.9	95.9	96.4	87.8	64.1	63.0	98.3	64.2	88.0
JFH1(2a)	2.5	3.1	8.1	−17.0	−10.8	66.5	63.8	77.3	88.5	50.2	68.0	52.1	87.3	95.4	69.9	38.4	30.0	95.6	68.2	80.3
J6(2a)	15.1	2.3	−7.9	−5.0	0.8	63.4	27.5	41.1	50.0	30.8	34.5	25.8	41.5	56.8	34.2	54.6	28.1	64.2	53.3	77.5
J8(2b)	3.5	8.5	7.3	−1.6	2.7	79.1	53.7	49.2	49.3	45.8	59.7	48.7	65.2	68.8	60.0	80.6	49.3	79.8	74.7	94.0
S52(3a)	−7.7	6.3	2.4	−0.9	−11.5	73.3	58.1	23.3	44.8	60.5	25.8	15.4	32.9	48.9	19.0	54.1	49.3	65.2	47.7	62.2
ED43(4a)	−5.5	−0.9	9.3	9.3	8.1	66.9	63.8	38.5	47.6	44.8	45.8	43.2	69.8	77.9	34.0	50.1	50.2	83.3	34.5	78.1
SA13(5a)	6.3	10.4	−12.9	2.0	5.6	60.9	40.3	38.7	16.3	24.4	18.0	33.4	47.3	60.9	11.1	59.5	34.2	67.9	54.6	61.0
HK6a(6a)	6.8	8.6	−8.2	−2.1	7.4	78.0	71.2	45.4	88.4	58.4	72.6	61.5	84.0	87.1	60.4	69.3	65.2	86.9	70.1	77.3
QC69(7a)	17.9	−13.8	6.3	−0.9	18.3	46.5	48.7	27.7	54.6	50.8	4.1	−9.9	12.8	42.8	−4.3	75.0	52.3	80.0	56.5	70.8
Neutralization breadth[a]	0	0	0	0	0	11	8	4	8	7	6	3	7	10	6	10	6	12	9	12

Macaque antisera were diluted 1:20 for neutralization tests. For a given antiserum, its neutralization potency was defined as "strongly neutralizing" (defined as ≥50% inhibition), "weakly neutralizing" (defined as 20%–50% inhibition), or "nonneutralizing" (defined as <20% inhibition).

[a]Neutralization breadth was defined as the number of HCVcc strains that can be strongly neutralized (defined as ≥50% inhibition) by a given antiserum.

Table 2. 50% Neutralization Titers (NT_{50}) of Select Macaque Antisera Against Genotypes 1–7 Cell Culture–Derived Hepatitis C Virus (HCVcc)

	1a	1b			2a		2b	3a	4a	5a	6a	7a
Antisera	H77	Con1	PR52 B6mt	PR79 L9	JFH1	J6	J8	S52	ED43	SA13	HK6a	QC69
A1	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
D1	10	80	80	80	20	20	20	40	20	20	40	160
D2	5	40	80	80	20	10	10	20	10	20	40	40
D3	40	320	320	320	640	40	20	40	320	20	80	80
D4	20	40	80	80	80	40	40	10	10	10	40	80
D5	80	640	160	80	1280	40	20	80	640	40	320	640

Antisera from 5 macaques from group D (the sE2/alum/MPL group) and 1 macaque from group A (the alum-alone group) were chosen to perform neutralization assays. NT_{50} was defined as the highest dilution of sera able to neutralize 50% of HCVcc infectivity. Data are representative results from 3 independent experiments.

which are either FDA approved (alum and alum plus MPL) [34] or in clinical trials (alum plus CpG). Among them, alum plus MPL appeared to be superior in terms of the magnitude and breadth of NAbs that were elicited. Specifically, in the sE2/alum/MPL group, antisera from 2 of 5 macaques at a 1:20 dilution showed ≥50% neutralization against all 12 HCVcc strains tested, and the other 3 antisera each neutralized 6–10 strains (Table 1). At a lower dilution (1:5), all individual antisera from this group exhibited ≥50% neutralization against all HCVcc tested (Table 2). In addition to the NAbs, sE2/alum/MPL of the 3 sE2/adjuvant combinations induced the highest number of IFN-γ– or IL-4–secreting intrahepatic cells, as well as balanced Th1 and Th2 responses (Figure 1D). Together, the results described above suggest that sE2/alum/MPL is the best combination for the induction of both NAbs and T-cell immunity against HCV. Since alum/MPL adjuvants have been approved for human use, this finding adds confidence to advancing the sE2/alum/MPL vaccine into clinical trials.

Tissue-resident memory T cells play a critical role in protective immunity [35, 36]. For example, vaccine-induced tissue-resident memory T cells in the initial infection site, such as lung [37] or cervical mucosal tissue [38], has been closely related to protection against virus infection. Likewise, Huang et al reported a liver-resident cytotoxic T-lymphocyte

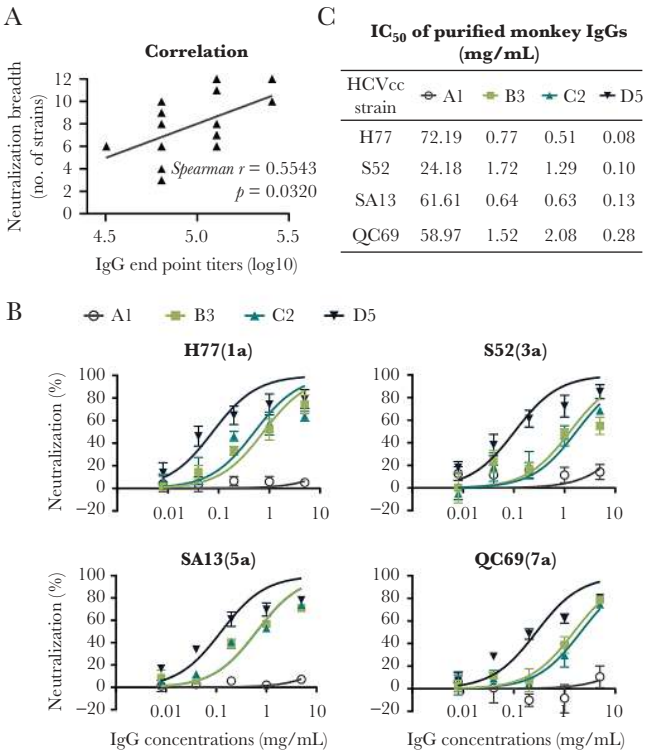

Figure 3. A correlation between sE2-specific immunoglobulin G (IgG) titer and neutralization breadths. *A*, Spearman correlation analysis between sE2-specific antibody titers and neutralization breadths. *B*, Dose-dependent neutralization by purified IgG. IgG was purified from antisera of 4 macaques, including 2 macaques (C2 and B3) with weak neutralizing potencies, macaque D5 (as a high-neutralization control), and macaque A1 (as a negative control). Neutralization assays were performed to analyze the neutralizing potencies of serial diluted IgG against H77, S52, SA13, and QC69 cell culture–derived hepatitis C virus (HCVcc). Mean values ± standard errors of the mean of 2 independent experiments performed in duplicate are shown. *C*, Fifty percent inhibitory concentrations (IC_{50} values; in mg/mL) of the individual IgGs.

population controlled viral infection of the liver after vaccination [39]. Of note, 2 previous studies demonstrated that intrahepatic T cells, particularly IFN-γ–producing cells, are relevant to protection from or delay of HCV infection in chimpanzees [23, 40], emphasizing the critical role of intrahepatic T-cell responses. In the present study, we found that the sE2 vaccine elicited a long-lasting T-cell response in macaques. Specifically, IFN-γ– and IL-4–secreting cells were detected upon sE2 stimulation of not only PBMCs and splenocytes (Figure 1B and 1C) but also intrahepatic cells (Figure 1D), indicating the establishment of both systemic and resident T-cell memory following vaccination. The induction of intrahepatic T-cell responses by vaccination may result from a migration of T cells from the peripheral circulation to the liver and differentiation to liver-resident memory T cells [35, 36]. Although it remains challenging to assess the protective effect of these long-lived hepatic T cells in macaques (as macaques are not susceptible to HCV infection), the establishment of hepatic T-cell memory is likely favorable for controlling and clearing HCV infection in the liver. Therefore, the ability to induce HCV-specific intrahepatic T-cell responses should be a desirable feature of the sE2 vaccine.

Overall, the present study demonstrates that insect cell–produced sE2 is able to elicit broadly NAbs and both systemic and intrahepatic T-cell responses in nonhuman primates, therefore representing a promising HCV vaccine candidate that warrants further preclinical and clinical development. Of importance, the neutralizing titers of monkey sera were lower than those that can be reached in mouse sera [28]. This difference can possibly be attributed to several factors, including but not limited to vaccine dose, formulation, administration routes, and the diverse genetic background in outbred rhesus macaques. Nonetheless, our study identifies a correlation between E2-specific IgG titer/dose and neutralization breadth (Figure 3A–C), suggesting that future efforts to improve the vaccine potential of sE2 should be focused on enhancing its immunogenicity.

Notes

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