Immunization with Hepatitis C Virus-Like Particles Induces Humoral and Cellular Immune Responses in Nonhuman Primates

Sook-Hyang Jeong,†‡ Ming Qiao,†‡§ Michelina Nascimbeni,† Zonyi Hu,† Barbara Rehermann,† Krishna Murthy,‡ and T. Jake Liang†‡

Liver Diseases Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892,† and Department of Virology and Immunology, Southwest Foundation for Biomedical Research, San Antonio, Texas, 78227‡

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We have previously reported the production of hepatitis C virus-like particles (HCV-LP) using a recombinant baculovirus containing the cDNA of the HCV structural proteins (core, E1, and E2). These particles resemble the putative HCV virions and are capable of inducing strong and broad humoral and cellular immune responses in mice. Here we present evidence on the immunogenicity of HCV-LP and the effects of novel adjuvant systems in a nonhuman primate model, the baboon. Three groups of four baboons were immunized with HCV-LP, HCV-LP and adjuvant AS01B (monophosphoryl lipid A and QS21), or HCV-LP and the combination of AS01B and CpG oligodeoxynucleotides 10105. After four immunizations over an 8-month period, all animals developed HCV-specific humoral and cellular immune responses including antibodies to HCV structural proteins and gamma interferon+ (IFN-γ+)CD4+ and IFN-γ+CD8+ T-cell responses. The immunogenicity of HCV-LP was only marginally enhanced by the use of adjuvants. The overall HCV-specific immune responses were broad and long lasting. Our results suggest that HCV-LP is a potent immunogen to induce HCV-specific humoral and cellular immune responses in primates and may be a promising approach to develop novel preventive and therapeutic modalities.

Hepatitis C virus (HCV) is a major public health problem; approximately 3% of the world population, about 170 million people, are infected by the virus (19, 22). HCV causes high rate of chronic infection, which can lead to severe complications of chronic liver disease such as liver cirrhosis and hepatocellular carcinoma. The efficacy of therapy for chronically infected patients is less than satisfactory. Development of an effective vaccine may hold the key in the control of HCV infection.

HCV not only causes chronic infection in the majority of infected people but also displays high genetic and antigenic diversities with at least six different genotypes and diverse quasispecies within the infected individuals (19, 22). In addition to this inherent difficulty, the lack of tissue culture systems can modulate the immune response toward different T-helper response (Th1 versus Th2) (1, 5, 8, 14, 17, 23, 38, 40). Vaccination of HCV-LP combined with adjuvant(s), AS01B (monophosphoryl lipid A and QS21), and/or CpG 10105 (oligodeoxynucleotides containing the immunostimulatory CpG motif) enhanced HCV-specific antibody production and promoted cellular immune responses with a Th1 bias in AAD mice (30).

In order to optimize the vaccine effect of HCV-LP for use in humans, we evaluated in this paper the safety and immunogenicity of HCV-LP in a nonhuman primate model, the baboon. In addition, we evaluated the effects of vaccine adjuvant AS01B and the combination of AS01B and CpG 10105 on the immunogenicity of HCV-LP in these animals. Although chimpanzees are the only animals susceptible to HCV infection (18) and have a >98% genomic sequence homology with human, they are an endangered species and difficult to work with because of high costs and other restrictions. Next to the great apes (chimpanzees, orangutans, gorillas, and gibbons) in the evolutionary distance are the Old World monkeys, such as baboons, mandrills, mangabeys, and macaques. Baboons are phylogenetically close to humans, have four immunoglobulin G (IgG) subclasses, and possess cross-reactive Ig and cluster of differentiation antigens similar to those of humans and chimpanzees (16). The overall profile of baboons, as a less costly
nonendangered species, more accessible animal model, and yet possessing immunology comparable to that of humans and chimpanzees, makes them a suitable animal model for preclinical studies of vaccine, although they are not susceptible to HCV infection (34).

**MATERIALS AND METHODS**

**Purified proteins and synthetic peptides.** E1 and E2 proteins were expressed in vvHCV.S-infected BSC-1 cells and purified by using a lectin column (4). HCV core protein (amino acids [aa] 1 to 115) was purchased from Microgen (München, Germany). Synthetic peptides spanning the whole HCV structural genome of HCV 1b genotype J strain synthesized by Chiron Mimotopes (Clayton, Victoria, Australia). Peptides were 15 aa in length with a 10-aa overlap. The overlapping peptide 1 (OLP1) consisted of a pool of 38 peptides spanning the HCV core sequence, OLP2 consisted of 38 peptides spanning the HCV E1 sequence, OLP3 consisted of 35 peptides spanning the N-terminal half of the HCV E2 sequence, and OLP4 consisted of 36 peptides spanning the C-terminal half of the HCV E2 sequence. Peptide stock solutions were prepared in 5% dimethyl sulfoxide in phosphate-buffered saline (PBS) at the concentration of 1 mg/ml and stored at −20°C.

**Assay of antigens, vvHCV.S, and the adjuvants.** Procedures for production and purification of HCV-LP were as described previously (3), with some modifications. Briefly, S99 cells (2 × 10^7) were infected with recombinant baculovirus (hvHCV.Sp7) at a multiplicity of infection of 5 to 10 and incubated at 27°C for 3 days in SF900 serum-free medium (Gibco-Invitrogen Corporation, Carlsbad, Calif.). Cells were harvested by centrifugation at 2,500 × g for 5 min at room temperature, and the cell pellet was washed once with PBS. The cell pellet was resuspended in 18 ml of prewarmed PBS and 3 ml of 90% glycerol containing 10 mM HEPES buffer, 1 mM phenylmethylsulfonyl fluoride [PMSF]; Sigma-Aldrich, St. Louis, Mo.) and a cocktail of EDTA-free protease inhibitors (PI; one tablet per 50 ml; Roche, Indianapolis, Ind.). The cell suspension was mixed and incubated at 37°C for 5 min. The process was repeated twice so that the final glycerol concentration in the cell suspension reached 30% (32). The cells were then chilled on ice for 5 min and centrifuged at 2,500 × g for 10 min at 4°C. The following purification steps were performed at 4°C unless specified.

The cell pellet was resuspended with 50 ml of lysis buffer (10 mM Tris-HCl [pH 7.4], 1 mM MgCl$_2$, 1 mM CaCl$_2$, 1 mM PMSF, PI [TNC-PI buffer]; containing 0.5% digitonin and allowed to sit on ice with gentle agitation. The percentage of cell lysis reached satisfactory levels after periodic monitoring for cell lysis by trypan blue exclusion, the cell lysate was centrifuged at 26,000 × g in an SW 28 rotor (Beckman) for 30 min to remove cell debris. To maximize the HCV-LP yield, the lysis process may be repeated once more by resuspending the cell pellet in fresh lysis buffer containing 0.5% digitonin. The supernatant was loaded onto 3 ml of a 20% sucrose cushion in TNC-PI buffer and centrifuged at 100,000 × g for 3 h by using an SW41 rotor (Beckman). The pellet was then resuspended in a small volume of TNC-PI buffer and then loaded onto 9 ml of a 20 to 60% sucrose gradient and centrifuged at 120,000 × g for 16 h in an SW41 rotor. One-milliliter fractions were collected from the top of the tube, and these were tested for core, E1, and E2 proteins by enzyme-linked immunosorbent assay (ELISA) (37) and Western blotting. Total protein was quantitated by using Coomasie Blue Plus protein assay reagent (Pierce, Rockford, Ill.), and ultrastructural study of HCV-LP was performed by electron microscopy.

Recombinant vaccinia virus (wild type, Western reserve) expressing HCV structural proteins (vvHCV.S) as positive controls for HCV-specific immune response. All animal experiments were conducted according to the criteria published by the National Institutes of Health (NIH publication 86-23, revised in 1985).

**Analysis of antiviral and antibody responses.** Baboons sera collected before vaccination (week 0) and 4 weeks after each vaccination (weeks 4, 8, 12, and 35) were tested for antiviral and antibody responses by ELISA. Briefly, 96-well microtiter plates (Nunc Immunoplates; Nunc Corp., Naperville, Ill.) were coated with purified E1 and E2 protein solution (5 μg/ml) or core protein (2 μg/ml; Microgen, Munich, Germany) diluted in 0.05 M bicarbonate buffer (pH 9.6) at 4°C overnight. The wells were washed three times with PBS to remove unbound proteins and then blocked 2 h at 37°C with 5% skim milk in PBS. After the plate was washed three times with PBS containing 0.05% Tween 20 (PBST), 100 μl of sera diluted 1:100 in PBST containing 5% skim milk was added to the wells and incubated for 1 h at 37°C. The wells were washed six times with PBST, and 100 μl of peroxidase-conjugated goat anti-human IgG (Sigma-Aldrich) diluted 1:2,000 in PBST with 5% skim milk was added and incubated for 45 min at room temperature. The wells were washed six times and incubated with a peroxidase substrate (ABTS, Microwell Peroxidase Substrate System; KPL Laboratories, Gaithersburg, Md.). The optical density at 405 nm (OD$_{405}$) of the plate was read. All assays were run in triplicates. Antibody titers were determined by the serial end point dilution method. End point titers were defined as the serum dilution giving an OD$_{405}$ reading for positivity was defined as two times the OD value of the preimmune sera (signal/noise ratio of ≥2).

**Titration of vaccinia virus neutralizing antibodies.** Detection and titration of vaccinia virus neutralizing antibodies was performed as previously described by using BSC-1 cells (9). Briefly, virus inoculum of wild-type vv-WR (Western Reserve) was mixed with fivefold serial dilutions of pre- and postvaccination sera (week 8) for 2 h at 4°C after heat inactivation of the sera. The virus-serum mixture was added to near-confluent BSC-1 cell monolayers in six-well plate and incubated for 1 h at 37°C. After being washed twice with 2× PBS, cells were incubated for 2 days in 37°C at 5% CO$_2$. Cells were then stained with 0.1% crystal violet for 5 min, and the plaques were counted. The dilution of serum capable of reducing the number of virus plaques by 50% compared to the virus control was set as the 50% inhibitory concentration.

**Ex vivo direct ELISPOT assay for IFN-γ.** Baboon peripheral blood mononuclear cells (PBMC) prior to immunization (week 0) and 4 weeks after each immunization (weeks 4, 8, 12, and 38) were used to measure the number of antigen-specific gamma interferon (IFN-γ)-producing cells by using an ELISPOT kit (U-CyTech, Utrecht, The Netherlands). Briefly, microtiter plates were coated with monoclonal antibody specific for mouse IFN-γ at 4°C overnight. After being washed five times with 0.05% PBST, the wells were blocked with 1% bovine serum albumin for 2 h at 37°C. PBMC (3 × 10^5) were added to the wells in duplicates and stimulated with soluble protein antigens (1 μg/ml of HCV core protein or 2 μg of HCV E1/E2 proteins/ml, or OLP pools of core, E1, and E2 (1 μg of each peptide/ml), respectively. As a negative control, media (without any antigen) was added and as a positive control, Concanavalin A (Sigma-Aldrich) was added at a concentration of 5 μg/ml. Cells were incubated for 24 h at 37°C and then lysed with distilled water for 10 min. After being washed 10 times with PBST, biotinylated detecting antibody was incubated for 1 h at 37°C. After being washed 10 times with PBST, antibiotic antibody was incubated for 1 h at 37°C followed by the addition of chromogenic substrate according to the manufacturer’s instructions. Tap water was added to the wells to stop the reaction, and the dried plate was read by use of the automated ELISPOT Reader System with KS software (Carl Zeiss, Thornwood, N.Y.). IFN-γ spot-forming units (SFU) representing single cells were counted and expressed as SFU for 3 × 10^5 PBMC. The numbers of antigen-specific SFU per well were calculated by subtracting background values (wells without antigen, typically less than 5). The sample was considered positive when the SFU (after subtracting the background) was greater than 10 and more than or equal to twice the mean SFU of the sample prior to immunization in the same animal. The PBMC were also tested against an irrelevant peptide (HBV core peptide, aa 18 to 27) as an additional control, and in all cases, the SFU were less than 10 (without subtracting the background).

**Intracellular cytokine staining for IFN-γ-Cy5** T cells. PBMC (10^6) cells in 0.5 ml of RPMI medium in 5 ml polytene tubes (Falcon; Becton Dickinson, Franklin Lakes, N.J.) were stimulated with OLP pools of core, E1, or E2 (1 μg of each peptide/ml) and costimulated with 1 μg of anti-human CD28 (clone CD28.2) and anti-human CD49d (9F10) (BD Biosciences)/ml for 1 h at 37°C and then for an additional 5 h in the presence of monensin (Golgi Stop; BD Pharmingen). As controls, cells were incubated without peptide (as a negative control) or stimulated with phorbol myristate acetate (PMA) (50 ng/ml) and iono-
mycin (1 μM) instead of peptides (as a positive control), respectively. After being washed twice with PBS–1% fetal calf serum, cells were incubated with fluorescein isothiocyanate-conjugated anti-CD8 (mouse anti-human monoclonal antibody [clone RPA-T8, 5×10^6 PBMC]) for 30 min at 4°C. The cells were then washed with permeabilization buffer using Cytofix/Cytoperm kit (BD PharMingen) and stained with 10^6/l of phycoerythrin-conjugated mouse anti-human IFN-γ (clone 4S.B3; BD Biosciences) for 30 min at 4°C. The stained cells were washed with PBS–1% fetal calf serum, fixed with 2% paraformaldehyde, and analyzed by flow cytometry by using CellQuest software (BD Biosciences). At least 100,000 events and in some cases, up to 300,000 events were acquired from each sample. The percentage of IFN-γ CD8^+ T cells was defined as the difference between the percentage detected in peptide-stimulated cells and the percentage detected in unstimulated cells in the PBMC of the same animal. The cutoff for positive percentage of IFN-γ CD8^+ T cells was set at greater than twice the mean percentage of sample prior to immunization in the same animal. As a negative control, the PBMC were also tested against an irrelevant peptide (HBV core peptide, aa 18 to 27), which typically gave 0.01% of IFN-γ CD8^+ T cells.

Statistical analysis. Comparisons between groups of baboons were analyzed by the Kruskal-Wallis test using software from the SAS Institute Inc (Cary, N.C.), and differences were considered significant when P values were ≤0.05.

RESULTS

Safety of HCV-LP and adjuvants in baboons. The 12 baboons subjected to HCV-LP immunization were housed together, and the remaining 2 baboons subjected to vvHCV.S immunization were housed separately. All baboons were healthy throughout the study period, and there were no signs of systemic or local reactions to HCV-LP, adjuvants, or vvHCV.S. Blood chemistry and complete blood counts performed before immunization and after each immunization were normal.

Humoral immune response. Anti-HCV-E1/E2- and core-specific humoral responses were analyzed by ELISA (20), and the results are shown in Fig. 1. Blood samples collected before immunization and 4 weeks after the third and fourth immunization were tested in parallel and in duplicate. Seroconversion was defined as an increase of twofold or more of the OD_405 of postimmunization sera over that of the preimmune sera at a 1:100 dilution.
All baboons immunized with HCV-LP developed an anti-E response (100% seroconversion rate) after the fourth immunization (week 38) (Fig. 1A). However, the seroconversion rate after the third immunization (week 12) was increased by the use of adjuvant(s) from 0% in the HCV-LP group to 50% in the HCV-LP + ASO1B group and 100% in the HCV-LP + ASO1B/CpG 10105 group. The combination of ASO1B and CpG 10105 seemed to be superior to no adjuvant or to single adjuvant ASO1B in promoting a faster and stronger anti-E1 or anti-E2 response. However, the overall anti-E titers after the fourth immunization were not significantly different in baboons immunized with HCV-LP or HCV-LP plus adjuvant(s) (Table 1).

In contrast to the anti-E1 and anti-E2 response, the anticore response was slow to develop in all animals (Fig. 1B and D) and no anticore antibodies were detected in any of the three groups of animals after three immunizations (week 12). However, all animals (with or without adjuvant) developed anticore antibodies after the fourth immunization and the antibody titers of the animals immunized with HCV-LP plus adjuvant(s) were only slightly higher than those of animals immunized with HCV-LP (P value not significant). End point titers of anti-E1 or -E2 and anticore among the three groups after the fourth immunization are summarized in Table 1. The anti-HCV antibodies induced in all three groups persisted (less than twofold reduction in titer) 8 months after the last immunization (Fig. 1C and D).

As immunization controls, two baboons were immunized twice with $5 \times 10^8$ PFU of purified recombinant vaccinia virus expressing HCV structural proteins (vvHCV.S) at weeks 0 and 4. ELISA results showed that these baboons developed low levels of anti-E response (titers of 50 and 100) after the second immunization while anticore response was not detected at all in either animal. We detected vaccinia virus-neutralizing antibodies in both animals (titers, 200 and 400) by virus neutralization assay.

**CD4$^+$ T-cell response.** To quantitatively examine the T-helper response against the HCV structure proteins in baboons immunized with HCV-LP alone or HCV-LP combined with adjuvant(s), we performed ELISPOT assays for IFN-γ. PBMC collected before immunization (week 0) and after the third and fourth immunization (weeks 8, 12, 30, and 38) were tested in parallel and in duplicate. These cells were stimulated with recombinant core or E1 and E2 proteins or with four pools of OLP (core, E1, and E2) as described in Materials and Methods. The number of HCV-specific SFU was calculated by subtracting the number of spots in the absence of stimulant from those in the presence of antigen or OLP. Figure 2 displays the IFN-γ ELISPOT results of all 12 animals after immunization with HCV-LP, HCV-LP + ASO1B, or HCV-LP + ASO1B/CpG 10105.

Immunization with HCV-LP (Fig. 2, far left column) resulted in IFN-γ secretion after stimulation of PBMC with HCV proteins or OLPs. The addition of ASO1B or ASO1B + CpG 10105 seemed to further enhance the number of IFN-γ producing T cells (Fig. 2, middle and far right columns), although the difference did not reach statistical significance. The IFN-γ responses of these animals suggested that HCV-LP immunization induced a Th1 response against HCV structural proteins and OLPs. The number of SFU was markedly increased after
the last booster immunization in all the baboons, indicating that the fourth immunization at month 8 (6 months after the previous immunization) is important in generating high-level HCV-specific cellular immune responses. There was no obvious trend in the three groups of animals in their responses to different HCV-specific stimulants (HCV proteins or OLPs).

The HCV-specific CD4⁺ response was maintained for an extended period of time after the fourth immunization. Blood samples obtained 8 months after the fourth immunization showed IFN-γ SFU results by ELISPOT similar to those obtained with the samples taken 1 month after the fourth immunization (Fig. 2).

**CD8⁺ T-cell response.** To quantitatively determine the CD8⁺ responses in HCV-LP immunized animals, we per-
FIG. 3. CD8⁺ T-cell response obtained by intracellular cytokine staining (ICS) in HCV-LP-immunized animals. PBMC collected before immunization (week 0) and 4 weeks after the second to fourth immunizations (weeks 8, 12, and 38, respectively) were stimulated in vitro with HCV OLPs spanning core, E1, N-terminal E2(1), and C-terminal E2(2). Negative control without peptide and positive control with phorbol myristate acetate and ionomycin were included. Representative fluorescence-activated cell sorter histograms of baboons (percentage of IFN-γ⁺ CD8⁺ cells shown in the right upper corner) from each immunized group after the fourth immunization are shown in panel A and the overall responses are summarized in panel B. For background determination, PBMC cultured without HCV-specific peptide stimulation were tested in parallel with those stimulated with HCV core and E peptides. The percentage of HCV core-specific or HCV E-specific IFN-γ⁺ CD8⁺ cells from each group of animals was determined after subtracting the percentage of IFN-γ⁺ CD8⁺ cells in the absence of HCV peptides from that in the presence of HCV peptides. The cutoff of the assay for each peptide pool is about 0.015%, which is set as twice the mean of multiple negative controls (without antigen).
formed ex vivo intracellular cytokine staining (ICS) for IFN-γ-producing CD8+ cells (Fig. 3). PBMC collected before immunization (week 0) and after the second, third, and fourth immunizations were stimulated with OLPs of HCV core protein and E1 and E2 proteins, respectively. As shown in Fig. 3B, all the immunized baboons demonstrated significant production by CD8+ cells of IFN-γ to one or more of the OLPs (greater than twofold over the baseline value) after the fourth immunization. The fluorescence-activated cell sorter histograms of representative immunized baboons after the fourth immunization are shown in Fig. 3A. Some of the animals also exhibited a positive response at earlier time points, either after the second or the third immunizations. However, many animals did not demonstrate any detectable IFN-γ-producing CD8+ response until after the fourth immunization, again supporting the importance of the final boost after an extended period of time from the third immunization. Combining all the OLP responses, the total frequency of the IFN-γ-producing CD8+ cells after the fourth immunization reached up to 0.45% in some animals. HCV-LP combined with adjuvants ASO1B and CpG (Fig. 3B) appeared to induce higher frequencies of IFN-γ-producing CD8+ T-cell response, but statistical significance was not observed among the three groups of baboons (Table 1).

**Cellular immune response in animals immunized with vvHCV.S.** As an immunization control for cellular immune response, we immunized two baboons with vvHCV.S. PBMC were collected and tested in the same way as those of HCV-LP-immunized animals. HCV-specific SFU with E protein stimulation was approximately 40 to 50 SFU/10^5 cells after two immunizations. Similarly, PBMC stimulated with OLPs of HCV core and E proteins yielded detectable SFU (Table 1). Intracellular cytokine staining of HCV OLP-stimulated PBMC did not show detectable induction of IFN-γ-producing cells among CD8+ cells after two immunizations with vvHCV.S (Table 1).

**DISCUSSION**

Here we present a study of the immunogenicity of HCV-LP and the effects of novel adjuvant systems in a nonhuman primate model, the baboon. Three groups of four baboons were immunized with HCV-LP or HCV-LP plus adjuvant(s), ASO1B (monophosphoryl lipid A and QS21) or the combination of ASO1B and CpG 10105. After four immunizations over an 8-month period, all animals developed strong and durable HCV-specific humoral and cellular immune responses, in particular, antibodies to HCV core protein and E1 and E2 pro-
teins, and virus-specific cellular immune responses including CD4+ (by ELISPOT assay for IFN-γ) and CD8+ (by ICS for IFN-γ) T cells. These responses were durable, showing similar strength 8 months after the last immunization. The immunogenicity of HCV-LP was only marginally enhanced by the use of adjuvant ASO1B and the combination of ASO1B and CpG 10105. The effect was most notable in the induction of an earlier immune response during the series of immunizations, but the effect was no longer evident by the end of the immunization schedule.

All the formulations, HCV-LP alone or HCV-LP plus adjuvant(s), were well tolerated and safe in baboons. The overall immune responses, as determined by the seroconversion rate and frequency of HCV-specific IFN-γ producing CD4+ and CD8+ T cells after three immunizations, were low. However, an additional boost at month 8 (6 months after the third immunization) markedly enhanced both humoral and cellular immune responses to significantly high levels. This suggests that boosting after an extended period of hiatus from the previous immunization is likely to be crucial in the activation and expansion of memory T- and B-cell responses to elicit a robust and long-lasting immune response in these animals. Further studies may be required to optimize the dose and schedule of the vaccination.

Previous studies of the topological features of the HCV envelope proteins on HCV-LP demonstrated that several conserved conformational epitopes were exposed on the surface of the HCV-LP (37). The production of anti-core antibody after HCV-LP immunization suggests that the HCV core protein may be partly exposed on the surface of HCV-LP or HCV-LP preparation may contain naked HCV core protein (4). Further structural study of HCV-LP is being conducted. While strong antibody responses to HCV structure proteins were induced in our subject baboons, the significance of these antibodies cannot be determined until tested by HCV challenge in chimpanzees. At present, the role of humoral immune response in the protective immunity against HCV infection is controversial (2, 24, 26, 29, 41).

Several studies have illustrated that viral clearance is associated with vigorous, multispecific (6, 11, 21, 31, 36), and long-lasting (35) CD4+ T-cell responses. Although we didn’t separate CD4+ cells from our baboons’ PBMC for ELISPOT assay because of the unavailability of baboon-specific-antibody-coated magnetic beads, the IFN-γ+ spot-forming cells responding to stimulation with HCV core or E1 and E2 proteins were presumably CD4+ T cells (11). After the fourth immunization with HCV-LP, the baboons produced 36 to 75 mean specific SFU, and this magnitude of response is comparable to that of patients recovered from HCV infection (35). When these cells were stimulated with OLPs in the ELISPOT assay, the spot-forming cells likely included a mixture of CD4+ and CD8+ T cells. This may explain the findings that the numbers of IFN-γ+ spot-forming cells after OLP stimulation were higher than those obtained after protein stimulation. The difference in the numbers of SFU responding to protein or peptide stimulation indicates that HCV E2 probably contain many CD8+ T-cell epitopes.

Spontaneous resolution of HCV is also associated with an early and durable cytotoxic T-lymphocyte response with IFN-γ production, and the presence of weak and/or functionally impaired CD8+ T cells is a characteristic feature of chronic infection (7, 13, 21, 36, 39). Our results of intracellular cytokine staining showed that up to 0.45% of the peripheral CD8+ T cells were HCV specific after four immunizations with HCV-LP and this level of response was maintained 8 months after the last immunization, suggesting that high-level, durable, and functional CD8+ T-cell responses can be induced by HCV-LP immunization. This level of HCV-specific CD8+ T-cell response is similar to that of patients recovered from acute HCV infection (0.1 to 0.6% in tetramer studies) (36), although these two methods (multiple-peptide response versus single-peptide response and different measurements of HCV-specific response) are not directly comparable.

What would be the mechanism of an exogenous protein such as HCV-LP inducing a CD8+ T-cell response? Typically, exogenous protein antigens are processed and presented by major histocompatibility complex class II molecules to CD4+ T cells, but some proteins and virus-like particles can induce CD8+ T-cell response by “cross-priming” (27, 33). Virus-like particles themselves can act as adjuvants by carrying peptide sequences inside the antigen-presenting cells and by feeding into the “endogenous” processing pathway (12). These characteristics make HCV-LP a powerful and promising vaccine candidate, as both CD4+ and CD8+ T-cell responses are important in inducing protective immunity of HCV infection (2, 26).

The adjuvants CpG 10105 and ASO1B had been demonstrated to enhance both humoral and cellular immune responses of HCV-LP in our mouse study (30). Mice immunized with HCV-LP plus the combination of ASO1B and CpG 10105 demonstrated additive or synergistic effects in HCV-specific antibody production and IFN-γ+ CD4+ T-cell responses to stimulation with core protein (30). In this study of baboons, we didn’t observe any additive or synergistic effects of these adjuvants on HCV-LP, suggesting probable differences according to species in the effects of these adjuvant(s).

The HCV-LP is noninfectious and exhibits morphological, biophysical, and antigenic properties similar to those of the putative HCV virions (4). Our study of baboons suggests that HCV-LP is a promising immunogen for the induction of HCV-specific immune responses in primates. In addition, HCV-LP immunization appears to be superior to immunization with recombinant HCV vaccinia virus in our study. In light of the relatively weak immunogenicity of HCV DNA vaccines in primates (10, 28), this approach with HCV-LP, if successfully tested in the chimpanzee model, may hold great promise in developing novel preventive and therapeutic modalities against HCV infection in humans.

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