Retraction

IMMUNOLOGY. For the article “Prophylactic DNA vaccine for hepatitis C virus (HCV) infection: HCV-specific cytotoxic T lymphocyte induction and protection from HCV-recombinant vaccinia infection in an HLA-A2.1 transgenic mouse model” by Tatsumi Arichi, Takafumi Saito, Marian E. Major, Igor M. Belyakov, Mutsunori Shirai, Victor H. Engelhard, Stephen M. Feinstone, and Jay A. Berzofsky, which appeared in number 1, January 4, 2000, of Proc. Natl. Acad. Sci. USA (97, 297–302), the undersigned authors note the following: “We deeply regret to report that we have discovered that the virus protection data in Figs. 4–6 of our PNAS paper have been fabricated and the paper must be retracted. We have not been able to reproduce the growth in vivo in mice of the NYVAC-based challenge recombinant vaccinia virus expressing the HCV core protein. We later learned that the NYVAC strain of vaccinia used in this particular study is attenuated in mice, in contrast to the WR strain-based vaccinia recombinants widely used elsewhere by us and others, that do grow well in mice. Dr. Arichi, the first author, has now admitted in writing that he spiked some of the ovary homogenates reported in Figs. 4, 5, and 6 with calculated quantities of stock virus, resulting in the titers reported in the paper. These actions were taken without the knowledge of the other authors. We deeply regret that this serious problem occurred, and we sincerely apologize to any of our colleagues who have been misled or inconvenienced by this publication.”

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www.pnas.org/cgi/doi/10.1073/pnas.101132998
Prophylactic DNA vaccine for hepatitis C virus (HCV) infection: HCV-specific cytotoxic T lymphocyte induction and protection from HCV-recombinant vaccinia infection in an HLA-A2.1 transgenic mouse model

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Communicated by Maurice R. Hilleman, Merck & Co., Inc., West Point, PA, November 8, 1999 (received for review August 31, 1999)

DNA vaccines express antigens intracellularly and effectively induce cellular immune responses. Because only chimpanzees can be used to model human hepatitis C virus (HCV) infections, we developed a small-animal model using HLA-A2.1-transgenic mice to test induction of HLA-A2.1-restricted cytotoxic T lymphocytes (CTLs) and protection against recombinant vaccinia expressing HCV-core. A plasmid encoding the HCV-core antigen induced CD8+ CTLs specific for three conserved endogenously expressed core peptides presented by human HLA-A2.1. When challenged, DNA-immunized mice showed a substantial (5–12 log10) reduction in vaccinia virus titer compared with mock-immunized controls. This protection, lasting at least 14 mo, was shown to be mediated by CD8+ cells. Thus, a DNA vaccine expressing HCV-core is a potential candidate for a prophylactic vaccine for HLA-A2.1+ humans.

There are about 170 million carriers of hepatitis C virus (HCV) in the world today and about 4 million in the United States alone (1). These persistently infected individuals are the source for most new infections. Because treatment of hepatitis C is not successful in most cases, and also is extremely expensive and requires prolonged therapy, it is not likely that the number of carriers will be significantly reduced by antiviral therapy in the near future. Therefore, it is desirable to develop a vaccine to prevent spread of HCV. However, the path to vaccine development is not clear. There is broad genetic heterogeneity of the virus, resulting in at least six genotypes (2), and the virus itself exists in any given carrier as a population of closely related viruses termed quasispecies. The virus grows poorly in cell culture, making the testing of antibodies in vitro for neutralizing ability difficult. Virus persistence may be related to the emergence of immune escape variants (3, 4), and even successful clearance of an acute HCV infection does not necessarily lead to protection from reinfection (5, 6).

Experimental vaccines, based on the two envelope glycoproteins, have so far had limited success in the only animal model for HCV infection, the chimpanzee (7). High levels of antibody to both the variable HCV envelope glycoproteins, termed E1 and E2, provided protection against only a very low-dose challenge with the same strain of virus from which the vaccine antigens had been derived. Therefore, in addition to E1 and E2, it seemed logical to expand the immune response of the vaccine to include specific cytotoxic T lymphocyte (CTL) epitopes, which may come from better conserved viral proteins such as the core or nonstructural proteins.

The core gene of HCV is one of the most conserved regions of the HCV genome (8). This conservation extends across different genotypes, and we have shown that this antigen includes at least three well-conserved (identical in 16/16 strains of HCV examined) HLA-A2.1-restricted CTL epitopes recognized by chronic HCV-infected patients who express HLA-A2.1 (9, 10), making it an ideal candidate for inclusion in a broadly protective vaccine. The extreme conservation of these epitopes makes them less likely to be susceptible to escape mutations. These epitopes have also been characterized by others in patients with chronic HCV infection (11), and our lab and others have found them to be immunogenic in HLA-A2.1-transgenic mice (12).

DNA vaccines represent a novel method to express antigens in vivo for the generation of both humoral and cellular immune responses (14–23) and have elicited protective immunity in a number of preclinical models of disease. In the first demonstration of protective efficacy of a DNA vaccine in an animal-challenge model, mice immunized with DNA encoding an internal conserved protein of influenza A, nucleoprotein, developed both specific antibodies and MHC class I-restricted CTL that lysed virally infected target cells (14, 15). This demonstrated a simple method to elicit MHC class I-restricted CTL to multiple epitopes, by enabling epitope selection from the whole protein to occur in the host. Therefore, to develop a simply administered vaccine to induce cellular immunity to suppress HCV replication, we have selected a DNA vaccine as a potential candidate to protect from HCV.

For use in humans, an HCV vaccine should elicit CTL specific for HCV epitopes presented by human class I MHC molecules. Although the chimpanzee can be used to model human HCV infections, and it expresses some class I alleles similar to humans (24), it is a very expensive and rare animal for research use; therefore, a surrogate model for the initial testing of vaccine candidates before chimpanzee experimentation is highly desirable. Transgenic mice expressing human HLA-A2.1 have been used as a preclinical model to examine the immunity in the

Abbreviations: CTL, cytotoxic T lymphocyte; HA, hemagglutinin; HCV, hepatitis C virus; rVV, recombinant vaccinia virus; pfu, plaque-forming unit.

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context of a human HLA-A2.1 molecule, before translation to humans (12, 13, 25–33). Here, these mice were used to evaluate the CTL response conferred by DNA vaccination. We previously showed that CTL responses of HLA-A2.1-transgenic mice for HCV peptides successfully predicted the epitopes recognized by CTL of humans carrying HLA-A2.1 and infected with HCV (12). Furthermore, we used HLA-A2.1 transgenic mice to evaluate a synthetic HCV-core-based peptide vaccine with enhanced CTL activity (25, 34). Thus, this mouse model may be valuable for testing the efficacy of a vaccine for eliciting human-like HLA-A2.1-restricted CTL.

We previously showed the induction of humoral and cellular immune responses, restricted by murine MHC molecules, in BALB/c mice to HCV-core DNA immunization, but protection was not assessed (35). In the current study, we demonstrate the protective potential of a DNA vaccine that induces CTL directed against conserved HCV epitopes in the core protein restricted by the human HLA molecule HLA-A2.1, rather than a murine MHC molecule. The vaccinated HLA-transgenic mice were challenged with a recombinant vaccinia virus (rVV) expressing the HCV-core protein as a surrogate for HCV, which does not infect mice.

Methods

Mice. Transgenic AAD mice (26), derived from breeders developed at the University of Virginia colony, were bred in our colony at BioCon (Rockville, MD). These mice express the α1 and α2 domains from the HLA-A2.1 molecule and the α3 domain from the murine H-2Dd molecule.

Plasmid Construct AC7. The vector AC7 encoding the entire nucleocapsid (core) region of HCV H (1a) strain (36) (amino acids 1–191) was constructed. The sequence was amplified by PCR and inserted into the HindIII and BamHI sites of the eukaryotic expression vector pWRG7020 under the transcriptional control of the cytomegalovirus early promoter, kindly provided by Deborah H. Fuller (Agracetus, Middleton, WI). Direct sequencing of the insert DNA by using the fluorescent dye provided by Deborah H. Fuller (Agracetus, Middleton, WI). Purified by reverse-phase HPLC, and their sequences were determined from supernatants of cells incubated without effector cells.

Plasmid AC7 was amplified in Escherichia coli, purified by using a Quiagen purification kit (Quiagen, Chatsworth, CA), and maintained at −20°C until use.

Transient expression of HCV-core protein in cell culture was confirmed in human 293 cells, as previously described (35). Briefly, the plasmid was transfected into 293 cells by using lipofectamine (GIBCO BRL), and protein expression was analyzed by indirect immunofluorescence and immunoblotting by using a specific mouse monoclonal antibody to HCV-core (6G7), kindly provided by Henry H. Hsu and Harry B. Greenberg (Stanford University, Palo Alto Veterans Administration Medical Center, Palo Alto, CA) (37). For immunization, AC7 was maintained at 3 M sodium acetate and ethanol and redisolved in phosphate buffered saline.

Peptides. Peptides were synthesized in an automated multiple peptide synthesizer (Symphony; Protein Technologies, Phoenix, AZ) by using fluorenylmethoxycarbonyl chemistry. They were purified by reverse-phase HPLC, and their sequences were confirmed on an automated sequencer (477A; Applied Biosystems). The three HCV-core peptides used in this study have been previously shown to be presented by HLA-A2.1 to human CTLs (9).

Cells. The cell line C1R-AAD, the human B lymphoblastoid cell line HMcyC1R transfected with the MHC chimeric molecules containing the α1 and α2 domains from HLA-A2.1 molecules and the α3 domain from the murine H-2Dd molecule, has been previously described (26).

Immunization. Mice were immunized i.m. in the quadriceps major on days 0, 14, and 28 with 100 μg AC7 or 100 μg pWRG7020 (mock) dissolved in PBS solution.

CTL Assay. Immune spleen cells were harvested at 2, 6, and 14 mo after the last immunization, and 2.5 × 10^6 cells/well were stimulated in 24-well plates with autologous naive spleen cells (5.0 × 10^6/well) pulsed with 10 μM peptide (for each of the three human CTL epitope peptides) for 2 hr in complete T cell medium: 1:1 mixture of RPMI 1640/Eagle–Hanks’ amino acid mixture containing 10% FCS, 2 mM l-glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, 5 × 10^-5 M 2-mercaptoethanol. One day later, 10% T-Stim (Collaborative Biomedical Products, Bedford, MA) was introduced to each well. Cytolytic activity of in vitro secondary CTLs was measured as previously described (12, 25), by a 6-hr assay with ^51Cr-labeled target cells after at least three in vitro stimulations with peptide-pulsed C1R-AAD cells. To test peptide specificity of CTLs, we mixed effectors and ^51Cr-labeled target cells with various concentrations of peptides. The percent specific ^51Cr release was calculated as 100 × ([experimental release − spontaneous release]/(maximum release − spontaneous release)). Maximum release was calculated from supernatants of cells that were lysed by addition of 5% Triton-X 100. Spontaneous release was determined from supernatants of cells incubated without effector cells.

Blocking of CTL Activity by Antibodies. Culture supernatants of hybridomas were used to examine whether these antibodies block CTL activity at the concentrations indicated in the figure legends. Monoclonal anti-CD8 (38), anti-CD4(39), and anti-HLA-A2.1 (BB7.2) (25) were used.

rVV Used for Protection Studies. The core sequence from the HCV-H strain (40) was inserted downstream of the vaccinia H6 promoter (41) in the SmaI site of the plasmid COPAK H6. Recombinants containing the HCV-core sequence were obtained by simultaneous infection of Vero cells with NYVAC and transfection by using Lipofectin (GIBCO/BRL) of the recombinant COPAK H6 plasmid followed by selection on rabbit (RK13) cells. After plaque purification, stocks of rVV-core were assessed for HCV-core expression by indirect immunofluorescence and Western blotting.

rVV-HA, expressing the hemagglutinin protein of measles virus (42) and used as a negative control in challenge experiments, was a kind gift from Fabian Wild (Institut National de la Santé et de la Recherche Médicale, U151, Lyon, France).

Protection Study from Vaccinia Virus/HCV-Core. At 2, 6, and 14 mo after immunization and 5 df before sacrifice, mice were challenged i.p. with 1 × 10^7 plaque-forming units (pfu) of rVV-core or control rVV-HA. Some mice were treated 7, 6, 5, and 4 d before sacrifice with 0.5 mg anti-CD8 antibody i.p. to determine the dependence of protection on CD8+ cells. The ovaries [where this vaccinia preferentially replicates (43, 44)] were harvested, homogenized, sonicated, and assayed for rVV-core or rVV-HA titer by plating 10-fold dilutions on BSC-1 indicator cells and staining with 0.075 wt% crystal violet. The minimal detectable level of virus was 10 pfu/ovary.

Results

Expression of HCV-Core by Recombinant Plasmids and rVV. Cytoplasmic expression of the HCV-core protein was detected by immunofluorescence staining of cells transfected with plasmid AC7 or infected with rVV-core. Subsequent immunoblotting of cell
lysates showed that the expressed antigen had a molecular mass of 21 kDa (data not shown).

**HCV-Core DNA Immunization of HLA-A2.1 Transgenic Mice Elicits CTLs, Specific for Three Conserved HCV-Core HLA-A2.1-Binding Peptides, Persisting at Least 14 Mo After Immunization.** We immunized HLA-A2.1-transgenic mice with DNA that encodes the HCV-core gene and induced CTL lines specific for HCV-core peptides binding to HLA-A2.1. CTL lines specific for three different HCV-core peptides (1073.07, C7A2, 939.20.1) were generated 2, 6, and 14 mo after immunization with the DNA vaccine. The peptide 1073.07 corresponds to residues 35–44, YLLPRRG-PRL; C7A2 corresponds to residues 132–140, DLMGYIPLV; 939.20 corresponds to residues 178–187, LLALLSCLTV (see refs. 9–11). After at least three in vitro stimulations with each peptide, we performed CTL assays of each peptide-specific CTL line against each peptide on C1R-AAD targets, respectively. CTL lines were peptide specific (lysis on no-peptide targets <5% as shown in Fig. 1 and ≤5% on targets with each of the other two peptides, data not shown), and activity increased with the peptide concentration (shown for the CTL raised 6 mo after immunization in Fig. 1). In contrast, spleen cells from mice immunized with mock vaccine (plasmid without HCV-core gene) similarly stimulated three times in parallel with the cells from the HCV-core DNA-immunized mice failed to show any CTL response (<5% lysis) (data not shown). These results suggest that HCV-core DNA immunization could induce CTLs specific for different peptides within the HCV-core region in the context of HLA-A2.1 molecules. Similar results (peptide-specific CTL for HCV-core DNA-immunized mice and none for control plasmid DNA-immunized mice) were obtained 2 mo and 14 mo after immunization (data not shown).

**The Activity of the CTL from HLA-A2.1 Transgenic Mice Was Blocked by Antibodies to CD8 and HLA-A2.1.** To clarify which molecules play an important role in lysis of target cells pulsed with peptide, and whether these CTLs were restricted by the HLA-A2.1 molecule, we performed a CTL assay in the presence of anti-CD4 antibody, anti-CD8 antibody, or anti-HLA-A2.1 antibody in vitro. Anti-CD8 antibody and anti-HLA-A2.1 antibody, but not anti-CD4 antibody, substantially reduced cytolytic activity on C1R-AAD cells to near background levels (≤5%) (Fig. 2). These data indicate that CTLs specific for all three peptides were CD8+ and restricted by HLA-A2.1.

**Fig. 1.** Specificity of CTL lines raised against three HLA-A2-binding peptides by immunization with the HCV-core DNA vaccine. Spleen cells from mice immunized 6 mo previously with the HCV-core DNA vaccine were stimulated in vitro with the indicated peptides at 10 μM. After three to four weekly in vitro stimulations, the effector CTL were tested for lysis of 3,000 C1R-AAD target cells/well in the presence of the indicated concentrations of each peptide, at an effector-to-target cell ratio of 100:1. Lysis on targets in the absence of peptide was <5%, as shown on the left axis. Furthermore, each line killed targets only with the homologous peptide, and lysis on targets with each of the other two respective peptides was ≤5% (data not shown). Similar results were obtained 2 mo and 14 mo after immunization. At all three time points, no specific CTL were detected after similar peptide stimulation of spleen cells from mice immunized with a control DNA vaccine plasmid (<5% lysis).

**Fig. 2.** Blocking of the CTL activity against three HCV-specific peptides by anti-CD8, anti-CD4, or anti-HLA-A2 antibodies. CTL were expanded by in vitro peptide stimulation of spleen cells from mice immunized 6 mo earlier with the HCV-core DNA vaccine. The CTL were tested for killing of C1R-AAD target cells in the presence of the indicated peptides at 10 μM. The effector-to-target ratio was 50:1. The blocking antibodies indicated were present throughout the 6-h 51Cr-release assay as a 1:5 dilution of hybridoma culture supernatant. Background lysis on targets without peptide was ≤5%.

**DNA Vaccine Confers Long-Lasting Protection Against Infection with rVV Expressing HCV-Core, and This Protection Is Mediated by CD8+ T Cells.** To examine the efficacy of HCV-core specific CTLs to clear virus and protect against virus infection, at 2, 6, and 14 mo after immunization, we challenged the mice i.p. with 1 × 107 pfu of rVV-core or the control rVV-HA. Five days later, mice were killed, and the ovaries were harvested for the evaluation of vaccinia virus as previously described, because this is the organ in which the
vaccinia preferentially replicates (43, 45). Some mice were treated with 0.5 mg anti-CD8 antibody i.p. 7, 6, 5, and 4 d before sacrifice to determine the dependence of protection on CD8 cells.

In two similar experiments at 2 mo after immunization, the groups immunized with the plasmid AC7 and not treated with anti-CD8 antibody showed a reduction in titer of rVV-core of 6–12 log10 but no reduction in titer of rVV-HA compared with the corresponding mock-immunized controls (Figs. 4 a and b).

On the other hand, in mice immunized with AC7, treatment with anti-CD8 antibody (reducing splenic CD8+ cells to <1%) abrogated most of the protection. In the groups immunized with mock vaccine, there was no effect of anti-CD8 antibody treatment in mice challenged with either rVV-core or rVV-HA (Fig. 4 a and b).

At 6 mo after immunization, we still saw a statistically significant difference between the immunized groups. There was a 7-log10 reduction of rVV-core titer in the group immunized with AC7, and the protection still depended completely on CD8+ cells (Fig. 5). Even 14 mo after immunization, the group immunized with AC7 showed a statistically significant 5-log10 reduction of rVV-core titer compared with mice immunized with mock vaccine. Thus, protection was still strong even 14 mo after immunization (Fig. 6).

These results indicate that the HCV-core DNA vaccine confers long-lasting protection against infection with rVV expressing HCV-core in vivo, and that this protection is mediated by CD8+ T cells, consistent with the induction of CTL activity as measured in vitro against three HCV-core epitopes presented by human HLA-A2.1.
Discussion

DNA vaccines have been shown to induce both humoral and cellular immune responses and to elicit protective immunity in a number of preclinical models of disease as well as in humans (14–23). Although the immune response to DNA vaccines in chimpanzees and humans has, in general, not yet been as robust as that seen in mice, successful immunizations have been achieved for both HBV and HIV (46–48), and further improvements may be achieved with improved delivery systems and immunoenhancing molecules.

We focused on the core protein because of its conservation and our mapping of three conserved CTL epitopes presented by HLA-A2.1 (9, 10), but other conserved HCV proteins might be useful also. In previous studies, we developed a DNA vaccine expressing the HCV-core gene (35) and showed that this elicited a strong CTL response in BALB/c mice (restricted by murine MHC molecules). We also developed a synthetic peptide vaccine with enhanced CTL activity in an HLA-A2.1 transgenic mouse model by modifying the sequence of one of the HCV-core epitopes (25, 34). However, protection was not examined in either of these studies.

In the current study, we successfully induced CTL responses specific for three highly conserved HLA-A2.1-restricted HCV-core epitopes by DNA immunization, and these persisted 2 mo, 6 mo, and even 14 mo after DNA immunization with a plasmid expressing the HCV-core antigen. These CTLs could recognize the endogenously synthesized protein in cells infected with an HCV-core vaccinia recombinant, and the CTL activity was blocked with anti-CD8 and anti-HLA-A2.1 antibodies but not with anti-CD4 antibody, indicating that the CTLs that recognize peptide and endogenously synthesized HCV-core protein were CD8\(^+\) and restricted by HLA-A2.1. These results suggest that DNA encoding HCV-core can induce long-lasting CTL memory.

There is evidence that CTLs can inhibit the outgrowth of hepatitis B and C viruses (49–51). Thus, we challenged mice with vaccinia virus expressing HCV-core as a surrogate virus to evaluate the protective ability of CTLs induced by DNA immunization. Although vaccinia infection in mice is not an exact model of HCV infection in humans, this model allows study of HCV-specific CTL-mediated antiviral protection in a small-animal model in the context of a human HLA molecule. In control DNA-immunized mice, we found a marked reduction of the titer of the rVV expressing HCV-core antigen compared with mock-immunized mice. HCV-core-vaccinated mice challenged were not protected against a control rVV expressing measles HA. We also found unexpected protective immunity even 14 mo after immunization. At all times tested, the protection was mediated by CD8\(^+\) cells, as shown by almost complete abrogation of protection by treatment of the mice in vivo with anti-CD8 antibodies. The fact that substantial protection can be observed in vivo 14 mo after vaccination, and CTL precursors can be detected after restimulation in vitro, suggests that there is a large expansion of memory CTL induced by the DNA immunization and that CTL may be very rapidly expanded clonally after exposure to virus (52–56) to afford protection as early as 5 d after infection. We have obtained similar protection using a peptide vaccine to induce mucosal immunity against HIV (44, 45) at 6 mo after immunization. These two results are very important for the design of useful vaccines for humans.

Although the importance of specific CD8\(^+\) cells in the protection of the mice used in this study has been demonstrated, the role of cytokines or antibody has not been assessed. It has been shown that protection against vaccinia virus can be mediated by IFN-\(\gamma\) (57). However, immunized mice showed no reduction of vaccinia virus expressing the measles HA protein, indicating that this cytokine does play a role in the protection we observed, it is mediated by the specific cellular response to HCV antigen. Because CD8\(^+\) CTL make IFN-\(\gamma\) as well as having lytic activity, we cannot distinguish which of these effector mechanisms is used by the CD8\(^+\) cells that we have shown to be responsible for the protection. The possibility that antibody, in addition to CTLs, may play a role in protection in this model is very unlikely. We previously showed that immunization with a DNA construct expressing HCV core could induce specific antibody (35). However, the core antigen is not an HCV surface protein, and even HIV glycoproteins expressed by vaccinia recombinants are not incorporated into virions but are expressed only in infected cells (58). Therefore, any anti-HCV-core antibody induced by immunization could not neutralize the vaccinia virus particles. Protection must be mediated by immune recognition of virus-infected cells.

Although we have demonstrated that the protection depends on CD8\(^+\) cells and that CTL specific for several HCV-core epitopes presented by the human HLA-A2.1 molecule are elicited, we cannot be sure that these are the only CTL that mediate protection. Any CTL that may also be elicited to core epitopes presented by murine class I molecules present in the HLA-A2.1 transgenic mice (on the C57BL/6, H-2\(^b\)) background could potentially contribute to protection. However, in previous studies with C57BL/6 mice immunized with a rVV expressing HCV-core, we did not detect CTL to any of a series of peptides tested (10), and in preliminary experiments involving DNA immunization with a different plasmid expressing HCV-core protein, we also did not detect core-specific CTL in C57BL/6 mice (ref. 35 and T.S., unpublished observations). Thus, C57BL/6 mice may, by coincidence, be relatively low CTL responders to HCV-core, in which case the bulk of the CTL response in the HLA-A2.1 transgenic mice may actually be the CTL restricted to HLA-A2.1. For this reason, the protection afforded by the HCV-core DNA immunization may depend heavily, if not exclusively, on CTL to epitopes presented by the human HLA molecule. Further studies will be required to clarify this issue.

A recent study by Large et al. describes a greater pathogenicity of a rVV expressing the HCV-core protein in BALB/c mice (H-2\(^d\)) and a reduced induction of anti-vaccinia CTL that may account for this greater pathogenicity (59). We have not seen such a greater pathogenicity of the HCV-core rVV used here in HLA-A2.1 transgenic mice on a C57BL/6 background (H-2\(^b\)). Part of the difference may be in the genetic background and the MHC type of the mice, which are very different. We have seen no mortality at 5 d after challenge with 10\(^5\) pfu of vaccinia, but we have not used the higher challenge doses of 10\(^9\) pfu that Large et al. report. Furthermore, in their study, they interpreted the results to be a suppression by the core protein of the induction of a CTL response to the vaccinia antigens expressed together with the core protein. In our case, the mice were immunized in advance with the DNA, so the CTL were preexisting at the time of challenge with rVV. We were not studying the induction of CTL against vaccinia itself induced by the challenge.

Together with CTL induction restricted by a human HLA molecule, the elimination by CD8\(^+\) T cells of vaccinia virus expressing HCV-core more than a year after immunization in an HLA-A2.1 transgenic mouse model suggests that an HCV-core-based DNA vaccine may be appropriate for translation to human clinical trials.

In conclusion, a DNA vaccine expressing HCV-core is a potential candidate for inclusion in a prophylactic vaccine, at least for HLA-A2.1\(^+\) humans (nearly half the population) and probably in a broader population, because of the conservation of these epitopes among HCV genotypes (conserved in 16/16 isolates tested; ref. 9) and the ability of the vaccine to induce core-specific CTLs and to confer long-lasting immunity.

We thank Drs. Barbara Rehermann and Suzanne Emerson for critical reading of the manuscript and helpful suggestions.