Hepatitis C Genotype 1 Mosaic Vaccines Are Immunogenic in Mice and Induce Stronger T-Cell Responses than Natural Strains

Karina Yusim,a Rebecca Dilan,b Erica Borducchi,b Kelly Stanley,b Elena Giorgi,a William Fischer,a James Theiler,a Joseph Marcus trigiano,c Bette Korber,a Dan H. Barouchb,c Los Alamos National Laboratory, Los Alamos, New Mexico, USAa; Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USAa; Ragon Institute of MGH, MIT, and Harvard, Boston, Massachusetts, USAa; Center for Advanced Biotechnology and Medicine, Department of Chemistry and Chemical Biology, Rutgers University, Piscataway, New Jersey, USAa

Despite improved hepatitis C virus (HCV) treatments, vaccines remain an effective and economic option for curtailing the epidemic. Mosaic protein HCV genotype 1 vaccine candidates designed to address HCV diversity were immunogenic in mice. They elicited stronger T-cell responses to NS3-NS4a and E1-E2 proteins than did natural strains, as assessed with vaccine-matched peptides.

One hundred fifty million people are chronically infected with hepatitis C virus (HCV) worldwide, and more than 350,000 people die from HCV-related liver diseases every year (1). Potent new therapies have allowed for dramatically improved success rates, but cost, side effects, and treatment failure still remain issues, underscoring the importance of developing prophylactic and therapeutic vaccines (2).

Immunological control of HCV is mediated by host T-cell responses (3–5), but the genetic diversity of the virus poses a major challenge to successful vaccine development (6, 7). Therefore, a vaccine that can elicit responses that cover a wide range of circulating variants is essential. Our group created a computational method that uses a machine learning strategy to design sets of vaccine antigens called mosaic proteins. Mosaic proteins closely resemble natural proteins, with the intent of preserving natural antigen expression and processing (8–10), but are recombinant proteins generated and selected in silico to maximize the coverage of potential T-cell epitopes. Potential epitopes are defined as all fragments with a length of 9 amino acids (i.e., all 9-mers), the most common CD8 T-cell epitope length, found in circulating populations; common 9-mers are favored in the design, while rare and unique 9-mers are minimized to avoid vaccine-specific responses (8). Thus, two- and three-valent mosaic antigens provide better 9-mer population coverage of HCV genotype 1 sequences with fewer unique 9-mers than their natural counterparts (Fig. 1) (6). HIV-1 mosaic vaccines have shown promise in animal models, eliciting responses with greater breadth and cross-reactivity than natural HIV vaccine proteins, and HIV-1 mosaic human phase 1 trials are planned (10–14). The mosaic concept was also very promising for filoviral vaccines (15). Here we demonstrate that mosaic HCV genotype 1 sequences (6) are immunogenic and provide responses similar to or better than those obtained with natural strains in a mouse model. The vaccines tested include the HCV NS3-NS4a proteins, a protease-encoding region (16), because NS3-directed T-cell responses play a critical role in natural and therapeutic viral clearance (17–20); the vaccines also include the NS3-NS4a.

FIG 1 Coverage of HCV genotype 1 by the vaccine candidates used in this study. Four vaccine cocktails were used: a two-valent natural cocktail made of two natural proteins (genotype 1a strain HCV-1 [accession number M62321] and genotype 1b strain HCV-1 [accession number D90208]), a two-valent mosaic cocktail made of two mosaic proteins (one mosaic protein optimized on genotype 1a and one mosaic protein serially optimized on genotype 1b, where the 1b mosaic protein was designed to maximize genotype 1b 9-mer coverage given that a genotype 1a mosaic protein is already in the cocktail), a three-valent natural cocktail (the two natural strains above and genotype 1b strain BID-V141 [accession number EU155337]), and a three-valent mosaic cocktail (the two mosaic proteins above and one mosaic protein optimized on genotypes 1a and 1b, considering that the other two mosaic proteins are already in the cocktail). Exact matches (red) and eight-of-nine (one off, orange) and seven-of-nine (two off, yellow) 9-mer coverages of genotype 1 E1-E2 and NS3-NS4a sequences were calculated. The lower panel shows the 9-mer coverage of 827 E1-E2 genotype 1 sequences (left) and 753 genotype 1 NS3-NS4a sequences (right) by each vaccine cocktail. The upper portion of the graph shows the number of unique 9-mers (defined as present in only one natural strain of the global genotype 1 alignments, so responses to these 9-mers would be likely to be strain specific) present in each vaccine cocktail.
matched 15-mer peptides, which provided 100% 9-mer coverage of each vaccine sequence (so that every single 9-mer in each vaccine sequence had an exactly matching 9-mer in a test peptide set) while at the same time maximizing the coverage of circulating HCV strains (Fig. 2).

All of the HCV vaccines used generated T-cell responses in BALB/c mice (Fig. 2). Responses to four of six peptide pools were found, including three NS3-NS4a pools, but a response to only one of three E1-E2 pools was found, although BALB/c mice expressed the major histocompatibility complex (MHC) H-2d haplotype (27) and there were H-2d binding motifs and known epitopes in all three E1-E2 pools (data not shown). Mosaic constructs induced significantly stronger responses than natural strains ($P = 0.001$) when nonparametric resampling algorithms were used to estimate how likely the occurrence of stronger responses by chance alone is (see the supplemental material). Three-valent mosaic vaccines induced stronger responses to all four pools than did three-valent natural vaccines ($P = 0.002$, Fig. 2), while two-valent mosaic vaccines were better than two-valent natural vaccines in three of four pools ($P = 0.1$, Fig. 2.). The mosaic-versus-natural distinction was also observed when mixed-effect linear models were used: the model was significantly improved by inclusion of the vaccine type ($P = 0.005$), and mosaic protein response levels were estimated to be 1.7-fold greater than natural-protein response levels. The responses to the pools were also significantly different, and there was no interaction between the pools and vaccine type ($P = 0.001$).
vaccine types. There was no statistically significant benefit in using three strains instead of two for either natural or mosaic proteins. A benefit in increasing the number of strains in a vaccine cocktail, however, might be too subtle to detect with just eight BALB/c mice per vaccine but could still be important for large-scale human vaccinations.

While the main purpose of this experiment was to determine whether the artificial HCV mosaic constructs were at least as immunogenic as natural strains (and they were), mosaic proteins, in fact, performed significantly better than natural strains (Fig. 2). Although mosaic proteins are designed to do better in the context of population diversity, in this experiment, test peptides covered 100% of both mosaic proteins and natural strains; thus, comparable performance of the vaccines tested was expected. The apparent superiority of mosaic proteins might be explained by several hypotheses. First, mosaic proteins provide more distinct 9-mers (potential epitopes) than natural strains. Three mosaic proteins, indeed, had 14% more distinct 9-mers than three natural strains, but three mosaic proteins also cover 30% more distinct 9-mers than two mosaic proteins do and were not statistically significantly better as immunogens in these mice. Thus, it is hard to believe that simply the number of potential epitopes in the different vaccine cocktails accounts for the improved responses, unless the greater dilution required for the three- versus two-valent vaccine strategy had a counterbalancing impact. Second, this could be a chance outcome due to the specific BALB/c MHC, if the mosaic proteins happened to deliver a few particularly good epitopes for this background that were missed by the natural strains. Third, mosaic proteins may be processed better if the evolution of natural strains in vivo includes immune escape through the acquisition of processing defects and these tend to be reset at transmission because of fitness costs. This argument is consistent with the high immunogenicity of HIV mosaic proteins (11, 13, 28) and a reported benefit of the central ancestral HCV sequence versus the natural strain (7). As this pilot study was limited to the assessment of responses to peptide pools in one mouse strain, further studies involving epitope mapping and testing in other MHCs are required to fully understand the observed benefits of mosaic HCV vaccines.

ACKNOWLEDGMENTS

This research was funded through Los Alamos National Laboratory internal LDRD funding; NIH grants AI066924, AI078526, AI096040, and AI080659; and the Ragon Institute of MGH, MIT, and Harvard.

We are grateful to Peter Hober for help with the figures and to Peter Abbink, Lori Maxfield, Diana Lynch, Nate Simmons, and Adam San-Miguel for help with the experiments.

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