Abstract
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Hepatitis C is an important cause of liver disease worldwide. WHO estimates that about 200 million people, 3% of the world’s population are infected with hepatitis C and 3 to 4 million persons are newly infected each year with a global 170 million chronic carriers at risk of developing liver cirrhosis and/or liver cancer. In the absence of an effective universal vaccine on account of sequence variability and high mutation rate of the virus within the host, genotype-specific vaccine development represents an alternate strategy. In India, although an estimated 12 to 13 million people are HCV infected with a predominance of genotype 3 (>70%), so far no attempts for indigenous vaccine development have been made in context of the Indian population. Evaluation of immunogenicity against regions of genotype 3 therefore becomes essential.

Induction of potent and sustained humoral and cellular responses is necessary to prevent and clear HCV infection. The 27 amino acid long N-terminal region of E2 glycoprotein (amino acids 384-410) termed as hypervariable region 1 (HVR1), is the most variable region of the whole HCV polyprotein and contains a neutralizing determinant. Studies have shown that HVR1 can serve as B and T cell epitope. Its genetic hypervariability is driven by host’s immune pressure. However, it is known that HVR1 alone is insufficient for the development of vaccine. Immune response to envelope proteins develops slowly and achieves only modest titres during primary infection. Use of an additional HCV protein was therefore considered necessary. In this context, non-structural protein-3 (NS3) eliciting strong humoral and cellular immune response was chosen. Importantly, the T-helper
immune response against NS3 is associated with viral clearance and absence of such response leads to viral persistence. We have attempted to develop a genotype 3 based, HCV vaccine with hypervariable region-1 (HVR1) and non-structural region-3 (NS3) components employing two adjuvants.

HCV RNA extracted from serum samples of 49 chronically infected patients was PCR amplified to obtain HVR1 region. These amplified products were cloned to obtain 20 clones per sample in order to identify the quasispecies pattern and further generate a consensus sequence. The HVR1 consensus sequence, along with three variants was reverse transcribed to obtain peptides. The peptides were checked for immunoreactivity individually, as a pool or as a single peptide tetramer interspersed with four glycine residues. Anti-HCV positivity varied from 42.6% (tetramer) to 92.2% (variant-4) upon screening of 115 anti-HCV positive sera representing genotypes 1, 3, 4 and 6. All the 95 anti-HCV negatives were scored negative by all antigens. Mice were immunized with different liposome encapsulated or Al(OH)$_3$ adjuvanted formulations of HVR1 variants or recombinant NS3 protein alone or in combination, and monitored for anti-HVR1 and anti-NS3 antibody titres, IgG isotypes and antigen specific cytokine levels. A balanced Th1/Th2 isotyping response with high antibody titres was observed in most of the liposome encapsulated antigen groups. The effect of liposomes and aluminium hydroxide on the expression of immune response genes was studied using Taqman Low Density Array. Both Th1 (IFN-gamma, Il18) and Th2 (Il4) genes were up regulated in the liposome encapsulated HVR1 variant pool-NS3 combination group. In-vitro binding of the virus to anti-HVR1 antibodies was demonstrated.
The optimum immunogen was identified to be combination of peptides of HVR1 consensus sequence and its variants along with pNS3 encapsulated in liposomes, which could generate both cellular and humoral immune responses in mice deserving further evaluation in a suitable cell culture system / non-human primate model.