in the absence of integration into the host genome, this approach has great promise for HCV and other infectious diseases.

HCV Vaccines Using Disabled Viral Vectors

The use of a defective or attenuated viral vector to deliver vaccines has several potential advantages. Firstly, a wide tropism of the host vector leads to the efficient delivery of the vaccine genes and encoded antigens. Preferably, this tropism includes antigen-presenting cells leading to a very effective priming of the immune response, thereby requiring only one immunization for long-lasting immunity. The use of a vector already used as a vaccine itself, offers further obvious advantages with respect to manufacturing, distribution, and user acceptance. Finally, many vectors allow the insertion of multiple genes thus facilitating the induction of a broad, cross-protective immune response, particularly useful against heterogeneous agents such as HCV.

One such promising approach for HCV has been the use of an attenuated rabies viral vector into which either the HCV gpE1–gpE2–p7 gene cassette was inserted, or just the ectodomain of gpE2 linked to the CD4, C-terminal TMR, and cytoplasmic domain. In the case of the latter construction, recombinant rabies virions were produced that actually contained the hybrid gpE2 within the virion. Virions expressing gpE1–gpE2–p7 were immunogenic in mice eliciting CTL responses to gpE2 (178). Similarly, defective Semliki Forest virions containing the HCV NS3 gene produced long-lasting NS3-specific CTLs after one immunization in mice transgenic for human HLA-A2.1 (179). As observed in HCV-infected patients, the immune response was directed to one immunodominant epitope within NS3. Defective, recombinant adenoviruses expressing the HCV C–gpE1–gpE2 gene cassette have also been shown to prime HCV-specific CTLs in mice immunized intramuscularly, although the induction of anti-gpE1/gpE2 antibodies required further immunization with purified gpE1/gpE2 glycoproteins (180). Replication-defective adenoviruses expressing C and gpE1 also primed long-lasting, specific CTL responses in mice (181). Recombinant canary pox viruses, expressing an HCV gene cassette containing C–gpE1–gpE2–p7–NS2–NS3, elicited HCV-specific humoral and cellular immune responses in mice, although the optimum immunization regimen required first priming with a plasmid DNA expressing the HCV genes prior to boosting with the recombinant canary pox virus (182).

It has been showed that vaccination with adenoviral vectors and electroporated plasmid DNA encoding the HCV non structural region NS3 to NS5B, protected chimpanzees from acute hepatitis induced by challenge with a heterologous virus differing from the vaccine sequence by more than 13% at the amino acid level (183). Four out of five vaccinated chimpanzees developed a cross-reactive T-cell response against the challenge virus that resulted in a low viremic state, although no difference was observed compared to the control group with respect to the number of animals that did not proceed into the chronic carrier state. It is likely that in the absence of neutralizing antibodies, cellular responses are unable to eradicate infection, thereby making this vaccine approach interesting to investigate as a therapeutic add-on to other therapies.

A recent interesting approach has been the use of recombinant HCV polypeptides combined with various Th1-type adjuvants and replication-defective alphaviral particles encoding HCV proteins (184). In this study, mice were immunized with defective chimeric alphaviral particles derived from the Sindbis and Venezuelan equine encephalitis viruses encoding either the HCV envelope glycoprotein gpE1/gpE2 heterodimer (E1E2) or nonstructural proteins 3, 4, and 5 (NS345), and strong CD8(+) T-cell responses but low CD4(+) T helper responses to these HCV gene products were detected. In contrast, recombinant E1E2 glycoproteins adjuvanted with MF59 containing a CpG oligonucleotide elicited strong CD4(+) T helper responses but no CD8(+) T-cell responses. A recombinant NS345 polyprotein also stimulated strong CD4(+) T helper responses but no CD8(+) T-cell responses when adjuvanted with Iscomatrix containing CpG. Optimal elicitation of broad CD4(+) and CD8(+) T-cell responses to E1E2 and NS345 was obtained by first priming with Th1-adjuvanted proteins and then boosting with chimeric, defective alphaviruses expressing these HCV genes. In addition, this prime/boost regimen resulted in the induction of anti-E1E2 antibodies capable of cross-neutralizing heterologous HCV isolates in vitro. This vaccine formulation and regimen may therefore be optimal in humans to recapitulate all of the cellular and humoral immune response in an ideal vaccine regimen.

SUMMARY

Data indicating the existence of natural immunity against the HCV and vaccine efficacy in the chimpanzee challenge model allow optimism for the development of at least a partially effective vaccine against this heterogeneous pathogen that is responsible for much of the chronic liver disease around the world.

A few years ago, prospects for effective vaccination against HCV were considered remote because of the high propensity of this virus to promote chronic persistent infections, evidence that convalescent humans and chimpanzees could be readily reinfected following reexposure as well as the considerable genetic heterogeneity of this virus. The situation today can be more optimistic for several reasons. First, we now know that the spontaneous eradication of virus occurring in a consistent fraction of acute infections is associated with specific immune responses to the virus. Recapitulation of such immune responses by appropriate vaccination therefore becomes a realistic option. Second, clear evidence for at least some natural immunity has emerged in both humans and chimpanzees. These studies have shown that convalescent humans and chimpanzees are protected from chronic infection against reexposure to virus in the majority of cases, even against very divergent viral strains.

Some issues surrounding clinical development of a prophylactic HCV vaccine remain to be solved. Not only is it difficult to identify the appropriate at risk population to enroll in an efficacy trial for a preventive HCV vaccine, but it may also be very difficult to conclude an efficacy trial designed to measure prevention of chronic infection. As discussed earlier, the great majority of acute infections are asymptomatic and without clinical consequences, and it is the manifestations of chronic HCV infection that lead to the clinically evident disease. Thus, a vaccine that allowed only a ''transient infection'' (either subclinical or of limited acuity), while preventing the development of chronic HCV infection could be as beneficial as one that provided sterilizing immunity. Indeed, vaccine efficacy data from the chimpanzee challenge model indicate that is possible to prevent the progression to chronic infection in vaccinees. However, this endpoint, in the absence of correlates of