Team # 2

Vaccination against Hepatitis C Virus with Recombinant Adenovirus on South Asia Populations

LITERATURE REVIEW

Epidemiology

Hepatitis C Virus (HCV) is globally distributed and a major cause of liver disease worldwide (Goedert et al., 2007; Shepard et al., 2005). Prevalence estimates of infection with HCV range widely across populations with some degree of geographic variability. Countries located in Africa and Asia report the highest prevalence estimates, and areas with lower prevalence include North America, Western Europe and Australia. Low seroprevalence estimates of HCV in developed countries have been reported in nations including Germany (0.6%), Canada (0.8%), France (1.1%), and Australia (1.1%), while slightly higher seroprevalence estimates have been reported in the USA (1.8%), Japan (1.5-2.3%), and Italy (2.2%) (Shepard et al., 2005).

In developing countries, fewer data are available making it harder to assess the burden of the disease. Of the most populous of these countries, China, which accounts for one-fifth of the world's total population, reported an estimated HCV seroprevalance of 3.2% in a nationally representative population sample (Shepard et al., 2005). In contrast, India, which also contributes one-fifth of the world's population has a relatively low reported seroprevalence of 0.9% (Chowdhury et al., 2003). However, prevalence has been reported as high as 22% in Egypt (Frank et al., 2000), which contributes an estimated 73 million people to the world population.

Using statistical modeling Salomon *et al.* (Salomon et al., 2002) determined three major periods of differing incidence of HCV in the United States. It was determined that there was a low incidence of HCV in the 1950s, there was a steep increase starting in 1965 and peaking in 1985 with gradual decline up until 1997. The Centers for Disease Control and Prevention reports incident cases of HCV in the U.S. decreased from an average of 240,000 per year in the 1980s to about 26,000 in 2004. Additionally, an estimated 4.1 million (1.6%) Americans have been infected with HCV, of which 3.2 million are chronically infected (CDC, 2006).

Several populations have been identified as at risk for acquiring HCV infection. Those at high risk of infection include injecting drug users and recipients of clotting factors made before 1987, when more thorough screening of blood and blood products was implemented. Those with an intermediate risk of infection include hemodialysis patients, recipients of blood and solid organs before 1992, people with undiagnosed liver problems, and infants of infected mothers (CDC, 2006). Low risk populations have been identified as healthcare and public health workers, people with multiple sexual partners, and people having sex with an infected sexual partner. Testing for HCV is recommended for people in the high and intermediate risk categories, while testing is only recommended for those in the low risk category if a known exposure to HCV has occurred.

Pathogen Structure

HCV is an enveloped, single-stranded RNA virus with an approximate size of 50 nm (Glenn, 2006). The structural organization of HCV, beginning with the outer structures and working to the interior is straightforward. On the outer-most part of the virus are envelope proteins E1 and E2 which are type I transmembrane glycoproteins. Their functions include membrane anchoring, endoplasmic reticulum (ER) localization and heterodimer assembly (Cocquerel et al., 2000; Pawlotsky et al., 2007). The E1 and E2 proteins form a tetramer complex consisting of two heterodimers (each dimer is an E1 complexed with an E2). These glycoproteins cover the surface of the virus and form a smooth protein layer approximately 3 nm thick with a very high density (Yu et al., 2007).

The E1 and E2 proteins are embedded on the outside of the viral envelope that is formed from the host cell's membrane during exocytosis from a previously infected cell. This lipid bilayer is distinctly inferior to the glycoprotein layer and is approximately 4 nm thick, but less dense than the glycoprotein layer. The membrane bilayer is constrained by the icosahedral formation of the protein layer and takes on a polygonal shape rather than the spherical shape normally associated with aggregating lipids (Yu et al., 2007). Inferior to the envelope lipid bilayer is the capsid core. This is composed of a nucleocapsid protein that interacts with various intracellular host cell signaling pathways. The nucleocapsid protein structure is highly conserved among different HCV strains (Glenn, 2006).

Within the capsid is the viral RNA genome. It is a positive-sense, single-stranded RNA virus, meaning it can directly translate RNA into protein without forming a complementary RNA strand. The viral genome is comprised of 9.6 kb that encode a single polyprotein with a primary structure approximately 3000 amino acids long. This polyprotein is processed by proteolytic enzymes from the host cell and the viral polyprotein. The cleaving of the polyprotein forms the structural (E1, E2, capsid core) and nonstructural (RNA polymerase, protease, helicase, etc.) proteins that form the virus (Glenn, 2006).

The viral RNA genome encodes for the long polyprotein but also contains non-translated and conserved regions at the 5' and 3' ends. These conserved regions form cis-acting signals that promote the replication and translation of the viral genome. The 5' NTR (non-translated region) allows for direct binding of the ribosome very close to the start codon for the reading frame of the polyprotein sequence (Glenn, 2006).

The replication process of the viral RNA genome involves an RNA polymerase that is encoded in the viral genome. During replication, a negative-sense strand of complementary RNA is made and serves as the template for the transcription of more positive-sense RNA genomes. This RNA polymerase lacks an editing function and thus the risk for spontaneous mutations increases. This results in genetic variation for the virus and increases the ability of the virus to adapt to any environmental or host immune system pressures. The genetic variation of hepatitis C is more common in the hypervariable region of the E2 glycoprotein (Glenn, 2006).

Disease Mechanism

HCV is a blood-borne pathogen and the major cause of chronic liver disease, cirrhosis and hepatocellular carcinoma (HCC). Potential transmission routes of HCV infection are: blood transfusions, occupational exposure such as nurses, sharing drug-injecting equipment, vertical transmission, sexual transmission, and household routes such as sharing razors and toothbrushes (Cocquerel et al., 2000; Pawlotsky et al., 2007).

The immune response, innate and adaptive, represents the first line of defense against viral replication (Caruntu and Benea, 2006). HCV has complex mechanisms to elude this immune response. HCV must enter target cells, once inside it can transcribe and translate the genetic material to produce structural and nonstructural components for new virion. This allows replication and packaging of the genetic material into the virion structure, and finally releasing the new viruses (Kaplan, 2006). At least six nonstructural proteins (NS2, NS3, NS4 A and B, and NS5 A and B) are involved in replication, transcription, and polyprotein processing (He et al., 1999).

The E1 and E2 glycoproteins are essential for fusion and entry into the target cells (Kaplan, 2006). E2 has a hypervariable region that contains the major neutralizing epitope (Farci et al., 1996; Penin et al., 2001; Zibert et al., 1997). Hypervariable regions are present among a variety of HCV strains suggesting that they may play an important role on the virus life cycle (Nielsen et al., 2004).

Virus attachment is initiated by E2 interactions with receptor molecules such as human CD81, glycosaminoglycans, scavenger receptor B type I, lipoprotein receptors, CD209, etc. HCV binds to hepatocyte receptors on the host cells; this triggers receptor mediated endocytosis (Caruntu and Benea, 2006). During endocytosis, fusion of the viral envelope to the host cell membrane allows for entry of the viral capsid into the cytoplasm. Once in the cytoplasm, the capsid uncoils and exposes the viral RNA genome. Exposed mRNA is translated on the ribosome for polyprotein synthesis, followed by polyprotein cleavage by the enzyme signalase in the host cell. Cleavage is required to produce mature glycoproteins to activate viral infectivity.

HCV replicates through the lytic cycle. Viral polymerase transcribes the negative strand RNA through the formation of a replication complex; synthesis of mRNA takes place at the nucleocapsid. The negative RNA strand serves as a template for numerous progeny positive RNA genomes (Glenn, 2006). Translation of the structural proteins E1, E2 and C (capsid protein) is achieved and is followed by the assembly of the progeny virion.

Very limited information is available to understand the mechanism of viral assembly and release of new virions. However, studies have shown that assembly and release is due to an interaction between the core proteins and the RNA (Pawlotsky et al., 2007). HCV envelope proteins interact with the ER through transmembrane domains suggesting E1, E2, C and the progeny positive strand RNA are assembled and are packaged prior to vesicle fusion at the host cell plasma membrane. At this point, progeny virions are released from the host cell via the constitutive secretory pathway (Pawlotsky et al., 2007).

Previous Treatments

Currently there remains over 3.2 million people that are chronically infected with HCV (CDC, 2006). Treatment for these individuals involves a tradeoff between cost and side effects. Viral elimination, which is the key to constraining HCV, involves targeting specific HCV genotypes and detecting the RNA that encodes the expression of this virus (Salomon et al., 2002).

Current progress of possible HCV treatment has centered on the study of interferon (IFN) signaling pathways. Specifically, IFN-α has been observed in having a significant affect on inhibiting HCV replication in Huh7 hepatoma cell lines (Salomon et al., 2002); targets for therapy include virus entry, and uncoating, translocation, protein processing, viral replication, viral assembly and release of mature particles from the hepatocyte. The actual "inhibiting" effect of IFN-α on Huh7 hepatoma cells is dependent of the intracellular signaling pathway that takes

place, and more importantly, has been observed on numerous HCV genotypes. Additionally, this IFN-α has been shown to inhibit HCV replication in primary human hepatocytes that are infected during incubation with infectious human plasma (Salomon et al., 2002).

Pegylated IFN-α has been specifically studied in treating chronic HCV in adults (Salomon et al., 2002). Pegylated IFN-α exists in two forms: pegylated IFN-α2a, and pegylated IFN-α2b, both of which have been approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) for treatment of HCV in adult patients. The introduction of the pegylated IFN-α as possible means of treatment is still undergoing clinical studies. In examining the details of the pegylated IFN-α, a cohort of 10 patients, each with chronic forms of non-A, non-B hepatitis was treated with pegylated IFN-α. Preliminary data showed that in eight of ten patients, there was a significant decline in alanine aminotransferase (ALT) levels (Salomon et al., 2002). After ten years, five of these ten patients exhibited absolutely no traces of infection.

In order to counteract the declince of ALT levels, further research investigated whether combination therapy of pegylated IFN-α and ribavirin would optimize treatment conditions, improve ALT levels, and eradicate the virus in 40% of patients they selected for this study (Salomon et al., 2002). With the use of ribavirin, the HCV replication was transiently inhibited *in vivo*, but efficiently prevented any relapse that may have occurred during the ribavirin combination therapy.

Combination therapy of pegylated IFN-α and Ribavirin is the current suggested treatment for chronic HCV. This combination of agents is effective in eliminating HCV infection in approximately 50% of patients who are able to tolerate therapy. However, currently there are no HCV-specific antiviral agents approved for the treatment of HCV infection (Davis et al., 2007). Clinical efficacy of pegylated IFN-α –ribavirin combination therapy treatment produced a successful virological response (SVR) rate of 54-56%. In patients who had the HCV in genotypes 2 or 3, the SVR ranged from 76-84%, and in patients that exhibited infection in genotype 1, the success of treatment ranged from 42-52%. Of this cohort, 10% of patients discontinued therapy because of adverse events, while dose reductions were required in 30% of the cases.

Despite high success rates of this source of HCV treatment, there are several problematic issues with this method. The major question about the mechanism of the IFN-*α* response against HCV is why a fraction of infected patients do not respond to IFN-*α* therapy (Davis et al., 2007). One possible reason may be due to patient-drug resistance towards IFN-α- ribavirin combination therapy. IFN does not specifically target the HCV gene but enhances host antiviral responses that, in turn, exert selection pressures on viral genome regions and epitopes. The targets of IFN actions are diverse and thus viral resistance to this therapy may be due to HCV mutants that escape specific host responses induced by IFN (Davis et al., 2007).

Another problem arose during application of this treatment and involved the chemical properties of the ribavirin. Upon activation, ribavirin is metabolized into RTP (ribavirin 5' triphosphate), a weak inhibitor of HCV in RNA dependent RNA polyermase. However, RTP is unlikely to play any major role in eliminating the HCV virus, during IFN-α –ribavirin combination therapy (Salomon et al., 2002) and instead act as an RNA mutagen.

Lastly, problems that arose during clinical efficacy examination included conditions of Neutropenia and Thrombocytopenia which are frequently associated with IFN-α administration. Additionally, hemolytic anemia, which is known to be an adverse effect of ribavirin administration is a severe form of anemia that was frequently observed among patients (Salomon

et al., 2002). Less frequent complications included acute fibrosis, cholestatic hepatitis, high viral loads, advanced fibrosis, and pre-existing cytopenia. Renal insufficiency and drug interactions were also quite common upon examination (Davis et al., 2007).

Pegylated IFNα –ribavirin combination therapy has proven to be effective, however the endless list of side effects raises concerns. Interferon therapy will remain the foundation of antiviral therapy for the foreseeable future; however, the fact that the other half of patients are not able to completely suppress HCV during interferon therapy and the current development of antiviral agents directed at the replicative machinery of HCV raise questions as to whether partial suppression of virus replication would be clinically beneficial (Davis et al., 2007).

Moreover, the manufacturing costs and administration expense of these drugs will make it difficult for patients to negotiate the cost in their budget, which may lead to infrequent and improper practice of taking the medications. It is definite to say that the future of HCV drug development will be challenged by designing optimal combination therapeutic strategies that will completely inhibit HCV replication (Davis et al., 2007).

Due to the potential complications associated with current HCV therapies it is important to have another means of dealing with the burden of disease associated with HCV. In addition, the cost and lack of effective treatment (in some cases) argues for a more stable preventative strategy to eliminate HCV. For these reasons, we propose the development of a recombinant vaccine against the Hepatitis C Virus.

VACCINE DESIGN AND PROTECTIVE IMMUNITY

Recombinant Vaccine

The purpose of the vaccine is to induce an immune response and immune memory to HCV antigens. In the course of viral infection presentation of virus-specific peptides on both MHC Class I and MHC Class II of infected cells occurs. Certain vaccine designs have been innovated to use viruses as the vectors for immunization to specific and select proteins from another pathogen. This design is called a recombinant vaccine (Bonnet et al., 2000).

Recombinant vaccines use viruses as the vectors and have an advantage over non-viral vectors (like plasmid DNA) because they can enter the cell and model natural infection, thus inducing a strong cell-mediated immune response. There are several types of viruses that are used as the recombinant vectors. They include retroviruses, poxviruses, adenoviruses, adeno-associated viruses, alphaviruses and herpes simplex viral vectors. They all have their advantages and disadvantages as viral vectors. Retroviruses are a two-strand positive sense RNA virus that replicates through a DNA intermediate. They transfer their genetic material to the genome of the host cell and thus have a high risk of insertional mutagenesis. They are not the safest of vectors. The next vectors, poxviruses, are large-enveloped viruses that contain a double stranded DNA genome. They can hold large inserts of DNA and thus have the capacity to encode for multiple antigens. Certain poxvirus vectors do not elicit an anti-vector immune response nor is their effectiveness as a viral vector hindered by individuals who might already have immunity to poxviruses (Bonnet et al., 2000).

The vector chosen was adenovirus. Adenoviruses are medium size, icosahedral in shape and contain a linear double-stranded DNA genome. They replicate in the nucleus of infected target cells without integrating their DNA into the cell's genome. Approximately 85 % of adults have antibodies to the most common adenovirus serotypes (Bonnet et al., 2000). Adenovirus also

elicits a strong cellular response. For adenovirus to be an effective vector it needs to be replication-defective; thus certain genes are deleted from the genome. These vectors can hold up to 7.5 kb of foreign DNA and can be amplified to high titers. Adenovirus entry into host cells is by means of receptor-mediated endocytosis (using the Coxsackie B virus and adenovirus receptor CAR) and the interaction of other molecules on the cell surface (Bonnet et al., 2000). Adenoviruses can transfer genes to both replicating and non-replicating cells.

For the proposed recombinant adenovirus vaccine genes are inserted for non-structural protein 3 (NS3), and structural proteins E1 and E2. In order to increase the effectiveness of the vaccine and reduce the amount of anti-vector immune response, the recombinant adenovirus is transduced into dendritic cells. Vaccination will occur via the transfected dendritic cells presenting HCV antigen (Zabaleta et al., 2007).

Adjuvants

Protein fragments of viruses and other parasites can be efficiently presented by Class I MHC molecules. Dendritic cells (DC) are able to present both MHC Class I and II proteins. They are considered the most important of all antigen presenting cells because they can activate naive T cells. DCs are the initiators of an immune response and function to sustain strong T cell responses. Additionally, they are the most powerful of all antigen presenting cells, expressing significant levels of MHC, costimulatory and adhesion molecules which effectively stimulate T cells.

Once activated, DCs travel to the closest lymph node, but before doing so they release chemokines to attract more effector cells into the tissue. Since DCs are short-lived they die within a few days of reaching the lymph nodes. Activation of new DCs continues to take place until the infection subsides, thus naive T cells are always presented with the most current antigen peptides.

Prior experiments show that DCs successfully prime naïve T cells *in vitro* against several known tumor antigens. Furthermore, it has been confirmed that “DCs obtained from normal healthy donors’ PBMCs, upon infection with recombinant adenoviral vectors containing HCV Core or NS3 genes, express these proteins in the cells and still have normal phenotype and functions”. There is definite evidence that DCs expressing HCV Core/NS3 antigens are able to stimulate CD4+ and CD8+ T cell responses *in vitro* (Li et al., 2006). As previously reported, DCs have been successfully used as adjuvants; therefore this is an appropriate adjuvant for this vaccine (Encke et al., 2005).

Immune Response

Humoral Immunity

HCV attains entry into the host cell via the E1 and E2 structural proteins. It is therefore proposed that in order to target these structural proteins, a humoral immune response is essential. Humoral immune response against E1 and E2 is pertinent in its function to combat HCV (Stamataki et al., 2007). Experiments show that neutralizing antibodies against surface glycoproteins, E1 and E2, play a key role in combating HCV (Stamataki et al., 2007). Furthermore, neutralizing antibody responses elicited during primary infection provided protection from re-infection, by participating in both viral clearance and control of viral replication in early stages of infection (Stamataki et al., 2007). Moreover, recombinant E2 and combined E1/E2 can induce a polyclonal antibody response with a cross-reactive neutralizing antibody (Stamataki et al., 2007).

The activation of humoral immunity will only occur upon receiving signal from the antigen cross linking of the B cell receptor (BCR) and the Th2 cell signal. B cell activation must first be initiated by cross linking the BCR with the HCV. The epitopes found on the virus must bind to their specific BRCs in order for proper cross linking to occur. The epitopes for antibody, however, are located in the hypervariable 1 regions of the E2 glycoprotein. Since antibody can neutralize homotypic virus, the challenges that have been previously faced concerned dealing with heterotypic virus, such as the HCV (Gowans et al., 2004). Antibodies to multiple virus strains are important. Furthermore, previous studies have shown that most HCV carriers develop antibody response to both E1 and E2 glycoproteins, rather than just one. The current methodology of the humoral response during infection is still unknown, largely due to the inability to propagate HCV in cell culture and to measure the functional antibody responses (Stamataki et al., 2007). Recombinant forms of E2 or E1E2/p7 have also been associated with inducing a polyclonal antibody response with cross-reactive neutralizing activity (Stamataki et al., 2007).

Cellular Immunity

Induction of a T-cell response to HCV is an important step in the resolution and prevention of infection with the virus. In acute infection with HCV, a strong T-cell response against viral antigens, mediated by CD4+ and CD8+ T-cells has been associated with viral clearance (Sarobe et al., 2003). In the case of chronically infected individuals, a T-cell response against viral antigens is low or negative . Additionally, individuals who spontaneously resolve the virus or are successfully treated exhibit potent CD4+ and CD8+ T-Cell responses against viral antigens (Zabaleta et al., 2007). Therefore, eliciting a cell mediated immune response is important for this vaccine.

In a mouse model, vaccination against HCV with dendritic cells (DC) transduced with a recombinant adenovirus expressing HCV non-structural protein 3 (DC-AdNS3) induced both CD4+ and CD8+ T-cell responses (Zabaleta et al., 2007). The CD4+ T-cells produced in this model were Th1. Th1 cells are important in activation of CD8+ T-cells through the production of cytokines necessary for priming of CD8+ cytotoxic T lymphocytes (CTL), which target virally infected cells (Sarobe et al., 2003). This illustrates the necessity of stimulating a T-cell response of both CD4 and CD8 backgrounds.

In contrast, when immunized with DCs expressing HCV core and E1 (CE1) proteins, mice had an impaired CD4+ T-cell response. Consequently, CTL responses were also suppressed, indicating that immunization with HCV CE1 reduced the ability of DC to activate CD4+ T cells. When compared to mice immunized with DC-AdNS3, mice immunized with DC containing recombinant HCV-CE1 in an adenoviral vector produced a weaker CD4+ and CD8+ T-cell response (Sarobe et al., 2003). This suggests that vaccination with DC-AdNS3 is necessary to produce a better cell mediated response, compared to HCV-CE1.

Two mechanisms are important in the CD8+ T cell-mediated response to HCV viral infection. The first mechanism involves direct lysis of virally infected cells through the Fas/FasL pathway, which activates granzymes and perforins (Balkow et al., 2001). The second mechanism is cytokine mediated and involves the production of IFN-γ, which inhibits viral gene expression without direct cell lysis (Guidotti et al., 1994). CD4+ T-cells are also capable of producing IFN-γ to assist in the second antiviral mechanism. This vaccine is intended to produce a cell mediated immune response by vaccination with DC-AdNS3, inducing a CD4+ and CD8+ T cell response.

VACCINE EFFICACY

Administration: Route, Timing and Target Population

The vaccine will be administered to a target young adult population of South Asia via route of injection. It is necessary to induce a systemic IgG response and not a mucosal IgA response because HCV is a blood-borne infection. The timing of the vaccine is difficult to determine, however it is most likely that the vaccine will require several doses to induce a strong, protective immune response. Thus, injection of the vaccine will be needed in an initial dose and in a booster at least 140 days later (Doria-Rose and Haigwood, 2003). It is important to ensure the safety of the vaccine by considering the toxicity of recombinant viruses on the brain, liver, lung, kidney, heart and spleen.

Measuring Protective Immunity

Humoral Immunity

In order to measure anti E1/E2 antibody responses, ELISA analysis must be conducted. In this study, a combination of $E1/E2_{75}$ antigen or $E2_{715}$ antigen, approximately 0.5 μg/ml or greater, will be placed in microplates, and will sit for 24 hours at 4°C. These plates can then be blocked with 1% BSA and then washed with PBS (which contains a blocking buffer for the antibodies). Serum samples extracted from the patients will then be diluted in the blocking buffer and transferred to blocked well plates. The plates will then incubate for one hour at room temperature, and then washed again to assure that any remaining unbound antibody is removed. Again, Chromogenic substrate can then be added to the well plate to generate color when bound to the antibody. The bound antibody will be detected by an enzyme-dependent color change reaction, and detect the amount of antibodies present (Stamataki et al., 2007).

Cellular Immunity

To measure the presence of CD4+ and CD8+ T cells, flow cytometry will be performed on PBMCs collected from immunized individuals. To measure T-cell activity, IFN-γ production will be measured using the enzyme-linked immunosorbent spot assay (ELIspot).

Flow cytometry will be carried as previously described (Zabaleta et al., 2007). Whole blood will be collected from immunized individuals 24 hours after immunization. Cells will be stained in phosphate buffered saline at 4°C containing 2% calf serum with anti CD4 and CD8 antibodies labeled with fluorescein isothiocyanate for 30 minutes. Cells will be washed and detection of surface molecules will be carried out on a flow cytometer. T-cell response will be measured after each subsequent immunization.

To assess immune memory, blood will be collected from immunized individuals at 30, 60, and 120 days after their final immunization. Cells will be stimulated with HCV NS3 *in vitro*. Approximately 24 hours after stimulation with HCV NS3, cell suspensions will be measured for the presence of CD4+ and CD8+ T-cells by flow cytometry as described above.

To measure production of IFN-γ by T-cells, the ELIspot assay will be performed (Zabaleta et al., 2007) using cells harvested after each immunization. Plates coated with anti-IFN-γ antibody are blocked for 2 hours with HL-1 medium containing 10% horse serum. Harvested cells will be cultured in the presence of NS3 overnight. Cells are then washed with phosphate buffered saline and incubated with biotinylated anti- IFN-γ antibody for 2 hours. After incubation, plates are washed and incubated with a 1/100 dilution of streptavidin-peroxidase for 1 hour. Finally, plates are washed and developed to convert substrate to

colorimetric product. The development reaction is stopped with distilled water and spots are counted using and ELIspot reader. A positive result indicates cytokine production.
NOTE: Although the above tests are useful for measuring general T cell response, they are not useful for measuring any levels of HCV antigen-specific T cells. This section will be altered to include how to stimulate HCV-specific CD4 and CD8 T cells and how to measure the levels of those antigen specific T cells (Lönard, 2007).

Clinical Efficacy

Several treatments have been developed in order to elicit an immune response against HCV. Recombinant pegylated INF-α and/or a combination of ribavirin are the only treatments available for HCV (Gowans et al., 2004). Although, neutralizing antibodies have been developed (monoclonal and polyclonal), they compete for attachment to the receptor molecules. Several problems have been identified with the current therapies such as IFN overstimulating the existing immune response. On the other hand, neutralizing antibodies present low levels of HCV mRNA (Pawlotsky et al., 2007), indicating that treatments fail on the suppression of the levels of mRNA. Since efficacy of current treatments against HCV have been questioned, it is important to consider the problems that may be encountered with this vaccine and how to minimize them and increase vaccine efficacy.

The vector: Several studies have shown that recombinant viruses are very efficient in penetration of cells and in mimicking an infection, therefore they are good tools to induce a good immune response. However, studies have shown adenoviruses have a strong immunogenicity when used for vaccination purposes, therefore is a limiting factor in patients with cancer. HCV has a high tendency to cause chronic hepatitis, liver cirrhosis and in the last stage of a chronic infection cause hepatocellular carcinoma (Bonnet et al., 2000). Therefore recombinant vaccine would be used for patients that have not presented early stages of chronic hepatitis infection.

Impaired Immunity: HCV structural antigens are known to interfere with the DC maturation process. Impaired immunity or incomplete activation of T cells allows persistence of the virus. Impaired immunity could be reduced by other effector molecules that might be required for control and prevention of viral development. Prevention of impaired immunity can be accomplished by allowing DC maturation prior to transfection with the viral vector encoding HCV non-structural proteins (Sarobe et al., 2003).

Autoimmunity: DC represents a unique antigen producing cell capable of sensitizing T cells to both new and previously processed antigens. It is possible that DC vaccination will result in liver disease through induction of a cytolitic CD8 T-cell response (Thimme et al., 2001). Although it is believed that cellular and humoral immune responses exist against adenoviral proteins, this could be minimized with co-administration of IL-12 which would prevent the induction of neutralizing antibodies (Bonnet et al., 2000).

Antigenic variation: The immunity that promotes viral persistence is poorly understood in HCV, and knowledge of whether adaptive immunity is sufficient to overcome antigenic variation is lacking. CD4 T-cell response is associated with a Th1 cytokine, suggesting that cell mediated immunity is associated with recovery. Patients who cleared the virus generated a Th1 response, whereas those who developed persistent infection had a Th2 response. Currently there are six HCV genotypes, the 5'UTR is the most highly conserved region and therefore used to determine genotype on infected individuals. HCV-infected patients could be infected with several HCV genotypes thus limiting HCV treatment. For this reason, development of a vaccine for HCV remains a major challenge due to the high sequence divergence among the six major genotypes.

Viral Escape: HCV in nature has great variability, this fact has been demonstrated when a patient presents an infection with several species of the virus that have been able to escape from the immune system by affecting antibody and T-cell recognition (Shimizu et al., 1994). In HCV, viral clearance is associated with an early, strong, multi-antigen T-cell response that probably prevents the emergence of variants. Chronic HCV-infected patients are known to possess a narrow and weak T-cell response which impairs T-cell recognition of the virus. Additionally, viral escape can be enhanced by mutation in cytotoxic T lymphocyte epitopes (Erickson et al., 2001).

REVIEWERS, PLEASE NOTE THE FOLLOWING:

We will be changing the design of our vaccine. We have decided that using dendritic cells as an adjuvant is not a practical approach to vaccinating the masses. Although it is feasible in the laboratory and it does produce excellent protective immunity, it is not possible to collect dendritic cells from every individual and to make a personal vaccine. We will be changing the original vaccine of dendritic cells transduced with recombinant adenovirus containing genetic information for HCV antigens (E1, E2 and NS3) to just the recombinant adenovirus.

REFERENCES

Balkow, S., Kersten, A., Tran, T.T., Stehle, T., Grosse, P., Museteanu, C., Utermohlen, O., Pircher, H., von Weizsacker, F., Wallich, R., Mullbacher, A. and Simon, M.M., 2001. Concerted action of the FasL/Fas and perforin/granzyme A and B pathways is mandatory for the development of early viral hepatitis but not for recovery from viral infection. J Virol 75, 8781-91.

Bonnet, M.C., Tartaglia, J., Verdier, F., Kourilsky, P., Lindberg, A., Klein, M. and Moingeon, P., 2000. Recombinant viruses as a tool for therapeutic vaccination against human cancers. Immunol Lett 74, 11-25.

Caruntu, F.A. and Benea, L., 2006. Acute hepatitis C virus infection: Diagnosis, pathogenesis, treatment. J Gastrointestin Liver Dis 15, 249-56.

CDC. 2006. Hepatitis C Factsheet.

Chowdhury, A., Santra, A., Chaudhuri, S., Dhali, G.K., Chaudhuri, S., Maity, S.G., Naik, T.N., Bhattacharya, S.K. and Mazumder, D.N., 2003. Hepatitis C virus infection in the general population: a community-based study in West Bengal, India. Hepatology 37, 802-9.

Cocquerel, L., Wychowski, C., Minner, F., Penin, F. and Dubuisson, J., 2000. Charged residues in the transmembrane domains of hepatitis C virus glycoproteins play a major role in the processing, subcellular localization, and assembly of these envelope proteins. J Virol 74, 3623-33.

Davis, G.L., Krawczynski, K. and Szabo, G., 2007. Hepatitis C virus infection--pathobiology and implications for new therapeutic options. Dig Dis Sci 52, 857-75.

Doria-Rose, N.A. and Haigwood, N.L., 2003. DNA vaccine strategies: candidates for immune modulation and immunization regimens. Methods 31, 207-16.

Encke, J., Findeklee, J., Geib, J., Pfaff, E. and Stremmel, W., 2005. Prophylactic and therapeutic vaccination with dendritic cells against hepatitis C virus infection. Clin Exp Immunol 142, 362-9.

Erickson, A.L., Kimura, Y., Igarashi, S., Eichelberger, J., Houghton, M., Sidney, J., McKinney, D., Sette, A., Hughes, A.L. and Walker, C.M., 2001. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. Immunity 15, 883-95.

Farci, P., Shimoda, A., Wong, D., Cabezon, T., De Gioannis, D., Strazzera, A., Shimizu, Y., Shapiro, M., Alter, H.J. and Purcell, R.H., 1996. Prevention of hepatitis C virus infection in chimpanzees by hyperimmune serum against the hypervariable region 1 of the envelope 2 protein. Proc Natl Acad Sci U S A 93, 15394-9.

Frank, C., Mohamed, M.K., Strickland, G.T., Lavanchy, D., Arthur, R.R., Magder, L.S., El Khoby, T., Abdel-Wahab, Y., Aly Ohn, E.S., Anwar, W. and Sallam, I., 2000. The role of parenteral antischistosomal therapy in the spread of hepatitis C virus in Egypt. Lancet 355, 887-91.

Glenn, J.S., 2006. Molecular virology of the hepatitis C virus: implication for novel therapies. Infect Dis Clin North Am 20, 81-98.

Goedert, J.J., Chen, B.E., Preiss, L., Aledort, L.M. and Rosenberg, P.S., 2007. Reconstruction of the hepatitis C virus epidemic in the US hemophilia population, 1940-1990. Am J Epidemiol 165, 1443-53.

Gowans, E.J., Jones, K.L., Bharadwaj, M. and Jackson, D.C., 2004. Prospects for dendritic cell vaccination in persistent infection with hepatitis C virus. J Clin Virol 30, 283-90.

Guidotti, L.G., Ando, K., Hobbs, M.V., Ishikawa, T., Runkel, L., Schreiber, R.D. and Chisari, F.V., 1994. Cytotoxic T lymphocytes inhibit hepatitis B virus gene expression by a noncytolytic mechanism in transgenic mice. Proc Natl Acad Sci U S A 91, 3764-8.

He, X.S., Rehermann, B., Lopez-Labrador, F.X., Boisvert, J., Cheung, R., Mumm, J., Wedemeyer, H., Berenguer, M., Wright, T.L., Davis, M.M. and Greenberg, H.B., 1999. Quantitative analysis of hepatitis C virus-specific CD8(+) T cells in peripheral blood and liver using peptide-MHC tetramers. Proc Natl Acad Sci U S A 96, 5692-7.

Kaplan, D.E., 2006. Propagation of hepatitis C virus infection: Elucidating targets for therapeutic intervention. Disease Mechanisms, Gastrointestinal disorders 3.

Li, W., Krishnadas, D.K., Li, J., Tyrrell, D.L. and Agrawal, B., 2006. Induction of primary human T cell responses against hepatitis C virus-derived antigens NS3 or core by autologous dendritic cells expressing hepatitis C virus antigens: potential for vaccine and immunotherapy. J Immunol 176, 6065-75.

Nielsen, S.U., Bassendine, M.F., Burt, A.D., Bevitt, D.J. and Toms, G.L., 2004. Characterization of the genome and structural proteins of hepatitis C virus resolved from infected human liver. J Gen Virol 85, 1497-507.

Pawlotsky, J.M., Chevaliez, S. and McHutchison, J.G., 2007. The hepatitis C virus life cycle as a target for new antiviral therapies. Gastroenterology 132, 1979-98.

Penin, F., Combet, C., Germanidis, G., Frainais, P.O., Deleage, G. and Pawlotsky, J.M., 2001. Conservation of the conformation and positive charges of hepatitis C virus E2 envelope glycoprotein hypervariable region 1 points to a role in cell attachment. J Virol 75, 5703-10.

Salomon, J.A., Weinstein, M.C., Hammitt, J.K. and Goldie, S.J., 2002. Empirically calibrated model of hepatitis C virus infection in the United States. Am J Epidemiol 156, 761-73.

Sarobe, P., Lasarte, J.J., Zabaleta, A., Arribillaga, L., Arina, A., Melero, I., Borras-Cuesta, F. and Prieto, J., 2003. Hepatitis C virus structural proteins impair dendritic cell maturation and inhibit in vivo induction of cellular immune responses. J Virol 77, 10862-71.

Shepard, C.W., Finelli, L. and Alter, M.J., 2005. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 5, 558-67.

Shimizu, Y.K., Hijikata, M., Iwamoto, A., Alter, H.J., Purcell, R.H. and Yoshikura, H., 1994. Neutralizing antibodies against hepatitis C virus and the emergence of neutralization escape mutant viruses. J Virol 68, 1494-500.

Stamataki, Z., Coates, S., Evans, M.J., Wininger, M., Crawford, K., Dong, C., Fong, Y.L., Chien, D., Abrignani, S., Balfe, P., Rice, C.M., McKeating, J.A. and Houghton, M., 2007. Hepatitis C virus envelope glycoprotein immunization of rodents elicits cross-reactive neutralizing antibodies. Vaccine 25, 7773-84.

Thimme, R., Oldach, D., Chang, K.M., Steiger, C., Ray, S.C. and Chisari, F.V., 2001. Determinants of viral clearance and persistence during acute hepatitis C virus infection. J Exp Med 194, 1395-406.

Yu, X., Qiao, M., Atanasov, I., Hu, Z., Kato, T., Liang, T.J. and Zhou, Z.H., 2007. Cryo-electron microscopy and three-dimensional reconstructions of hepatitis C virus particles. Virology 367, 126-34.

Zabaleta, A., Llopiz, D., Arribillaga, L., Silva, L., Riezu-Boj, J.I., Lasarte, J.J., Borras-Cuesta, F., Prieto, J. and Sarobe, P., 2007. Vaccination Against Hepatitis C Virus With Dendritic Cells Transduced With an Adenovirus Encoding NS3 Protein. Mol Ther.

Zibert, A., Kraas, W., Meisel, H., Jung, G. and Roggendorf, M., 1997. Epitope mapping of antibodies directed against hypervariable region 1 in acute self-limiting and chronic infections due to hepatitis C virus. J Virol 71, 4123-7.