DEFECTIVE RECOMBINANT ADENOVIRUSES AS A TOOL TO INDUCE ANTI-HCV IMMUNE RESPONSES

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Introduction

Cytotoxic T lymphocytes (CTL) have been shown to play a major role in the control of many viral diseases. HCV infection has a strong tendency to chronicity suggesting that the cytotoxic T cell reaction against HCV antigens is poor or ineffective. Thus, the development of efficient ways of inducing CTL *in vivo* are important steps towards prevention and/or treatment of HCV infection. Up to date, different methods have been used to study as well as to induce cytotoxic responses, among which those based on recombinant vaccinia viruses have been the most extensively developed. While replication-competent vaccinia recombinants entail substantial risks in men, replication- deficient adenoviruses do not appear to be hazardous. These recombinant viruses are able to express foreign antigens very efficiently inside non-permissive cells without spreading the infection. Based on these principles, we constructed a recombinant adenovirus containing core and E1 genes of HCV and studied its ability to express these proteins in infected cells, and to induce an anti-HCV cytotoxic immune response in mice. We studied the effect of coimmunization of the recombinant adenovirus in conjunction with other defective adenovirus expressing IL-12-p35 and p40 subunits on the immune response against HCV proteins.

Materials and Methods

Construction of the adenovirus and expression of HCV proteins

A replication-defective recombinant adenovirus (RAdCMV-CE1) containing core and E1 genes of hepatitis C virus (HCV) was constructed. The genes were cloned from a patient with chronic hepatitis C infected with genotype 1b. Immunofluorescence experiments in human fibroblasts infected with RAdCMV-CE1, using different anti-HCV (+) human sera, a monoclonal anti-core antibody and a monoclonal anti-E1 antibody indicate a high efficiency of expression of recombinant HCV proteins by our adenoviral vector. Another defective double-recombinant adenovirus expressing IL-12-p35 and p40 subunits was constructed in order to analyze the immunopotentiating effect of IL-12.

Immunological studies

Immunization of BALB/c mice with 1 x 10^8 pfu of RAdCMV-CE1 induced a specific cytotoxic T cell (CTL) response against the two HCV proteins. This response was characterized using a panel of 60 synthetic 14 or 15-mer overlapping peptides (10 amino acids overlap) spanning the entire sequence of these proteins. Cytotoxicity assays were performed using mouse spleen cells incubated with target cells labelled with 51Cr and preincubated with each singte peptide or with different recombinant adenoviruses.

Results and Discussion

Five main epitopes were found in core, four of which had been previously described either in mice or humans. One single novel epitope was found in E1 (p312-326). This epitope, encompassing residues 312-326 from E1 protein corresponds to a highly conserved motif among all HCV isolates. Using 10-mer overlapping peptides we identified the sequence GHRMAWDM as the minimal epitope identified by CTL. These cytotoxic responses were mediated by the classic CD4- CD8+ phenotype, were H-2d restricted and lasted for at teast 100 days. Immunization of BALB/c with the double recombinant defective adenovirus expressing both p35 and p40 subunits induced *in vivo* the production of bioactive p70 IL-12 cytokine and the increasing of serum IFN-gamma levels. One single immunization with RAdCMV-CE1 does not induce detectable levels of antibodies against HCV core or E1 antigens. However, immunization of RAdCMV-CE1 in conjunction with a defective double-recombinant adenovirus expressing IL-12-p35 and p40 subunits enhanced humoral and cellular immune response against HCV proteins. This work demonstrates that replication-defective recombinant adenoviruses can efficiently express HCV proteins and are able to induce an *in vivo* cytotoxic T cell response against a diversity of epitopes from HCV antigens and that IL-12 can act as an adjuvant to potentiate the immune responses against HCV. These vectors should be taken into consideration in the design of vaccines and also as a mean to stimulate specific T cell responses in HCV carriers.