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• BASIC RESEARCH •

Induction of hepatitis C virus-specific cytotoxic T and B cell responses by dendritic cells expressing a modified antigen targeting receptor

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Abstract

AIM: To find a novel antigen (Ag) presentation strategy to improve the immune responses induced by dendritic cell (DC) vaccine expressing hepatitis C virus (HCV) core antigen (pcDNA 3HCV C-Fc) in Balb/c mice (H-2d).

METHODS: pcDNA 3HCV C-Fc plasmid and eukaryotic expression vector pcDNA3 were injected into mice sc. Immune responses to pcDNA 3HCV C-Fc were studied. Meanwhile the effect of pcDNA 3HCV C-Fc on anti-translated subcutaneous tumor of SP2/0 cells stably expressing HCV C Ag (SP2/0-HCV C-FC) was also studied. Anti-HCV C in serum was detected by enzyme-linked immunoadsordent assay (ELISA) and HCV specific cytotoxic T lymphocyte (CTL) activity was measured by LDH release assay. After 3 wk of DNA immunization, the cells of SP2/0-HCV C-FC were inoculated into mice subcutaneously and tumor growth was measured every 5 d. The survival rate and living time of mice were also calculated.

RESULTS: After 4 wk of DC immunization, the $A_{450\ nm}$ values of sera in mice immunized with pcDNA 3HCV C-Fc-DC and pcDNA3-DC were 0.56±0.17 and 0.12±0.03 respectively. The antibody titres in mice codeliveried with pcDNA 3HCV C-Fc with DC were significantly higher than those of mice injected with pcDNA3-DC. The HCV specific CTL activities in mice coinjected with DC and pcDNA 3HCV C-Fc or empty expression vectors were(73.2±3.1) % and (24.4±8.8) % , which were significantly higher than those of mice injected with water. The DC vaccine could evidently inhibit tumor growth, prolong the survival time of mice and improve the survival rate of mice and these effects could be improved by HCV C-Fc (pcDNA 3HCV C-Fc) gene codelivered.

CONCLUSION: DC vaccine has a strong antigenicity in humoral and cellular immunities, which can be promoted by transduced pcDNA 3HCV C-Fc expressing HCV C or Fc. Thus, pcDNA 3HCV C-Fc-transduced DCs may be a promising candidate for a CTL-based vaccine against HCV.

Key words: Hepatitis C virus; Dendritic cell vaccine; Cytotoxic T-Lymphocytes

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INTRODUCTION

Hepatitis C virus (HCV) chronic infection is characterized by low or undetectable cellular immune responses to HCV antigens. Some studies have suggested that HCV proteins manipulate the immune system by suppressing the specific antiviral T-cell immunity. Antiviral immunity requires recognition of viral pathogens and activation of cytotoxic and Th cells by innate immune cells.[1-5] In this study, we designed a novel antigen (Ag) presentation strategy by transducing DCs to produce and secrete a fusion protein consisting of a HCV core protein HCV C Ag fused with cell-binding domains such as the Fc fragment of IgG. The secreted fusion proteins, in addition to inducing antibody (Ab) responses, are transported back to DCs via receptor-mediated internalization. It has been demonstrated that Ag presentation by receptor-mediated internalization of DCs can be enhanced up to 1 000-fold, compared with fluid phase Ag pinocytosis. Thus, this receptor-mediated Ag presentation strategy is able to induce vigorous Th cell, CTL, and B cell responses to the model HCV nucleocapsid protein in mouse models[6-10].

MATERIALS AND METHODS

Construction of expression vectors

Plasmid expressing HCV core antigen (pcDNA3-HCV)[3-5] was constructed by Professor Feng (in this Department). Plasmids expressing human IgG cDNA Fc (pCMVsFc)[5-7] were a generous gift from Dr. Chen (USA). Human IgG cDNA Fc fragment was generated by PCR amplification with plasmid pCMVsFc containing human IgG heavy chain cDNA as a template. The pair of primers for PCR was 5'-primer: 5' ATA TAC TCG AGG AAA ACT CAC ACA TGC 3' corresponding to the nucleotide sequence 956-970 of the heavy chain with an additional *Xho* I site,and 3'-primer: 5' GTT TCT AGA TCA TTT ACC CGG AGA CAG, corresponding to the nucleotide sequence 1 572-1 589 of the heavy chain with an additional *Xba* I site . Fc cDNA with a leader sequence was cloned into the *Xho* I/ *Xba* I cut pcDNA 3HCV. These resultant vectors were identified by restriction enzyme analysis and confirmed by DNA sequencing.

Production of vectors and DC transduction

To generate DC, bone marrow (BM) cells were flushed from the bones of mouse limbs, passed through a nylon mesh, and depleted red cells with ammonium chloride. After extensively washing with RPMI 1640, cells were incubated with rabbit complements and a mixture of mAbs consisting of anti-CD4, anti-CD8 in RPMI 1640 at 37 ℃ for 40-60 min. Then, 5×10^8/L in

RPMI 1640 supplemented with 100 mL/L FBS, 80 ng murine GM-CSF (mrGM-CSF) (Gibco), and 20 U/L IL-4 (Gibco) were plated in 12-well culture plates (2.5 mL/well), incubated at 37 ℃ in 50 mL/L CO_2 overnight, and then refed with fresh medium. After 48-h incubation, the cells were spun down. The bone marrow-derived dendritic cells were cultured in 100-mm culture dishes with RPMI1640 containing 100 mL/L heat-inactivated FBS (Life Technologies) and transfected with 10-15 μg of vector plasmids by lipofectin (Life Technologies). After an overnight incubation, the medium was replaced with RPMI1640 containing 100 mL/L FBS. Forty-eight hours later, the cells were incubated at 37 ℃ in 50 mL/L CO_2 for 3-4 h. The transduction procedure was repeated 2-3 times. After the final transduction, the cells were washed and cultured in the medium containing mGM-CSF and mIL-4 for several days to allow further DC differentiation. DC was further enriched by using a 500 g/L FCS-RPMI 1640 sedimentation procedure as described previously.

HCV C Ag-specific antibody assay

Sera samples were collected by tail bleeding at different times, beginning at 1 wk after immunization, and the presence of HCV C Ag-specific antibody was analyzed by ELISA. Briefly, microtitre plates coated with a mixture of recombinant HCV C Ag (each 50 ng/well) were incubated with serially diluted sera in a blocking buffer at room temperature for 2 h. Bound Ab was detected after incubation with peroxidase or alkaline phosphatase-conjugated Abs against mouse IgG (Sigma). A polyclonal anti-HCV C Ab was used as a positive control and nonimmunized mouse sera as a negative control. Ab titre was defined as the highest dilution with A_{450} or A_{405}>0.2. The background A_{450} or A_{405} of normal mouse sera was <0.1.

CTL assays

To determine whether immunization with HCV C-Fc-DC could induce strong CTL responses, LDH release cytotoxic assay was performed. The assays were performed in triplicate with 1×10^5 targets/well at various effector cell/target cell (E:T) ratios of 100:1, 50:1, 25:1. Pooled immunized mice were restimulated *in vitro* in RPMI 1640 containing synthetic peptide HCV C Ag (1 μmol/L) for 4-6 d. SP2/0 (H-2d) target cells were incubated with a synthetic peptide, HCV C Ag at a concentration of 10 mg/L overnight. Different numbers of effector cells were incubated with a constant number of target cells (1×10^4/well) in 96-well V-bottom plates (200 μL/well) at 37 ℃ for 3 h. The supernatants (100 μL) from triplicate cultures were collected. The percent of lysis was defined as (experimental release-spontaneous release)/(maximum release-spontaneous release) ×100. Maximum release was determined by cell lysis with 1% Triton X-100. Spontaneous release was always <5% of the maximum release in the assays.

Tumor challenge studies

Tumor cell line SP2/0 (Balb/c, H-2d) was transfected with plasmid pcDNA3-HCV C Ag by using lipofectin (Life Technologies) and then selected in the presence of 1 g/L G418 (Life Technologies). G418-resistant clones were subcloned and then screened for HCV C Ag expression by immunofluorescence and PCR. SP2/0-HCV C Ag cells expressing HCV C Ag were maintained at 37 ℃ in 50 mL/L CO_2 in RPMI1640 containing 100 mL/L heat-inactivated horse serum and 1 g/L G418. In the tumor protection experiments, Balb/C mice were immunized by iv injection with 1×10^5 transduced DCs on d 0 and 3, and then intradermally challenged with 3×10^6 exponentially growing SP2/0-HCV C Ag cells 1 wk after the first immunization. Tumor sizes were measured every 2-3 d, with tumor volumes calculated as the longest diameter×the shortest diameter.

Statistical analyses

All data were presented as mean±SE. Different groups were compared by Student-Newman-Keuls test with SigmaStat 2.03 software (SPSS). P<0.05 was considered statistically significant.

RESULTS

Construction and expression of fusion proteins

By using immunofluorescence and PCR, it was found that HCV C Ag-Fc proteins (HCV C-Fc) were efficiently produced and secreted from transfected cells (Figure 1). Transduced and untransduced BM-derived DCs were stained with an anti-HCV-FC Ab (Figure 2). When followed by incubation with FITC-conjugated anti-mouse IgG (Sigma) on ice for 30-60 min, the percentages of the cells positive for HCV-FC markers were indicated.

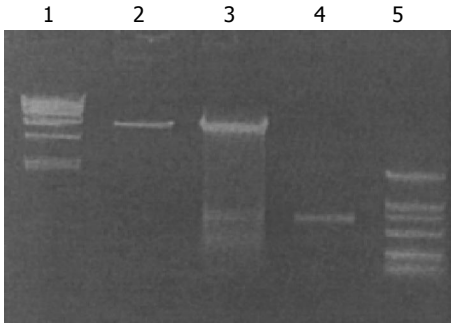

Figure 1 pcDNA 3HCV-Fc vectors identified by restriction enzyme analysis. Lane 1: Marker (*Ecoli*); lane 2: pcDNA3; lane 3: pcDNA 3HCV-Fc was cut by restriction enzyme *Xho* I/*Xba* I; lane 4: Fc cDNA fragment was cloned into the vector; lane 5: Marker (DL-2000).

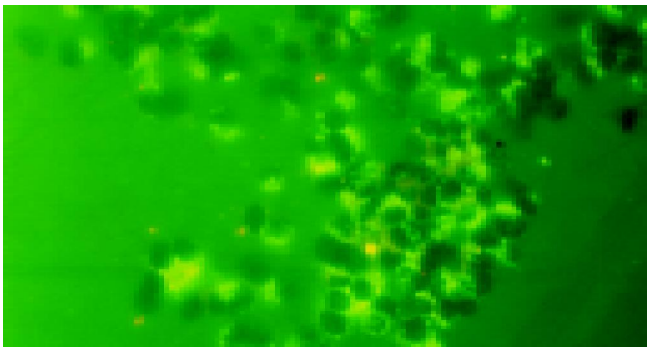

Figure 2 Expression of HCV C-Fc fusion proteins.

Culture and identification of BM-derived DC

After 7 d of culture, a large number of cells with typical characteristics of DCs were observed. Characteristics of the cultured cells were observed by SEM (Figure 3) and the expression of DEC205 was detected by FACS.

Induction of strong B cell responses in vivo

The pcDNA 3HCV C-Fc transduced DC showed a strong antigenicity in humoral immunity and anti-HCV C could be detected in sera of mice after pcDNA 3HCV C-Fc transduced DC vaccination. The serum titers of anti-HCV C in mice increased with the times of immunization in a period of time. The titres of anti-HCV C in sera of mice were significantly promoted by genes expressing HCV C-Fc fusion proteins (Figure 4).

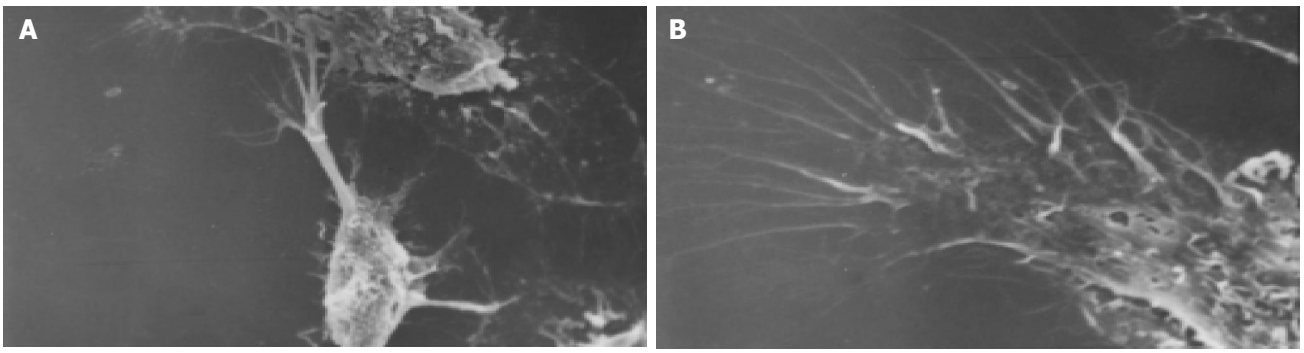

Figure 3 BM-derived DCs. A: BM-derived DC (SEM×500); B: BM-derived DC (SEM×700).

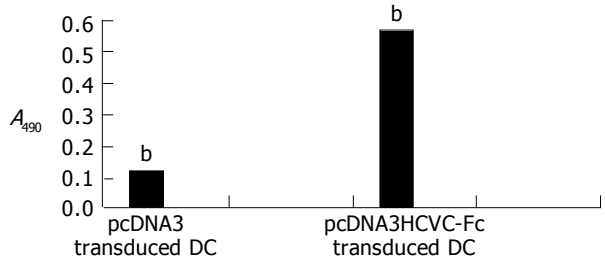

Figure 4 Serum anti-HCV C level in Balb/c mice. $^{b}P<0.01$ *vs* pcDNA3

Induction of strong CTL responses in vivo

HCV specific CTL activities were developed in the mice after pcDNA 3HCV C-Fc transduced DC immunization. As shown in Table 1 splenocytes from mice immunized with HCV C-Fc-DCs demonstrated significantly higher target cell killing than those from mice immunized with DCs alone. The specificity of the killing was demonstrated by the inability of splenocytes to kill HCV C Ag-pulsed p815 target cells with H-2^{d}. The superior CTL responses induced by HCV C-Fc-DCs might be due to the enhanced Th and the direct MHC-I presentation of internalized HCV C-Fc by DCs.

Table 1 Effect of pcDNA 3HCV C-Fc on CTL activity induced by DCs (n = 5; %; mean±SD)

Group	100:1	50:1	25:1
PcDNA 3HCV C-Fc transduced DC[ac]	73.2	46.8	23.2
PcDNA3 transduced DC[a]	24.4	13.7	3.9
PBS control	0.5	0.3	0.2

[a]$P<0.05$ *vs* PBS control; [c]$P<0.05$ *vs* pcDNA3 transduced DC.

Protective immunity induced by fusion construct immunization

A common tumor cell line (SP2/0) might grow rapidly in syngeneic mice as the target cell line for transfection and challenge experiments. SP2/0 clones transfected with the HCV C Ag expression vector were generated and shown to express HCV C Ag by PCR and immunoprecipitation assays. After inoculated with SP2/0-HCV C-FC, all six mice with or without pcDNA3 (100%) formed the tumor. The rate of tumor formation was 16.7% (1/6). No tumor was formed in mice coimmunized with pcDNA 3HCV C-Fc and DCs. The survival rate of mice immunized with pcDNA 3HCV C-Fc alone or coimmunized with DCs increased significantly and the tumor growth was evidently slower than that of mice immunized with or without pcDNA3. The antitumor activity induced by HCV C-Fc-DCs was specific because HCV C-Fc-DC-immunized mice challenged with SP2/0 cells also developed lethal tumors and died within 4 wk.

DISCUSSION

The majority of HCV infections would become chronic, despite the presence of HCV-specific cellular and humoral immune responses. Inadequate Ag presentation by APC might contribute to the failure of the human immune system to mount effective immune responses to chronic infections[11-16]. Accumulating evidence indicates that a vaccine or an immunotherapy, which can induce combined $CD4^{+}$ and $CD8^{+}$ T and B cell immune responses, might be the most effective one to prevent or control chronic infections such as HIV-1, hepatitis virus infection, or *Mycobacterium tuberculosis*, and tumors[17-20]. The results of this study demonstrate that this receptor-mediated Ag presentation strategy, which uses a unifying mechanism to efficiently present Ag to both MHC-I and -II, can potentially activate Ag-specific Th cells, CTL, and B cells. Thus, the receptor-mediated Ag presentation strategy with the ability to induce all arms of the adaptive immunity may have broad applications in the treatment and prevention of cancer, infection, and even autoimmune diseases.

DCs are the most potent APC for initiating primary and secondary immune responses[21-23]. Thus, for effective vaccines or immunotherapies, Ag must be acquired and displayed by DCs. Many investigators have tried to use the potential efficacy of DCs to develop effective immunotherapies and vaccines[24-26]. For exemple, some investigators transduced Ag genes into DCs, which allow the constitutive expression of Ag proteins leading to prolonged Ag presentation of multiple or unidentified epitopes in the context of MHC. Because the Ag-presenting pathway to MHC-I is distinctly different from that to MHC-II, it is difficult for Ag to be presented to both MHC-I and MHC-II by DCs. Thus, developing a strategy for DC to present Ag to both MHC-I and -II may lead to more effective immunotherapies and vaccines, because Th cells play a central role in the activation of CTL, B cells, NK cells, and macrophages[27-29].

The receptor-mediated Ag presentation strategy, which could efficiently activate not only Th cells, but also cytotoxic T and B cells, has unique and superior features. First, by using the receptor-mediated endocytosis pathway, fusion proteins could be efficiently captured, processed, and presented to MHC-II by DCs in both autocrine and paracrine modes to vigorously induce Th cells. Moreover, high level of cytokines produced by primed Th cells can be directly responsible for the control of viral infection and tumor growth. Second, this strategy could efficiently induce CTL, because Fcγ R-mediated internalization could directly present internalized Ag to MHC-I (cross-priming) as well as activate DCs. This strategy should be superior to transient peptide-pulse DC strategies because transduced DCs could continuously produce, as well as process. Finally, this strategy is versatile because of its adaptability for use with any Ag or many cell-binding domains and for incorporation into the design of almost all vaccines and immunotherapies. Indeed, this strategy has been shown to significantly enhance the potency of DNA

vaccines to induce immune responses. Thus, the receptor-mediated Ag presentation strategy may provide a generic and powerful means for the development of effective immunotherapies, therapeutic and preventive vaccines.

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