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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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 immunoadsorbent 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.

RESULTS: After 4 wk of DC immunization, the A600 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 codelivered 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 cojected 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.

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RPMMI 1640 supplemented with 100 mL/L FBS, 80 ng murine GM-CSF (Gibco), and 20 u/mL IL-4 (Gibco) were plated in 12-well culture plates (2.5 mL/well), incubated at 37 °C in 50 mL/L CO2 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 RPMMM1640 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 RPMM1640 containing 100 mL/L FBS. Forty-eight hours later, the cells were incubated at 37 °C in 50 mL/L CO2; 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-RPMM1640 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, microwell 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 A405 or A490<0.2. The background A405 or A490 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 1×105 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 RPMM1640 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 (1×105/well) in 96-well V-bottom plates (200 μL/well) at 37 °C for 3 h. The supernatants (100 μL) from triplicate cultures were collected. The percent of lysis was defined as (experimental 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 °C in 50 mL/L CO2 in RPMM1640 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 1×106 transfected DCs on d 0 and 3, and then intradermally challenged with 3×105 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](image1.png)

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

![Figure 2](image2.png)

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 transfused DC showed a strong antigenicity in humoral immunity and anti-HCV C could be detected in sera of mice after pcDNA 3HCV C-Fc transfused DC vaccination. The serum titers of anti-HCV C in mice increased with the times of immunization in a period of time. The titers of anti-HCV C in sera of mice were significantly promoted by genes expressing HCV C-Fc fusion proteins (Figure 4).
expression vector were the direct MHC-I was demonstrated from mice immunized with DCs alone. The specificity of the killing experiments.

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 SP2/0-HCV C-FC, all six mice with or without pcDNA3.

The antitumor activity induced by HCV C-Fc-DCs was slower than that of mice immunized with or without pcDNA3. pcDNA 3HCV C-Fc alone or coimmunized with DCs (100%) formed the tumor. The rate of tumor formation was significantly higher than that of mice immunized with or without pcDNA3. pcDNA3 transduced DC.

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

**Figure 4** Serum anti-HCV C level in Balb/c mice. bP<0.01 vs pcDNA3 transduced DC.

**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^b^. The superior CTL responses induced by HCV C-Fc-DCs might be due to the enhanced Th and 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 |
|--------------------------------------------|-------|------|------|
| PcDNA3 3HCV C-Fc transduced DC             | 73.2  | 46.8 | 23.2 |
| PcDNA3 transduced DC                       | 24.4  | 13.7 | 3.9  |
| PBS control                                | 0.5   | 0.3  | 0.2  |

P<0.05 vs PBS control; bP<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 immunized with pcDNA 3HCV C-Fc or DCs. The survival rate of mice immunized with pcDNA 3HCV C-Fc alone or communized 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 example, 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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