Vaccination With Protein-Transduced Dendritic Cells Elicits a Sustained Response to Hepatitis C Viral Antigens

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Background & Aims: **Professional antigen-presenting dendritic cells are capable of eliciting a vigorous antiviral response in naive T cells. The administration of antigen-loaded dendritic cells offers a potential approach to induce high-level immunity against hepatitis C virus.** ***Methods:*** **The dendritic cell population in mice was expanded in vivo by hydrodynamic delivery of naked DNA that encoded the secreted form of human fms-like tyrosine kinase 3 ligand. The CD11c-enriched dendritic cell population obtained from the spleen was transduced in vitro with recombinant hepatitis C virus core and nonstructural 5 proteins by using macromolecular-based protein delivery. Vaccine efficacy was assessed with a cytotoxic T-lymphocyte assay, cytokine enzyme-linked immunosorbent assays, and intracellular cytokine staining in vitro and by a tumor challenge model in vivo.** ***Results:*** **Relative to mice inoculated with nontransduced dendritic cells, splenocytes derived from mice immunized with either hepatitis C virus core-transduced or nonstructural 5–transduced dendritic cells showed 3- to 5-fold greater antigen-specific cytotoxic T lymphocyte activity. The $CD4^+$ T cells obtained from mice immunized with nonstructural 5–transduced dendritic cells produced interferon γ, but not interleukin 4, when stimulated with nonstructural 5. In contrast, T cells derived from mice immunized with hepatitis C virus core-transduced dendritic cells produced neither interferon γ nor interleukin 4 when stimulated with core protein. Mice vaccinated with nonstructural 5–transduced dendritic cells, but not a nonstructural 5–expressing plasmid, showed a sustained antiviral response to nonstructural 5 as evidenced by reduced growth of nonstructural 5–expressing tumor cells inoculated 10 weeks after vaccination.** ***Conclusions:*** **These findings suggest that vaccination with protein-transduced dendritic cells may constitute an important antiviral strategy for hepatitis C virus.**

Hepatitis C virus (HCV) is a major cause of liver diseases, including chronic hepatitis, cirrhosis, and hepatocellular carcinoma.[1] Approximately 200 million people worldwide (4 million individuals in the United States) are chronically infected with HCV.[2,3] Standard treatment consists of the combined administration of interferon (IFN) and ribavirin; a sustained virological and histological response is observed in approximately 40% of HCV-infected patients after treatment withdrawal.[4,5] Therefore, new approaches in the prevention and treatment of HCV are needed.

HCV RNA consists of a large open reading frame that encodes an approximately 3000–amino acid polyprotein precursor, which is cleaved by cellular and viral proteinases to yield the core, envelope (E1 and E2), and nonstructural (NS) proteins (NS2–NS5).[6–8] Although several HCV proteins are candidates for vaccine development, T cells obtained from individuals with self-limited HCV proliferate primarily in response to NS proteins.[9–11] These findings imply that NS proteins serve as important immunogens and should be considered in vaccine strategies to develop a protective host immune response. We previously reported that administration of a DNA construct encoding NS5 induced T-cell proliferation, cytotoxic T lymphocyte (CTL) activity, and NS5-specific tumor immunity in a mouse model.[12] In general, however, the capacity of DNA vaccines to generate a robust immune response in humans has been relatively modest.[13,14]

Although the mechanisms underlying DNA-based immunization remain to be clarified, dendritic cells (DCs) seem to play an important role.[15,16] DCs are pro-

Abbreviations used in this paper: CTL, cytotoxic T lymphocyte; DC, dendritic cell; ELISA, enzyme-linked immunosorbent assay; Flt3L, fms-like tyrosine kinase 3 ligand; IFN, interferon; IL, interleukin; MHC, major histocompatibility complex; NS, nonstructural; PTDs, protein transduction domains.

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fessional antigen-presenting immune cells that capture and process antigens into immunogenic peptides that are subsequently presented with products of the major histocompatibility complex (MHC) to T cells.[17] DCs have been used as cellular adjuvants to elicit protective T cell–mediated immunity against pathogens and tumors.[17,18] Thus, the use of DCs as cellular vectors for vaccination may be a promising approach to the prevention and/or treatment of HCV infection. DCs are highly resistant to methods of DNA transfection in vitro.[19,20] As an alternative approach, recombinant viral vectors have been successfully used to transfect DCs with genes that encode foreign antigens.[21] Use of attenuated viruses for vaccination, however, may cause substantial problems, including damage to mitochondrial DNA and induction of liver failure.[22]

Small protein domains (protein transduction domains; PTDs) cross biological membranes and promote the uptake of peptides and proteins by cells. Because they facilitate the intracellular delivery of biologically active proteins, in vivo PTDs, such as that associated with the human immunodeficiency virus transcriptional transactivator (TAT) protein, have generated considerable interest for their potential use in protein therapeutics.[23–25] PTD vectors, however, display a number of limitations, including a requirement for cross-linking to the target protein. Moreover, protein transduction by PTD/TAT fusion protein methods often requires protein denaturation before delivery to increase the accessibility of the PTD/TAT domain.

Recently, Morris et al[26] developed a new strategy for protein delivery based on a short amphipathic peptide carrier, Pep1, which rapidly delivers a variety of peptides and proteins into cells (within 2 hours) without denaturation or cross-linking. In this study, a mature DC population was generated in vivo by hydrodynamic delivery of DNA that encoded the secreted form of human fms-like tyrosine kinase 3 ligand (Flt3L). DCs were substantially transduced with recombinant HCV core or NS5 protein by using a protein delivery based on a short amphipathic peptide carrier, Pep1.[26] Relative to nonimmunized mice, splenocytes derived from mice immunized with transduced DCs showed 3–5-fold greater antigen-specific CTL activity. It is interesting to note that vaccination with transduced DCs led to disparate antigen-specific $CD4^+$ T-cell responses that were dependent on the protein transduced. Relative to conventional DNA-based vaccination, immunization with NS5-transduced DCs resulted in a significantly stronger, sustained immune response, thus suggesting that transduced DCs offer a novel antiviral approach for HCV infection.

Materials and Methods

Animals

Specific pathogen–free female BALB/c mice (Harlan Sprague Dawley, Indianapolis, IN) were maintained in accordance with the guidelines set forth by the Institutional Animal Care and Use Committee of Rhode Island Hospital (Providence, RI). Animals between 6 and 24 weeks of age were used in the experiments described.

In Vivo Generation of Dendritic Cells

The murine DC population was expanded by hydrodynamic delivery of naked DNA encoding the secreted form of human Flt3L. Briefly, mice were inoculated twice (days 0 and 6) via tail vein with 10 μg of pUMVC3-hFlt3L (Vector Core Laboratory, University of Michigan, Ann Arbor, MI) in 2 mL of saline administered within a 5-second period according to the method of He et al.[27] The spleens were dissected on day 11; single-cell suspensions were prepared, and erythrocytes were depleted by ammonium chloride treatment. DC-enriched populations were obtained by positive selection by using anti-CD11c–conjugated MicroBeads and the protocols provided by the manufacturer (Miltenyi Biotec, Auburn, CA).

Vaccination

Purified ($CD11c^+$) DCs were suspended at 1×10^6/mL in HEPES-buffered RPMI 1640 medium (BioWhittaker, Walkersville, MD). Subsequently, cells were transferred to 24-well tissue culture plates coated with 0.1% polyhydroxyethyl methacrylate to prevent attachment[28] and transduced by using 2.5 μg/mL recombinant HCV core of genotype 1b (amino acids 2–192; Biodesign, Saco, ME) or NS5 protein (amino acids 2054–2995; Austral Biologicals, San Ramon, CA) of genotype 1a and incubating the mixture with a peptide carrier complex for 2 hours according to the Chariot method of protein transduction (Active Motif, Carlsbad, CA). Cells were collected, washed, and resuspended in RPMI 1640 with 5% fetal calf serum. Groups of animals were inoculated subcutaneously 3 times at 2-week intervals with 200 μL of medium alone or medium that contained 1×10^6 DCs transduced or not transduced with HCV protein (core or NS5). For comparison purposes, additional groups were inoculated in both sides of the quadriceps muscle with 100 μg of (total) plasmid DNA containing pAp031-NS5 of genotype 1a or pAp031-CORE of genotype 1b; pAp031 empty plasmid served as a control.[12] At 2 weeks after the final inoculation, mice were killed. Erythrocyte-depleted splenocyte suspensions were prepared as described previously, and antigen-specific T-cell reactivity was determined in vitro.[29] Alternatively, at 10 weeks after inoculation, the mice were challenged with stably expressing HCV NS5 myeloma cells (SP2/21) or nonexpressing parental cells (SP2/0; detailed below).

Cytotoxic T Lymphocyte Assay

CTL activity was determined after 3 days of in vitro stimulation. Isolated splenocytes were cultured in HEPES-