Cellular Immunology 281 (2013) 1–10

Contents lists available at SciVerse ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

The dominant roles of ICAM-1-encoding gene in DNA vaccination against Japanese encephalitis virus are the activation of dendritic cells and enhancement of cellular immunity

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ARTICLE INFO

Article history:
Received 31 May 2012
Accepted 14 January 2013
Available online 29 January 2013

Keywords:
Cytokines
DNA vaccines
Encephalitis
Intracellular adhesion molecule-1

ABSTRACT

We investigated the cellular immune responses elicited by a plasmid DNA vaccine encoding prM–E protein from the Japanese encephalitis (JE) virus (JEV) with or without various forms of intercellular adhesion molecule (ICAM)-1 gene to maximize the immune responses evoked by the JE DNA vaccine. We observed that co-immunization with the construct containing murine ICAM-1 gene (pICAM-1) resulted in a significant increase in the percentage of $CD4^+$T cells, high level of JEV-specific cytotoxic T lymphocyte response, and high production of T helper 1 (Th1)-type cytokines in splenic T cells. Furthermore, the co-expression of ICAM-1 and DNA immunogens was found to be more effective in generating T cell-mediated immune responses than those induced by immunization with pJME in combination with pICAM-1. Our results suggested that ICAM-1 enhanced T cell receptor signaling and activated Th1 immune responses in the JEV model system by increasing the induction of $CD4^+$Th1 cell subset and activating dendritic cells.

1. Introduction

DNA vaccine production is a milestone in contemporary vaccine development, and has considerably offset many shortcomings of conventional vaccines. In recent years, plasmid DNA vaccines have gained considerable attention because of their abilities to elicit wide-ranging immune responses (such as antibody induction as well as CD4 T helper (Th) and CD8 cytotoxic T lymphocyte (CTL) generation), and to impart protection against a number of viral infections [1]. In DNA vaccination, plasmid endocytosis occurs followed by endogenous antigen production, which enables antigen presentation by major histocompatibility complex (MHC) class I and leads to the generation of the $CD8^+$ CTL response. The uptake of soluble antigen by professional antigen-presenting cells (APCs) also leads to the generation of the $CD4^+$Th response by MHC class II presentation [2]. Furthermore, DNA vaccines for use against various infectious diseases, for cancer therapies [3–6], as well as for autoimmune disease and allergy treatments [7,8] are now undergoing a variety of human clinical trials.

Japanese encephalitis (JE) is the most important cause of acute viral encephalitis and continues to spread to hitherto unaffected regions such as Indonesia, Pakistan, and Australia. Approximately 60% of the world's population inhabits JE endemic areas. Despite its restricted range being mostly in developing countries, a high annual incidence of 50,000 cases and approximately 10,000 deaths have been reported. The magnitude of the problem is of great concern because the survivors are left with serious, long-term neuropsychiatric sequelae. No antiviral treatment is available for JE, although three vaccines against JE exist, including inactivated mouse brain-derived, inactivated cell culture-derived, and cell culture-derived live-attenuated JE vaccines. Both inactivated and live-attenuated JEVs are widely used with significant success in providing first-generation vaccines to many Asian countries [9]. However, inactivated JEVs are limited by their poor availability, high production cost, lack of long-term immunity, and possibility of allergic reactions. Meanwhile, live-attenuated vaccines carry a possible risk of reverting to virulence [10,11]. These problems may be solved by immunization with epitope-based vaccines comprising rationally designed protective epitopes that are appropriately presented, easy to deliver, and capable of stimulating effective B cell, T

Abbreviations: APC, antigen-presenting cell; CFSE, 5-(and 6-)carboxyfluorescein diacetate succinimidyl ester; CTL, cytotoxic T lymphocyte; DC, dendritic cell; FBS, fetal bovine serum; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; ICAM, intercellular adhesion molecule; IFN, interferon; IL, interleukin; JE, Japanese encephalitis; JEV, Japanese encephalitis virus; LDH, lactate dehydrogenase; LFA-1, lymphocyte function-associated antigen-1; MFI, mean fluorescence intensity; MAb, monoclonal antibody; PBS, phosphate-buffered saline; PE, phycoerythrin; Th, T helper.

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http://dx.doi.org/10.1016/j.cellimm.2013.01.005

cell, and cytotoxic immune responses without the potentially hazardous and undesirable effects [12]. Over the past few years, DNA vaccines against JEV have been developed using both structural and non-structural genes and evaluated in animal models with different efficacies [13–21]. Recombinant DNA vaccines are currently being synthesized.

The DNA immunization mechanism is under continuous investigation. Whether immunogenic plasmids directly transfect APCs or antigens expressed in muscle cells are uptaken and presented by APCs has been the subject of considerable debate [22]. Nonetheless, APCs, particularly dendritic cells (DCs), are clearly important in the generation of immune responses following plasmid DNA immunization [23], and the co-stimulation of DCs and T cells is critical to the initiation of immune responses [24]. A number of strategies for adjuvanting plasmid DNA immunogens have focused on the use of various immunostimulatory molecules, including cytokines [25] and co-stimulatory molecules [26,27].

One such membrane-bound candidate molecule is the intercellular adhesion molecule (ICAM)-1. During antigen presentation, ICAM-1 expressed in DCs binds with lymphocyte function-associated antigen-1 (LFA-1) on Th cells. ICAM-1/LFA-1 interaction has a pleiotropic effect; it is important in both T cell recirculation, inflammation [28,29], and activation [30]. In particular, ICAM-1-mediated adhesion is critical to establishing and strengthening physical contact between DCs and Th cells, which leads to optimal Th cell activation [31].

Compared with the co-stimulatory molecules B7-1 and B7-2, the specific roles of adhesion molecules in the process of immune T cell activation remain to be clarified. Therefore, this study aimed to investigate the adjuvant effects of various forms of ICAM-1-encoding gene in the cell-mediated immunity and Th1/Th2 immune response balance against JEV. The relative contribution of ICAM-1 gene to the function of spleen DCs in BALB/c mice was also examined. We explored as well the relationship of changes in DC functions with various immunogen-mediated immune responses. Our findings suggested that DCs may drive the cell-mediated Th1 immune response induced by JE DNA vaccine with different forms of ICAM-1 gene.

2. Materials and methods

2.1. Cell cultures

Chinese hamster ovary (CHO) cells and P815 cells were purchased from Shanghai Institutes for Biological Sciences, China. CHO and P815 cells were maintained at 37 °C in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS; Gibco-BRL, Carlsbad, CA, USA), 100 U/ml penicillin, and 100 mg/ml streptomycin. CHO cells were used for the transfection experiment and P815 cells were used as target cells.

2.2. Plasmid construction

The eukaryotic vector pcDNA3.1(+) with a strong eukaryotic promoter derived from human cytomegalovirus and T7 bacteriophage promoter was purchased from Invitrogen (Carlsbad, CA, USA) for plasmid construction. pJME containing JEV gene encoding prME protein [32] was developed and stored in our laboratory. The coding sequence of murine cellular ICAM-1 (1614 bp) [33] was amplified by nested RT-PCR from RNA isolated from stimulated mouse splenocytes. The reverse transcription primer was 5′-TGGCTGAGGGTAAATGCTGT-3′. The forward orientation primer used in the first PCR reaction was 5′-CCCTGCAATGGCTTCAACCC-3′ and the reverse orientation primer was 5′-TGGCTGAGGG-TAAATGCTGT-3′. The forward orientation primer used in the second PCR reaction was 5′-gaattcGGTGGAGGCGGTTCAGGTG-GAGGTGGTTCAGGAGGAGGTGGATCG ATGGCTTCAACCCGTGCCA-AG-3′, which contained an *Eco*RI site (lower case letters) and a Gly-linker consisting of 15 aa (underlined letters) at the 5′ end. The reverse orientation primer was 5′-gcggccgcTCAGGGAGG TGGGGCTTGT-3′, which contained a *Not*I site (lower case letters) at the 5′ end. A 1659 bp fragment of murine ICAM-1and Gly-linker was digested with restriction enzymes *Eco*RI and *Not*I from pMD19-T simple-ICAM-1 and subcloned into the pcDNA3.1(+) vector at the *Eco*RI/*Not*I site. The construct containing murine ICAM-1 gene was designated pICAM-1. To construct a DNA vaccine encoding fusion protein of prME and ICAM-1 (pJME/ICAM-1), a 2001 bp fragment of JEV prME isolated from pJME by *Bam*HI and *Eco*RI digestion was inserted into the *Bam*HI/*Eco*RI site of pICAM-1. Competent *Escherichia coli* DH5α cells were transformed with the above recombinant and plated overnight on Luria broth agar plates containing 100 μg/ml ampicillin (Sigma, St. Louis, MO, USA). Single colonies were picked and inoculated with 2 ml Luria broth liquid cultures containing 100 μg/ml ampicillin. pJME, pICAM-1, and pJME/ICAM-1 were purified with a Qiagen Plasmid Mega DNA (Qiagen GmbH, Hilden, Germany) kit using the endotoxin-free buffer system, and then dissolved in ddH_2O at a concentration of 1 mg/ml. To identify the recombinant, pJME/ICAM-1 and pICAM-1 were digested with the restriction enzymes *Bam*HI/*Not*I and *Eco*RI/*Not*I, respectively, and then sequenced by T7 promoter primer from pcDNA3.1(+). The endotoxin content from purified plasmid DNA was less than 5 EU/mg.

The expression of ICAM-1 and fusion protein prME/ICAM-1 were analyzed by transfection into CHO cells as previously described [34]. Cells were harvested after stable transfection and tested for expression by Western blot analysis with goat polyclonal anti-ICAM-1 (1:200; Santa Cruz Biotechnology) [34].

2.3. Mouse experiments

BALB/c mice (4 weeks old, female) were obtained from the Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences, and maintained in sterile cages under specific pathogen-free conditions. The mice were divided into five groups ($n = 8$ per group) as follows: pJME, pJME/ICAM-1, pJME + pICAM-1, positive control, and negative control. The quadricep muscle mass on the left hind leg of mice were injected with the following: 100 μg of pJME for the pJME group, 100 μg of pJME/ICAM-1 for the pJME/ICAM-1 group, and 100 μg of pJME with 100 μg of plasmid pICAM-1 for the pJME + pICAM-1 group. Three and five weeks after the primary injection, the mice in these three groups received two booster doses in the same muscle (containing the same amount of plasmid as in the primary dose). The positive control group comprised mice immunized with an inactivated vaccine, i.e., a formalin-inactivated mouse brain-derived JEV vaccine (Beijing-1 strain) obtained from the Liaoning Province Center of Disease Control and Prevention. Each mouse in this inactivated-vaccine group was also given an injection of 100 μl (1/5 of the recommended adult dose) of inactivated vaccine and boosted with the same dose 3 and 5 weeks after the first immunization. The negative control group comprised mice immunized with pcDNA3.1(+). The volume of the vaccine solution injected into each thigh was adjusted to 100 μl per mouse with phosphate-buffered saline (PBS). For cell immunity evaluation, spleens were collected from groups of four immunized mice 3 weeks after the final immunization. This study was conducted in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Shengjing Hospital of China Medical University.