Vaccine 28 (2010) 5533–5542

Contents lists available at ScienceDirect

Vaccine

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

J-LEAPS vaccines initiate murine Th1 responses by activating dendritic cells

P.R. Taylor[a], G.K. Koski[b], C.C. Paustian[b], E. Bailey[a], P.A. Cohen[c], F.B.-G. Moore[d], D.H. Zimmerman[e], K.S. Rosenthal[a,*]

[a] Northeastern Ohio Universities Colleges of Medicine and Pharmacy, Rootstown, OH, United States
[b] Cleveland Clinic Foundation, Cleveland, OH, United States
[c] Mayo Clinic, Phoenix, AZ, United States
[d] The University of Akron, Akron, OH, United States
[e] CEL-Sci Corp., Vienna, VA, United States

ARTICLE INFO

Article history:
Received 8 March 2010
Received in revised form 29 April 2010
Accepted 10 June 2010
Available online 25 June 2010

Keywords:
LEAPS
Vaccines
Dendritic cells
IL12
Th1
HSV
HIV
Adoptive transfer

ABSTRACT

The Ligand Epitope Antigen Presentation System (LEAPS) converts a peptide containing a T cell epitope as small as 8 amino acids into an immunogen and directs the nature of the subsequent response. Tandem synthesis of the J peptide (a peptide from the beta-2-microglobulin) with peptides of 15 or 30 amino acids from HSV-1 or HIV made them immunogenic and promoted Th1 immune responses. Immunization of A/J or C57BL/6 mice with J-LEAPS heteroconjugates containing an epitope from the HSV-1 glycoprotein D (JgD) or an epitope from the HIV gag protein (JH) emulsified with Seppic ISA51 induced increased levels of IL-12p70 by day 3 and increased levels of interferon gamma (IFN-gamma) on days 10 and 24. Interestingly, levels of IL-10, TNF-alpha, and IL-6 did not change. Neither the H nor the gD peptides alone elicited responses and only weak responses followed immunization with the J peptide. Bone marrow (BM) cells became CD86 and CD11c positive within 48 h of treatment with JgD or JH. JH or JgD treatment promoted IL-12p70 production and expression of CD8 denoting the maturation and activation of a subclass of myeloid DCs. Pure cultures of immature myeloid DCs also responded to JgD treatment, forming clusters, developing dendrites, and producing IL-12p70 within 24 h. The JH or JgD treated bone marrow cells (JgD-DC) were necessary and sufficient to activate splenic T cells to produce IFN-gamma and the JgD-DC provided an antigen specific booster response to T cells from JgD immunized mice. Adoptive transfer of JgD-DC was also sufficient to initiate protective antigen specific immunity from lethal challenge with HSV-1. The J-LEAPS vaccines appear to act as an adjuvant and immunogen on DC precursors in a unique manner to promote activation and maturation into IL-12p70 producing DCs which then can initiate sufficient Th1 immune responses to elicit protection without production of acute phase cytokines.

1. Introduction

LEAPS™ is an acronym for Ligand Epitope Antigen Presentation System that converts small peptides into immunogens and at the same time steers the resulting immune response to either a Th1 or Th2 type of response. For the LEAPS approach, the antigenic peptide is covalently attached to an immune cell binding ligand (ICBL) from a peptide of human beta-2-microglobulin (aa 38–50) (DLLKNGERIEKVE) [1], termed "J," [2–6], from a peptide corresponding to a CD4 binding site on the beta chain of the MHC class II molecule (aa 135–149 (NGQEEKAGVVSTGLI)), termed "G," [2,3–6], or other ICBL. The original hypothesis stated that the ICBL binds to T cells to activate and initiate an immune response [4]. An antibody response is not generated to the J or G peptides [4,5]. The J-ICBL is from an exposed region of the beta-2-microglobulin component of MHC I molecules as identified by a monoclonal antibody [1] and antibodies to this peptide block allogeneic T cell responses in a mixed lymphocyte reaction [7].

Heteroconjugate vaccines combining the J-ICBL and peptides from herpes simplex virus type 1 ICP27 protein (JH1) [8], glycoprotein B (JgB) [3], or glycoprotein D (JgD) [9] administered as oil in water adjuvant emulsions elicited DTH responses and protection from lethal viral challenge consistent with Th1 immune responses. These J-LEAPS vaccines induced immune protection and did not require antibody. Antibody ablation studies indicated that CD4 and CD8 cells were important for initiating immunity by JgD but CD4 cells and IFN-gamma were necessary for delivering protection following lethal HSV-1 challenge. Immunogens consisting of a relevant antigen attached to the J-LEAPS ICBL also have the potential to provide immunotherapy by immunomodulating an

* Corresponding author at: Dept. of Integrative Medical Sciences, NEOUCOM, 4209 SR 44, Box 95, Rootstown, OH 44272, United States. Tel.: +1 330 325 6134; fax: +1 330 325 5914.
E-mail address: ksr@neoucom.edu (K.S. Rosenthal).

0264-410X/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.vaccine.2010.06.043

ongoing autoimmune disease, as shown for experimental autoimmune myocarditis and rheumatoid arthritis [10,11].

Immunoglobulin subtype analysis [5] and induction of protection from HSV challenge indicated that J-LEAPS vaccines activate immune responses and also direct the nature of immunity to a Th1 response. In this study we asked the following questions: do J-LEAPS vaccines activate Th1 related cytokine responses? What cell does the J-LEAPS vaccine interact with, a dendritic cell (DC) or a T cell? Is JgD treatment of DC precursors sufficient to induce an effective immune response (e.g. protection from viral challenge)? We show for JgD and JH (JH incorporates a peptide from the HIV gag protein [12]) that these immunogens interact with precursors of DCs obtained from bone marrow and the immunogens are sufficient to promote maturation of the cells into IL12 expressing DCs without induction of acute phase cytokines. The JgD or JH activated DCs (JgD-DC, JH-DC) can promote interferon gamma production from splenic T cells while JgD-DC are capable of promoting an antigen specific boost in IFN-gamma production from splenic T cells obtained from immunized mice. Most significantly, adoptive transfer of the JgD-DC is sufficient to initiate protective responses against lethal challenge from HSV-1 strain 129 infection. These studies demonstrate that J-LEAPS immunogens act as both an adjuvant and an immunogen to generate antigen carrying DCs that can promote and steer immunity towards a specific Th1 response and that this mechanism is responsible for inducing protection from HSV-1 infection upon immunization with JgD.

2. Materials and methods

2.1. Mice

For immunization studies, female A/J or C57BL/6 mice (Charles River, Wilmington, MA) were immunized, serum was obtained and pooled for analysis by cytokine protein array. Female C57BL/6 mice were used (A) to prepare bone marrow cells (Jackson Laboratories, Bar Harbor, ME) and (B) for generating pure DC cultures (Biological Testing Branch, Frederick Cancer Research and Development, National Cancer Institute, Frederick, MD). All animals were treated in accordance with Institutional Animal Care and Use Committee (IACUC) approved policies and procedures.

2.2. Peptide

The JgD and JH heteroconjugate peptide vaccines consist of an immune cell binding ligand, "J", ((DLLKNGERIEKVE), amino acid 38–50 from the beta-2-microglobulin) conjugated to a peptide from the N-terminus of HSV-1 glycoprotein D (SLKMADPNRFRGKDLP (amino acid 8–23)) or the HGP-30 (H) peptide from the p17 HIV gag protein (YSVHQRIDVKDTKEALEKIEEEQNKSKKKA (aa 85–115)) through a triglycine linker [6]. The vaccine peptides were synthesized by UCB (Atlanta, GA) and supplied by Cel-Sci (Vienna, VA).

2.3. Immunization

The peptides were dissolved in Hanks Balanced Salt Solution (HBSS) to produce a stock solution with a concentration of 2 mM adjusted to neutral pH. Each of the vaccine solutions was tested by a Limulus Amoebocyte Lysate assay as per manufacturer's instructions (Cambrex Biosciences Walkersville, MD) and shown to be endotoxin free. The vaccine peptide was administered to mice as a 1:1 (vol.) emulsion in Seppic ISA-51 (Seppic, Fairfield, NJ) as has been done in previous studies [9]. A/J or C57BL/6 female mice were immunized once with the JgD, JH, J, H, or gD peptides subcutaneously with two 50 μl injections of a 2 mM solution in the scruff of the neck and in the abdomen. The control mice were injected with HBSS in Seppic ISA-51 adjuvant.

2.4. Cytokine protein array following immunization

Serum collected from three mice were pooled on days 3, 10, and 24 after immunizations and analyzed for 21 different cytokine and chemokine proteins using RayBio® Mouse Cytokine Antibody I array membranes as per manufacturer instructions (RayBiotech, Inc., Norcross, GA). The following treatment groups were included in the analysis: (1) only adjuvant as a control group; (2) JgD in adjuvant; (3) JH in adjuvant; (4) J in adjuvant, (5) gD in adjuvant or (6) H in adjuvant. The serum taken at each bleed was pooled such that it represents the weighted response of three animals. Presence of cytokine was detected by chemiluminescence of the membranes and the duplicate spots on film for each cytokine were analyzed by densitometry (Total Lab Array Analysis, Nonlinear Dynamics). Densitometric results were standardized for each membrane by dividing the measured value of each spot by the average values for VEGF (which should not be influenced by the treatments).

Statistical sampling was designed to maximize discovery of trends within the cytokine array results. On each membrane, 2 spots (samples) for each cytokine were measured. The replicate spots were treated as a nested source of variance rather than as replicates in the analysis to avoid pseudo-replication. Post hoc sets for significance were performed using 2-way nested ANOVAs (SAS software system; SAS Institute, Carey, NC) with treatment and day as main factors. Replicate spots were not a significant source of variation. A third factor in the comparison of JgD values to adjuvant control, the strain of mouse, was not a significant source of variation for any of the 21 cytokines. A shared hypothesis (that there would be a response to a treatment) sequential Bonferroni adjustment was performed to allow for multiple comparisons. Critical alpha levels are adjusted to allow for the cumulative probability of type 1 error by this method [13]. The data presented in Fig. 1B includes both the uncorrected p-values for differences between peptide and adjuvant treated mice over the 24-day period (values for days 3, 10 and 24) and indication (bold box) of statistical significance after the Bonferroni adjustment.

2.5. Preparation of bone marrow (BM) cells

Bone marrow (BM) cells were prepared as previously described [14,15]. BM cells were suspended in tissue culture medium (TCM) (RPMI 1640 with glutamine plus 100 mg/nl PenStrep, 50 μM 2-mercaptoethanol, and 5% fetal calf serum) at approximately 5×10^6 cells/ml and incubated for 1 h at 37 °C in a 5% CO_2 atmosphere in plastic tissue culture flasks to remove adherent, mature macrophages. Decanted non-adherent cells were resuspended in TCM and 1.5×10^6 BM cells in 1 ml were placed into each well of a 24-well tissue culture plate (Falcon) and either left untreated or treated with 14.5 μmol of J, gD, JgD or JH vaccines. After incubation for 48 h at 37 °C, cells were viewed and photographed for changes in morphology, tissue culture supernatants were removed and the cells were prepared for flow cytometric analysis.

2.6. Generation of immature mouse dendritic cells

Immature DCs were generated from the bone marrow of five normal C57BL/6 female mice as previously described [16]. BM cells were harvested as before and cultured at 5×10^5/ml in 75 cm^2 flasks at 37 °C, 10% CO_2 for 6 days in a complete medium (CM) containing RPMI 1640, 10% fetal bovine serum, 2 mM glutamine, 0.1 mM nonessential amino acids, 100 units/ml sodium pyruvate,