Veterinary Immunology and Immunopathology xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

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

Research paper

Expanding specificity of class I restricted CD8$^+$ T cells for viral epitopes following multiple inoculations of swine with a human adenovirus vectored foot-and-mouth disease virus (FMDV) vaccine

Lasse E. Pedersen[a,b], Jared R. Patch[a,c], Mary Kenney[a], Raisa A. Glabman[a,c], Morten Nielsen[b,d], Gregers Jungersen[b], Soren Buus[e], William T. Golde[a,*]

[a] Plum Island Animal Disease Center, Agricultural Research Service, USDA, Greenport, NY, USA
[b] National Veterinary Institute and Centre for Biological Sequence Analysis, Technical University of Denmark, Lyngby, Denmark
[c] Department of Animal and Veterinary Sciences, University of Vermont, Burlington, VT, USA
[d] Instituto de Investigaciones Biotecnológicas, Universidad Nacional de San Martín, San Martín, Buenos Aires, Argentina
[e] University of Copenhagen, Copenhagen, Denmark

ARTICLE INFO

Article history:
Received 12 February 2016
Received in revised form 18 July 2016
Accepted 25 July 2016

Keywords:
Cytotoxic T lymphocytes
MHC
SLA
Class I
MHC tetramers
T cell epitopes

ABSTRACT

The immune response to the highly acute foot-and-mouth disease virus (FMDV) is routinely reported as a measure of serum antibody. However, a critical effector function of immune responses combating viral infection of mammals is the cytotoxic T lymphocyte (CTL) response mediated by virus specific CD8 expressing T cells. This immune mechanism arrests viral spread by killing virus infected cells before new, mature virus can develop. We have previously shown that infection of swine by FMDV results in a measurable CTL response and have correlated CTL killing of virus-infected cells with specific class I major histocompatibility complex (MHC) tetramer staining. We also showed that a modified replication defective human adenovirus 5 vector expressing the FMDV structural proteins (Ad5-FMDV-T vaccine) targets the induction of a CD8$^+$ CTL response with a minimal humoral response. In this report, we show that the specificity of the CD8$^+$ T cell response to Ad5-FMDV-T varies between cohorts of genetically identical animals. Further, we demonstrate epitope specificity of CD8$^+$ T cells expands following multiple immunizations with this vaccine.

1. Introduction

Foot-and-mouth disease is a highly contagious viral infection of livestock causing significant loss of meat and milk productivity and restriction of trade of livestock in endemic countries. Many strains of the virus cause myocarditis and death, particularly in young animals. Control and eradication of this disease are high priority to enable smallholder farmers in developing countries to have a reliable source of protein for consumption as well as supplemental income. In disease free countries, such progressive control reduces the risk of accidental or purposeful introduction of FMDV into the naïve herds and the economic impact of such an event (Grubman and Baxt, 2004).

Present killed virus vaccines have limited value in eradication efforts due to narrow protection across the many clades of viral strains and minimal duration of immunity. The recently licensed virus vectored vaccine for FMDV utilizes replication defective human adenovirus 5 to deliver an "empty capsid" (Moraes et al., 2002 and Grumban et al., 2010). This recombinant vaccine suffers the same performance shortfalls as the widely used killed virus vaccine. However, this vaccine platform presents an intriguing opportunity for addressing these performance deficits.

We have developed bioinformatic algorithms for the human leukocyte antigens (HLA) (*NetMHCpan*) to predict peptides within any pathogen proteome that hold the potential to be T cell epitopes within a given individual (Nielsen et al., 2007; Nielsen et al., 2008; Zhang et al., 2009; Lundegaard et al., 2011). This is based on the HLA molecules of that individual to bind pathogen derived peptides and "present" those to T cells. We have now applied this technology to the homologues of these proteins in both swine (swine leukocyte antigens; SLA) (Pedersen et al., 2011, 2013) and cattle (bovine leukocyte antigens; BoLA) (Hansen et al., 2014; Pandya et al., 2015; Svitek et al., 2015). Analysis of the proteome corresponding to structural proteins (capsid) of FMDV, strain A24,

* Corresponding author.
E-mail address: wtgolde@aol.com (W.T. Golde).

http://dx.doi.org/10.1016/j.vetimm.2016.07.012
0165-2427/

identified 60 potential T cell epitopes for CD8+, cytotoexic T lymphocytes (CTL) predicted to be bound by two swine class I SLA proteins. Biochemical analysis determined that 20 of the 60 potential T cell epitope peptides were efficiently bound by the swine SLA proteins (Pedersen et al., 2011, 2013).

We altered the adenovirus vectored FMDV vaccine to target CTL responses by eliminating the 3C protease, the enzyme that processes the primary polypeptide product of the FMDV structural genes into the final protein components of the capsid (Patch et al., 2011). This new construct was termed Ad5-FMDV-T, as the unprocessed polypeptide was proposed to be targeted to the proteasome for digestion into peptides. Because this vector has the processing protease mutated, more than 95% of the P1 precusor protein remains unprocessed and this becomes a substrate for ubiquitination and degradation by the proteosome. The result of this is the generation of FMDV P1 peptides that can be bound by newly synthesized class I MHC (SLA-1, SLA-2 and SLA-3) proteins. Once the trimolecular complex of SLA alpha chain, beta-2 microglobulin (β_2m) and the peptide forms, the protein can be transported via the golgi apparatus and expressed on the cell surface. This cell now can induce a response from FMDV specific CD8+ T cells, and with other signals from primarily antigen presenting cells, expand that FMDV specific, CD8+ T cell population (Yewdell and Haeryfar, 2005; Rock et al., 2010).

Following vaccination of genetically defined pigs (NIH mini) with the Ad5-FMDV-T vaccine, we have previously documented MHC restricted, antigen specific CTL killing of virus infected target cells specific for FMDV (Patch et al., 2011). These trials showed limited detection of T cell specificity using class I MHC tetramers, usually 2 or 3 of the twenty identified potential T cell epitope specificities. We further showed that animals infected with FMDV developed a clear and measurable CTL response, confirming the results generated using the altered Ad5-FMDV-T vaccine construct (Patch et al., 2013).

Here, we present further analysis of the fine specificity of reacting T cells from MHC homozygous pigs using MHC tetramers to characterize T cell specificities at a single cell level. The methodology was refined during these studies, leading to more sensitive and efficient detection of T cells. Results show that with each boost, the antigen specificity of T cells at this level of detection, expanded. Further, the specificities observed in the different trials were different, even though the genetics of the responding animals was identical in all trials and the same lot of vaccine was used throughout these studies. These differences may indicate immunological history can influence specificities upon exposure to new antigens. This data supports the concept that targeting T cell responses, at least under a protocol of repeated vaccination, results in the antigen reactivity broadening within population of responding T cells over the course of vaccine application.

2. Materials and methods

2.1. Animals

National Institute of Health miniature (NIH mini) pigs, homozygous for MHC haplotype 4a.0/4a.0 (*0401) (previously d/d) were provided by Scott Arn and David Sachs (Transplantation Biology Research Center, Massachusetts General Hospital, Boston, MA). Animals ranged in age between 10 and 14 months, and weighed approximately 100 lbs. Experiments were performed in a secure biosafety level three laboratory on Plum Island Animal Disease Center (PIADC) with approval by the Institutional Animal Care and Use Committee, and according to guidelines of the United States Public Health Service, Department of Health and Human Services and the Animal Plant Health Inspection Service (APHIS) of the USDA.

2.2. Vaccinations

Animals described as vaccinated, boosted and then challenged with FMDV (shown in Fig. 1) were vaccinated as previously reported (Patch et al., 2013). Briefly, animals in this cohort (Cohort 1) were vaccinated with Ad5-FMDV-T at 5×10^9 per animal, intradermally with the DermaVac, needle free vaccination device. These animals were boosted 12 weeks after vaccination. Eight days following the boost, animals were challenged with 10^5 $TCID_{50}$ of FMDV, strain A24 Cruzeiro, by intradermal needle inoculation in the heel bulb (Patch et al., 2013).

In the subsequent experiments (Cohorts 2 and 3), all animals were vaccinated with Ad5-FMDV-B, "empty capsid" vaccine at 2×10^9 PFU per animal and Ad5-FMDV-T at 5×10^9 per animal, intradermally with the DermaVac, needle free vaccination device (Patch et al., 2013). Boost vaccinations were done identically as the first vaccination at the times indicated for each experiment. When to do boost vaccinations was determined by the antibody titer to Adenovirus. These titers needed to return to low levels so that circulating anti-adenovirus antibody did not interfere with boost vaccinations. This timing varied between experiments.

2.3. Preparation of peripheral blood mononuclear cells (PBMC)

Porcine blood was collected in Vacutainer® tubes containing heparin, then diluted with PBS and underlain with Lymphoprep (Axis-Shield, Oslo, Norway). After centrifugation at 1400*g* for 20 min at room temperature, the band of PBMC was removed and washed three times with PBS. Cells were finally suspended in Roswell Park Memorial Institute (RPMI)-1640 medium (Invitrogen, Carlsbad, CA) containing 10% FBS and antibiotics/antimycotics.

2.4. CTL killing assay

Measurement of CTL cytolytic activity was carried out according to previously published procedures (Patch et al., 2011, 2013). PBMC were cultured in the presence of the porcine kidney cell line, PK(15) cells infected with Ad5-FMDV-T for 3 days. PK(15) cells are class I SLA homozygous and match the SLA-1 haplotype of NIH d/d mini pigs. Following this stimulation, PBMC were gradient purified with Lymphoprep as described above, and then CD6+ cells were positively selected by incubating cells with anti-porcine CD6 antibody (clone MIL8, AbD Serotec, Raleigh, NC) followed by goat anti-mouse antibody coated microbeads, and then passaged through an LS column using the MidiMACS™ magnetic separator (Miltenyi Biotec, Gladbach, Germany). Purified CD6+ cells were incubated for 4 h with target cells, PK(15) stably transfected with green fluorescence protein (PK(15)-EGFP cells) that had been infected overnight with Ad5-FMDV-T, Ad5-VSV-G, or mock infected. Effector and target cells were plated in E:T ratios of 50:1, 25:1, and 12:1, and incubation took place in the presence of 7-amino-actinomycin D (7-AAD) (BD Biosciences, San Jose, CA). Following incubation, percent killing was determined using flow cytometry by gating on EGFP+ cells and then measuring the percent 7-AAD+ cells. Flow cytometry was done using a FACS-Calibur flow cytometer with a high-throughput sampler (HTS) (BD Biosciences, San Jose, CA). Background lysis of target cells alone was subtracted from all data, and then for each time point and animal, the greatest mean lysis of control target cells (mock infected or Ad5-VSV-G infected) was subtracted from mean lysis of the antigen-specific target cells.

2.5. Tetramer construction and staining

Peptides and recombinant SLA-1*0401 and human beta-2-microglobulin ($h\beta_2m$) were produced and complexed into tetramers as described previously (Leisner et al., 2008; Pedersen

Please cite this article in press as: Pedersen, L.E., et al., Expanding specificity of class I restricted CD8+ T cells for viral epitopes following multiple inoculations of swine with a human adenovirus vectored foot-and-mouth disease virus (FMDV) vaccine. Vet. Immunol. Immunopathol. (2016), http://dx.doi.org/10.1016/j.vetimm.2016.07.012