Cellular Immunology 266 (2011) 130–134

Contents lists available at ScienceDirect

Cellular Immunology

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

Differential requirements for CD80/86–CD28 costimulation in primary and memory CD4 T cell responses to vaccinia virus

Shinichiro Fuse [1,2], Ching-Yi Tsai [2], Leah M. Rommereim, Weijun Zhang, Edward J. Usherwood *

Department of Microbiology and Immunology, Dartmouth Medical School, Lebanon, NH 03756, United States

ARTICLE INFO

Article history:
Received 30 July 2010
Accepted 28 September 2010
Available online 30 October 2010

Keywords:
Vaccinia virus
T cell memory
CD4 T cell
B7
CD80
CD86
CD28
Secondary response
Recall response
Smallpox

ABSTRACT

Vaccinia virus infection can confer immunity to smallpox by inducing potent T cell and antibody responses. While the CD8 T cell response to vaccinia virus has been well characterized, less is known about factors required for priming and memory for the CD4 T cells. Focusing on two recently described epitopes, we show that after intranasal infection, both I1L and L4R epitopes are co-dominant during the acute response, but the I1L epitope dominates during memory. CD4 T cell priming was intact in the absence of CD80/86, however secondary responses were reduced. This contrasts with our previous data showing CD80/86–CD28 interaction is required for optimal primary and memory CD8 T cell responses. The absence of CD80/86 also changed the immunodominance hierarchy during memory, with the I1L and L4R responses becoming co-dominant in knockout mice. These data highlight different costimulatory requirements for primary CD4 and CD8 T cell responses to vaccinia virus.

1. Introduction

Vaccinia virus (VAVC) is a double-stranded DNA virus belonging to the *Poxviridae* family and shares high similarities to other orthopoxviruses such as variola virus, the causative agent of smallpox. Vaccinia virus infection induces potent cellular and humoral responses [1–3], and intradermal inoculation with this virus provides protective immunity against smallpox infection. While the CD8 T cell response to this virus has been studied extensively, less is known regarding the CD4 T cell response. CD4 T cells are crucial for protection from primary vaccinia virus infection, largely by providing help for the antibody response [4]. Recently several CD4 T cell epitopes on the virus were mapped, mostly on structural proteins, and these are highly correlated with antigens recognized by the antibody response [5]. The pattern of responses to these epitopes during infection has not been reported in detail, nor have the requirements for effective primary and secondary responses to these epitopes. Understanding the secondary response is particularly critical for vaccine responsiveness, as rapid T cell expansion promotes containment of virus replication in a timely manner.

CD80 and CD86, the most well-studied costimulatory molecules, are expressed on antigen-presenting cells (APCs), upregulated upon cell activation, and bind to CD28 on the T cell, delivering a crucial signal for T-cell activation together with the T cell receptor [6]. CD28 signaling is mediated through the phosphatidylinositol 3-kinase-protein kinase B (Akt) and growth factor-receptor-bound protein 2 (Grb2) pathways, enhancing the production of interleukin-2 and other cytokines, upregulating anti-apoptotic molecules (such as *Bcl-x_L*), promoting energy metabolism (glucose uptake and rate of glycolysis), and facilitating cell-cycle progression [7–9]. Once T cells are activated, cytotoxic T-lymphocyte antigen 4 (CTLA-4), another receptor for CD80/CD86, is upregulated. CTLA-4 negatively regulates T cell responses by several mechanisms: sequestering CD80/CD86 because of the higher affinity for CTLA-4 compared with CD28, recruiting phosphatases such as Src homology region 2 domain-containing phosphatase 1 (SHP-1), to dephosphorylate downstream signals, and transducing signals into the APCs to induce indoleamine 2,3-dioxygenase and the catabolism of tryptophan, resulting an inhibitory environment for the T cell [10–12].

The effects of CD80/CD86 costimulation on T cell responses depends on the strength of the TCR signal, and whether the cell is a naïve, activated or memory T cell [13]. T cell responses to some viruses, such as LCMV, do not require costimulation through this pathway [14,15], whereas with other viruses, such as influenza or VSV, the response is impaired in the absence of CD28 signaling

* Corresponding author. Address: 608E Borwell Building, 1 Medical Center Drive, Lebanon, NH 03756, United States. Fax: +1 603 650 6223.
E-mail address: EJU@Dartmouth.edu (E.J. Usherwood).
[1] Present address: Clarion Healthcare Consulting LLC, 99 Summer Street, Suite 520, Boston, MA 02110, United States.
[2] These authors contributed equally to this work.

0008-8749/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cellimm.2010.09.008

[16,17]. The dogma has been that CD80/86–CD28 interactions are needed to initiate a response from naïve T cells, however this is not necessary for activating memory T cells. Work by our lab and others in recent years, focusing on the CD8 T cell response, has shown that, while CD80/86 is not necessary for a secondary T cell response, the magnitude of the response is significantly reduced without this costimulatory signal [18–21]. However CD80/86 is always necessary for a high-avidity neutralizing antibody response, including in the vaccinia virus system (EJU, unpublished data). Given the virus-specific nature of costimulatory requirements for the T cell response, and the importance of vaccinia virus as a vaccine, we wished to determine whether CD80/86 was necessary for efficient primary and secondary CD4 T cell responses to vaccinia virus.

Here we show that after intranasal infection with VACV, responses to the two immunodominant epitopes for CD4 T cells were initially of similar magnitude. One month after infection the I1L-specific response was dominant over the L4R-specific response. The magnitude of neither response was affected by the absence of CD80/86, although the response to secondary infection was sub-optimal. These data provide a kinetic profile of the CD4 T cell response to VACV, and highlight the key role for CD80/86–CD28 interactions in memory but not primary CD4 T cell immunity in this infection.

2. Materials and methods

2.1. Mice and virus

The Western Reserve strain of vaccinia virus (VV-WR) was originally obtained from Dr. William R. Green (Dartmouth Medical School, Lebanon, NH). C57BL/6 mice were purchased from The National Cancer Institute (Bethesda, MD). CD80/CD86$^{-/-}$ mice on the C57BL/6 background were bred in the Dartmouth–Hitchcock Medical Center mouse facility. Mice were infected with 10^3 PFU of VV-WR intranasally under anesthesia with 2,2,2-tribromoethanol. At day 35 after infection, mice were rechallenged intranasally with 6×10^5 PFU of VV-WR. All animal experiments were approved by the Animal Care and Use Program of Dartmouth College.

2.2. Tissue preparation

For the primary response, lungs and spleen were taken at day 10, 14, 22 and 29 after infection. For the recall responses, lungs and spleens were taken at day 8 post re-challenge. Single-cell suspensions of spleen and lung lymphocytes were prepared as described previously [22]. Briefly, spleens were prepared by passing through cell strainers. Lungs were injected with 2 ml of minimal essential medium containing 417.5 μg/ml Liberase CI and 200 μg/ml DNase I (both obtained from Roche, Indianapolis, IN), minced with scissors, and then incubated for 30 min at 37 °C and passed through cell strainers. Suspensions were resuspended in 80% isotonic Percoll and subsequently overlaid with 40% isotonic Percoll. Samples were then centrifuged at 400*g* for 25 min at 4 °C, and the cells at the 80%/40% interface were collected, washed, and counted.

2.3. Antibody staining and flow cytometric analysis

Cells were stained with APC-conjugated anti-CD4 (RM4-5; Biolegend). Stained samples were analyzed using a FACS Calibur flow cytometer and CellQuest software (BD Immunocytometry Systems).

2.4. IFN-γ ELISPOT assay

The number of IFN-γ secreting cells was determined after stimulation with peptides in an ELISPOT assay. Epitopes derived from I1L (7–21, QLVFNSISARALKAY) and L4R (176–190, ISKYAGINILNVYSP) proteins were synthesized as peptides and used in this study. Peptides were purchased from New England peptide. In brief, 96-well Multiscreen HTS nitrocellulose plates (Millipore) were coated overnight at 4 °C with 100 μl per well of rat anti-mouse IFN-γ antibody (R4–6A2; BD Pharmingen), at a concentration of 2 μg/ml. The plates were then washed and blocked before the addition of irradiated (3000RAD) normal C57BL/6 spleen cells (5×10^5 cells/well), a graded number of responder spleen cells, 2 μg/ml of each peptide and 10 U/ml recombinant human IL-2 (Tecin, National Cancer Institute). Plates were then incubated for 24 h at 37 °C and developed for 2 h with a biotinylated rat anti-mouse IFN-γ antibody (XMG1.2; BD Pharmingen) at a concentration of 2 μg/ml, followed by streptavidin–alkaline phosphatase (Biolegend) at a 1/500 dilution for 1 h at room temperature. Following addition of the chromogenic substrate 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium (BCIP/NBT; Sigma–Aldrich), visible spots were enumerated using a dissecting microscope. The frequency of CD4 T cells producing IFN-γ was calculated, together with the total number per spleen or set of lungs.

2.5. Statistical analysis

Student's *t*-test was used to compare experimental groups. A *P* value of <0.05 was considered significant.

3. Results

3.1. Quantification of lymphocyte and CD4 T cell populations in spleen and lung

Previous studies identified 14 VAVC-specific CD4 T cell epitopes in C57BL/6 mice. Two epitopes that induced the strongest responses were derived from the I1L and L4R proteins, which are both structural proteins with core-DNA binding activity [5,23]. We wished to profile the kinetics of responses to these two epitopes following intranasal infection with VAVC, and measure the effect of CD80/CD86–CD28 costimulation on the response using CD80/86-deficient mice. The intranasal route was chosen as it approximates the natural route of infection for smallpox virus. Our previous studies in mice lacking the CD28/CD80/86 pathway infected by VACV by the i.n. route show this pathway does not affect the clearance of the virus [20]. In the lungs at early times post-infection there was no difference in either the number of lymphocytes in the lung (Fig. 1A) or the number of CD4 T cells (Fig. 1B), however at later times there were larger numbers of cells in the wild-type animals. A similar pattern was observed in the spleen, with significantly larger cell numbers present in wild-type animals at later timepoints, although in this organ there was an earlier divergence in the size of the response between the two mouse strains (Fig. 1A and B).

3.2. Primary CD4 T cell responses to VACV epitopes

Next we analyzed the epitope-specific CD4 T cell response to VACV using IFN-γ ELISPOT analysis. We used the L4R and I1L epitopes in these studies, as these were shown to elicit the strongest responses among the MHC class II-restricted epitopes identified [5,23]. A strong CD4 T cell response was detectable in the lungs (Fig. 2) and spleen (Fig. 3) at 10 days post-infection, which slowly declined to a lower, but readily detectable level by day 29 days post-infection. The absence of CD80/86 did not reduce the magnitude of the response, in fact in some cases the response was higher in the knockout animals (Fig. 2A and B, Fig. 3A and B). Interestingly, the absence of CD80/86 altered the relative strength of the