Biochemical and Biophysical Research Communications 424 (2012) 635–640

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Repetitive peptide boosting progressively enhances functional memory CTLs

Kendra Smyth [a], Karla Garcia [a], Zhifeng Sun [a], Wenbin Tuo [b], Zhengguo Xiao [a,*]

[a] Department of Animal and Avian Sciences, University of Maryland, College Park, MD 20742, USA
[b] Animal Parasitic Diseases Laboratory, USDA/ARS, Beltsville, MD 20705, USA

ARTICLE INFO

Article history:
Received 2 July 2012
Available online 15 July 2012

Keywords:
T cells
Cytotoxicity
Peptide
Memory
Adjuvant
Boosting

ABSTRACT

Cytotoxic T lymphocytes (CTLs) play a critical role in controlling intracellular pathogens and cancer cells, and induction of memory CTLs holds promise for developing effective vaccines against critical virus infections. However, generating memory CTLs remains a major challenge for conventional vector-based, prime-boost vaccinations. Thus, it is imperative that we explore nonconventional alternatives, such as boosting without vectors. We show here that repetitive intravenous boosting with peptide and adjuvant generates memory CD8 T cells of sufficient quality and quantity to protect against infection in mice. The resulting memory CTLs possess a unique and long-lasting effector memory phenotype, characterized by decreased interferon-γ but increased granzyme B production. These results are observed in both transgenic and endogenous models. Overall, our findings have important implications for future vaccine development, as they suggest that intravenous peptide boosting with adjuvant following priming can induce long-term functional memory CTLs.

1. Introduction

Immunological memory is the cardinal feature of adaptive immunity. This intrinsic characteristic of the immune system grants long-lasting and effective protection from reinfection and is the foundation for vaccination, which is the most effective tool for combating or even eradicating infectious disease. CD8 T cells, or cytotoxic T lymphocytes (CTLs) play an important role in controlling virus infections [1], and memory CTLs possess unique functional properties which make them an essential defense against repeat infection by the same or a similar pathogen. Appropriately, a major goal of vaccination is to generate memory CD8 T cells of sufficient quality and quantity to protect against infection. Yet, induction of functional memory CTLs remains a major challenge for conventional vector-based vaccination strategies [1–3], and to date no vector-based vaccines have been licensed for human use in the United States [4]. Priming with a live vector is superior to priming with a killed or subunit vaccine, in that a live vector mimics natural infection by stimulating both the innate and acquired immune systems to achieve the optimal orchestrated immune response [5,6]. However, even after priming with a live vector, a CTL response is normally not induced to a protective level, and subsequent boosting is required to generate a sufficient level of functional CTLs [7–9].

Repeated vaccination, or boosting, using the same viral or bacterial vector is one way to establish strong humoral, but not cellular, immunity to specific pathogens [7]. In this case, the pre-existing immunity to the vector accelerates its clearance after secondary exposure, limiting the immune response by impairing antigen presentation and the production of inflammatory cytokines [7]. On the other hand, prime-boosting with different vectors is effective at generating memory CTLs [7]. This strategy involves priming the immune system to an antigen expressed by one vector followed by boosting with a second vector containing the same antigen. This circumvents the issue of inducing strong immunity against the vectors themselves and focuses the immune system on the common antigen [7,9,10]. However, prime-boosting with different vectors generates memory CTLs with potentially impaired functions. For example, immune senescence has been observed in memory CTLs after multiple boosts [9,11]. KLRG1 expression, which is indicative of short-lived effectors [12], increases in secondary and tertiary memory [9], and IL-7Rα, which is related to memory CTL survival [13], is down regulated in secondary memory CTLs [8]. These studies also suggest that the memory phenotype may vary when different vectors are used in boosting [8,9,14,15] and stress the need to characterize function directly. Despite these challenges, the biggest obstacle to future applications of the vector-based prime-boost strategy is limited availability of appropriate vectors, especially when multiple boosts are needed to elicit protective immunity [10,16]. Although vectors are required for optimal priming in vaccination, a new strategy for effective and repeatable boosting without vectors is urgently needed.

One promising strategy is the use of peptide vaccines instead of vector-based vaccines to generate memory CTLs. A peptide vaccine allows for focused induction of peptide-specific CTLs. However,

* Corresponding author. Fax: +1 301 405 7980.
E-mail address: xiao0028@umd.edu (Z. Xiao).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.07.018

peptides are poorly immunogenic and, by themselves, induce immune tolerance or deletion of peripheral CTLs [17,18]. When adjuvant is co-administered with peptide in a single subcutaneous immunization, low levels of memory CTLs are generated and the resulting immunity is relatively weak [19–22]. It is feasible that repeated peptide vaccination with adjuvant could be used to progressively enhance this memory. Therefore, the goal of this study was to determine if vaccination with peptide and adjuvant is an effective repeatable boosting method – generating memory CTLs of sufficient quantity and quality to protect against infection.

2. Materials and methods

2.1. Mice reagents and immunization

OT-I mice, gifts from MF Mescher, University of Minnesota, MN, have a transgenic TCR specific for H-2K^b and OVA$_{257–264}$ [23]. Mice were maintained under specific pathogen-free conditions at the University of Maryland, and these studies have been reviewed and approved by the Institutional Animal Care and Use Committee (protocol ID R-09–22). C57BL/6 mice were purchased from the National Cancer Institute. All conjugated fluorescent Abs were purchased from BD Biosciences (San Diego, CA), eBioscience (San Diego, CA) or Biolegend (San Diego, CA). Lipopolysacharide (LPS) was purchased from Invivogen (San Diego, CA), and used at 50 ug per mouse. SIINFEKL peptide was purchased from New England peptide (Gardner, MA), and was used at 50 ug per mouse. Boosting was performed through i.v. tail injection in a total volume of 300 µl in DPBS. Tetramer was a gift from Dr. Jameson in University of Minnesota.

2.2. Viruses and bacteria

Recombinant VV-GFP-JAW-OVA (VV-OVA) expressing the OVA$_{257–264}$ epitope and recombinant LM expressing full-length secreted OVA (LM-OVA) were gifts from SC Jameson, University of Minnesota, MN, the same as we described before [23,24]. Mice were infected i.p. with VV-OVA at 5×10^6 PFUs and LM-OVA at 10^4 CFU/mouse i.v. for primary infection, and $5x10^5$ CFU/mouse i.v. for test of protection.

2.3. Naive T cell purification

Inguinal, axillary, brachial, cervical, and mesenteric lymph nodes (LN) were harvested from wild-type (WT) OT-I mice, pooled, and disrupted to obtain a single-cell suspension. CD8$^+$CD44^{low} cells were enriched by negative selection using MACS magnetic cell sorting (Miltenyi Biotec, Auburn CA) as we described before [23,24].

2.4. Adoptive transfer and flow cytometric analysis

Purified naive OT1 cells were adoptively transferred into normal C57BL/6NCr mice by i.v. tail injection at the numbers indicated for each experiment. One day after transfer, mice were infected. Single-cell suspensions were prepared, viable cell counts were done (trypan blue). Since OT-1 T cells possess the congenic marker Thy1.1, we can identify them in a B6 host by flow cytometry staining with anti-Thy1.1 and CD8, as described before [23,24]. Memory CTLs are formed at the end of the contraction phase[1], and the memory population remains stable after a few weeks following acute virus infection or immunization [25]. As is common practice in immunology [26], we use one month as a cutoff point to examine memory CTLs as we reported previously [23,27,28]. Analysis was performed using a FACSCalibur flow cytometer and CellQuest software (BD Biosciences) to determine the percentage of total OT-I cells in the samples.

2.5. Tissue harvest and digestion

Mice were euthanized by CO_2 and peripheral lymph nodes and spleens were directly picked up and homogenized using 15 ml glass grinders. Lungs were perfused using 1× PBS at about 30 ml per mouse, cut into small pieces (1 mm^3), homogenized with a 10 ml pipette and resuspended in 4 ml Collagenase D (Roche, Indianapolis, IN). For complete digestion, lung tissues were kept in a water bath (37 °C) for 25 min. Digestion was stopped by the addition of 0.1 M EDTA, and digested tissues were homogenized using glass grinders. Bone marrow was harvested by flushing cut bones with 1× PBS.

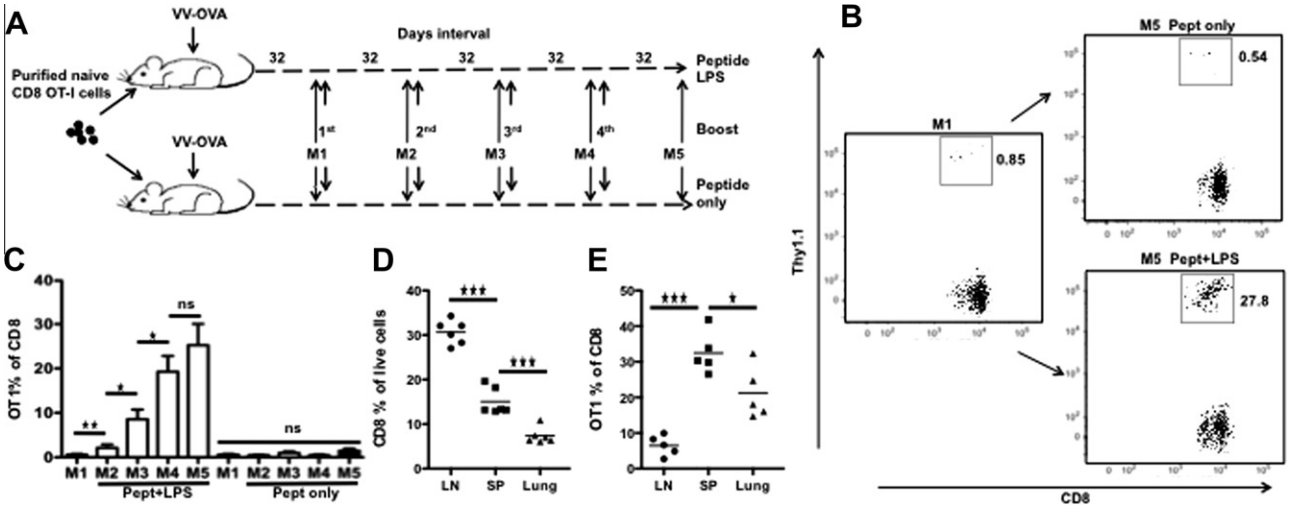

Fig. 1. Repetitive boosting with peptide and adjuvant drives memory CTLs progressively to high levels. (A) Scheme for experimental design. (B) Representative dot plots of M1 and M5. (C) OT1 percentage of total CD8 T cells in the blood from memory mice. (D–E) CD8 T cell percentage of live cells and OT1 percentage of total CD8 T cells in the tissues of M5 memory mice. Data represent mean ± SEM of six to ten animals. Asterisks indicate statistical significance. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ns: not significant. These will be followed in the rest of paper. Similar results were obtained from three experiments.