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The transcriptional program of vaccine-induced CD8$^+$ T cell memory

Rahul Roychoudhuri[a,1], Francois Lefebvre[b], Mitsuo Honda[a,1], Li Pan[b], Yun Ji[c], Christopher A. Klebanoff[c], Carmen Nichols[b], Slim Fourati[b], Ahmed N. Hegazy[d], Jean-Philippe Goulet[b], Luca Gattinoni[c], Gary J. Nabel[a,1], Michel Gilliet[e], Mark Cameron[b], Nicholas P. Restifo[c], Rafick P. Sékaly[b,*,2], Lukas Flatz[e,f,*,2]

[a] Vaccine Research Center, National Institute for Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA
[b] Vaccine and Gene Therapy Institute, Port St. Lucie, FL 34987, USA
[c] National Cancer Institute (NCI), National Institutes of Bethesda, Bethesda, MD 20892, USA
[d] Translational Gastroenterology Unit, Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom
[e] Department of Dermatology, University Hospital CHUV, 1011 Lausanne, Switzerland
[f] Institute of Immunobiology, Kantonsspital St. Gallen, St. Gallen, Switzerland

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ABSTRACT

The generation of CD8$^+$ T-cell memory is a major aim of vaccination. While distinct subsets of CD8$^+$ T-cells are generated following immunization that differ in their ability to confer long-term immunity against infection, the transcriptional profiles of these subsets within endogenous vaccine-induced CD8$^+$ T cell responses have not been resolved. Here, we measure global transcriptional profiles of endogenous effector (T_{EFF}), effector memory (T_{EM}) and central memory (T_{CM}) CD8$^+$ T-cells arising from immunization with three distinct prime-boost vaccine regimens. While a proportion of transcripts were uniquely regulated within distinct CD8$^+$ T cell populations, we observed progressive up- or down-regulation in the expression of a majority of differentially expressed transcripts when subsets were compared in the order $T_N > T_{CM} > T_{EM} > T_{EFF}$. Strikingly, when we compared global differences in gene expression between T_N, T_{CM}, T_{EM} and T_{EFF} cells with known transcriptional changes that result when CD8$^+$ T cells repetitively encounter antigen, our analysis overwhelmingly favored a model whereby cumulative antigen stimulation drives differentiation specifically from $T_N > T_{CM} > T_{EM} > T_{EFF}$ and this was common to all vaccines tested. These findings provide insight into the molecular basis of immunological memory and identify potential biomarkers for characterization of vaccine-induced responses and prediction of vaccine efficacy.

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1. Introduction

Following immunization or infection, a small number of naïve (T_N) CD8$^+$ T-cells bearing specificity for pathogen-associated antigens proliferate and differentiate to generate an acute response. Following clearance of antigen there is a contraction in the size of the acute response and only a fraction of cells remain to form long-lived memory cells. Upon infection with a pathogen bearing similar antigens, these memory cells expand to control the infection, often to a better extent than the primary response. Consequently, the generation of CD8$^+$ T-cell memory is an important aim of vaccination.

Phenotypically distinct subsets of CD8$^+$ T cells have been defined with differential capacity for proliferation, lymphoid homing and effector function [1–3]. In particular, naïve (T_N), central memory (T_{CM}), effector memory (T_{EM}) and effector (T_{EFF}) CD8$^+$ T cells can be distinguished based upon the expression of surface proteins relating to lymphoid homing and activation status [2,4]. Distinct subsets of memory CD8$^+$ T cells confer differential levels of protective immunity in a manner that is pathogen-dependent. For example, adoptive transfer of T_{CM} specific for a recombinant virally encoded model antigen resulted in better protection against vaccinia virus when compared with T_{EM} while a similar number T_{EM} cells provided better protection against lymphocytic choriomeningitis virus infection, respectively, when compared with T_{CM} [5]. Moreover, a persistent recombinant viral vector based on rhesus

* Corresponding authors at: Institute of Immunobiology, Rorschacher Strasse 95, 9007 St. Gallen, Switzerland. Tel.: +0041 79 425 4113.
E-mail addresses: roychoudhuri@mail.nih.gov (R. Roychoudhuri), rpsekaly@vgtifl.org (R.P. Sékaly), lukas.flatz@gmail.com (L. Flatz).
[1] These authors contributed equally to this work.
[2] These authors contributed equally to this work.

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cytomegalovirus encoding antigens derived from simian immunodeficiency virus (SIV) predominantly generating T_{EM} responses that homed to peripheral tissues exhibited superior protective capacity against subsequent infection with SIV [6]. Resolving the molecular basis for differences in the protective capacity of distinct $CD8^+$ T cell subsets is therefore a priority for the development of better vaccines.

Much has been learnt about the kinetics and composition of $CD8^+$ T-cell responses from the study of antiviral responses in mice [7–10]. Two major models exist to explain the observed predominance of cells with an effector phenotype during acute phases of the response and cells with a memory phenotype at later phases: According to the linear differentiation hypothesis, naive cells uniformly differentiate into effector cells upon encounter with antigen. Upon pathogen clearance, effector cells then either undergo apoptosis or differentiate into central/effector memory T cells [11]. According to the progressive differentiation hypothesis, $CD8^+$ T cells differentiate along a single continuum from T_N to T_{CM} to T_{EM} to T_{EFF}. Moreover, the model proposes that T cells acquire irreversible changes while differentiating that result from antigenic or inflammatory signals [12,13]. While there is a predominance of effector cells during the acute phase, central memory cells generated at this time survive while effector cells die upon withdrawal of antigen resulting in a predominance of central memory cells at late time points.

While fate-mapping experiments using artificial *Gzmb* promoter sequences to induce expression of a heritable marker in effector cells and their progeny indicated that cells that had once been effector cells could contribute to the memory pool [14,15], these studies did not rely on the activity of the endogenous *GzmB* promoter that may have been subject to further epigenetic or other modulation. Data from adoptive transfer models, on the other hand, support the progressive differentiation model. By performing adoptive transfers of central memory, effector memory and effector T-cell receptor (TCR) transgenic $CD8^+$ T cells arising at an acute phase of an antiviral response into infection-matched recipients, Kaech et al. were able to demonstrate a reduced capacity of effector cells to form memory cells [2]. Thus, there is contradictory evidence in the literature regarding lineage relationship between the $CD8^+$ T cell subsets.

In this study, we have measured global transcriptional profiles of distinct $CD8^+$ T cell subsets arising endogenously from vaccination of mice with three distinct prime-boost vaccine regimens. By using tetrameric peptide/major histocompatibility complex (MHC)-based sorting and highly sensitive microarray platforms, we were able to analyze endogenous vaccine-induced responses, avoiding the need for adoptive T cell transfer and the use of T cell receptor-transgenic model systems. Once fractionated into subsets, transcriptional profiles were remarkably similar between distinct vaccines. This enabled calculation of core gene expression profiles associated with distinct $CD8^+$ T cell subsets independent of vaccine used. While a proportion of transcripts were uniquely regulated within distinct $CD8^+$ T cell subsets, we observed progressive up- or down-regulation in the expression of a majority of differentially expressed transcripts when subsets were compared in the order $T_N > T_{CM} > T_{EM} > T_{EFF}$. When the transcriptional relationships of the $CD8^+$ T-cell subsets were compared in an unbiased fashion with known global transcriptional changes that result when T-cells repeatedly encounter antigen, our results favored a model whereby cumulative antigenic stimulation drives differentiation specifically from $T_N > T_{CM} > T_{EM} > T_{EFF}$. We have established transcriptional profiles of endogenous vaccine-induced $CD8^+$ T-cell responses that provide insight into molecular basis of immunological memory following vaccination and identify potential biomarkers for prediction of vaccine efficacy.

2. Materials and methods

2.1. Animals and immunization protocols

Female BALB/c mice between the ages of 6 and 10 wk (NCI/DCT, Jackson Laboratories or Charles River) were used for our experiments. They were housed in the animal facility of the Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Bethesda, MD. All animal experiments were reviewed and approved by the Animal Care and Use Committee, Vaccine Research Center, National Institute of Allergy and Infectious Diseases, NIH and were performed in accordance with all relevant federal NIH guidelines and regulations. All immunizations were administered intramuscularly. Recombinant replication-defective adenovirus (rAd5) vectors are replication-defective E1-, E3-, and E4-deleted human adenovirus serotype 5-derived vaccines generated as described previously [16]. Three intramuscular priming immunizations using plasmid DNA vaccines were used as previously described [17]. Recombinant lymphocytic choriomeningitis virus (rLCMV) vectors were generated as described previously [18].

2.2. Flow cytometry and cell sorting

Splenocytes were stained with H2-Dd/PA9-PE tetramers for 15 min at 4 °C in PBS prior to staining with fluorescently conjugated antibodies. Tetramers were produced by the NIH tetramer core facility. All other fluorochrome-coupled antibodies were obtained from Becton–Dickinson and used as directed by the manufacturer: CD3 Alexa Fluor 700, CD8a PerCP-Cy5.5, CD127-PECy7, CD62L-APC-Cy7, CD16-PacBlue and CD32-PacBlue. Sorting for microarray analysis was performed using a FACS Aria directly into cold RNALater (Ambion, Inc.) before freezing at −80 °C.

2.3. Intracellular cytokine staining

Briefly, spleens were harvested from mice 3 wk after the final immunization, and dissociated over nylon gauze. Single-cell suspensions were washed twice in PBS and 2 million cells per well were distributed into 96-well conical-bottomed plates, in RPMI medium supplemented with 10% FCS containing Brefeldin A (10 μg/mL) and PA9 or an irrelevant peptide (0.1 μg/100 μL). Cells were incubated at 37 °C for 5 h before being washed and stained with VIVID dye (Invitrogen) and fluorescently conjugated antibodies against CD3, CD8, and CD4. Cells were then fixed and permeabilized using BD Cytofix/Cytoperm (BD Pharmingen) and stained with antibodies against IFN-γ, TNF-α, and IL-2. Cells were analyzed using an LSRII flow cytometer (Becton–Dickinson) and resultant data were analyzed using FlowJo software (Treestar, Inc.).

2.4. Microarray cDNA hybridization analysis

Quantification was performed using a spectrophotometer (NanoDrop Technologies) and RNA quality was assessed using the Experion automated electrophoresis system (Bio-Rad Laboratories). Total RNA was amplified and labeled using the Illumina TotalPrep RNA Amplification kit, which is based on the Eberwine amplification protocol. This protocol involves a first cDNA synthesis step followed by *in vitro* transcription for cRNA synthesis. The biotinylated cRNA was hybridized onto Illumina Mouse Chips at 58 °C for 20 h and quantified using an Illumina BeadStation 500G× scanner and Illumina BeadStudio v3 software. Illumina probe data were exported from BeadStudio as raw data and screened for quality. Samples failing chip visual inspection and control examination were removed.

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