Vaccine-Induced CD8⁺ Central Memory T Cells in Protection from Simian AIDS

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Vaccine-Induced CD8+ Central Memory T Cells in Protection from Simian AIDS

Monica Vaccari,* Christopher J. Trindade,* David Venzon,† Maurizio Zanetti,‡ and Genoveffa Franchini*1*

Critical to the development of an effective HIV vaccine is the identification of adaptive immune responses that prevent infection or disease. In this study we demonstrate in a relevant nonhuman primate model of AIDS that the magnitude of vaccine-induced virus-specific CD8+ central memory T cells (TCM), but not that of CD8+ effector memory T cells, inversely correlates with the level of SIVmac251 replication, suggesting their pivotal role in the control of viral replication. We propose that effective preventive or therapeutic T cell vaccines for HIV-1 should induce long-term protective central memory T cells. The Journal of Immunology, 2005, 175: 3502–3507.

A major goal of immunization against cell-associated pathogens, such as HIV, is to generate long-lived protective memory T cells. After the initial exposure to Ags, the expansion of specific CD8+ T cells is followed by a contraction phase by which Ag-specific CD8+ T cells are distinguished into two subsets, central memory T cells (TCM) and effector memory T cells (TEM), on the basis of phenotypical and functional features (1). TCM express lymph node homing receptors (CD62L and CCR7), whereas TEM are mainly located at effector sites (2), express β1 and β2 integrins, chemokines such as CCR1, CCR3, and CCR5, and homing receptors such as CD103 and CLA (3). Although both the TCM and TEM subsets acquire effector functions such as cytokine production and lytic activity, TCM functions are characterized by a greater capacity for in vivo expansion after re-exposure to Ag (4). In mice, TCM are more efficient in conferring protection in the control of either viral replication or disease (4, 5), suggesting that effective T cell-based vaccines should be programmed to elicit a higher frequency of TCM rather than TEM. We hypothesized that the same may be true for primates and performed a retrospective analysis of cryopreserved samples obtained from macaques vaccinated before or after SIVmac251 infection, because the virological outcome in those studies was already known. In one of these studies, we have shown that a regimen of DNA priming followed by a boost with the highly attenuated NY-VAC-based SIV vaccine candidate (6) induced high virus-specific helper responses, increased the frequency and durability of CD8+ T cell responses, and, importantly, was more effective in protecting macaques from disease than NYVAC-SIV alone (7, 8). In a second study we have demonstrated that vaccination with NYVAC-SIV alone of SIVmac251-infected macaques treated with antiretroviral therapy (ART) also resulted in better containment of viral replication (9, 10), i.e., reduced virus level at set point. Viral levels expressed as RNA copies per milliliter after either primary viremia or viral rebound after ART suspension are predictive of disease development (5, 10–12). Interestingly, in both studies an inverse correlation was found between CD4+ Th responses, virus-specific CD8+ T cell responses to a dominant Gag peptide, and plasma virus level at set point (7–10, 13). However, which of the two CD8+ memory response subpopulations, TCM or TEM, boosted/elicted by vaccination contributed to containment of viral replication was not established.

In this study we demonstrate that virus-specific TCM correlated inversely with containment of viral replication in both the preventive and therapeutic vaccine studies, suggesting that the goal of an effective vaccine for HIV should primarily be to elicit central memory cells rather than CTLs.

Materials and Methods

Detection of Gag tetramer-specific CD8β+ T lymphocytes by flow cytometry in cryopreserved PBMCs of immunized macaques

The animals used in this study were housed and maintained in accordance with the standards of the Association for the Assessment and Accreditation of Laboratory Animal Care. The macaques were chronically infected with SIVmac251, as previously described (7, 8). The Abs used were anti-human CD8β Ab (clone 2ST8.5H7) from Beckman Coulter; anti-human CD28 Ab (clone 2ST8.5H7; Beckman Coulter), anti-human CD95 Ab (PE-Cy5 labeled, clone 2ST8.5H7; Beckman Coulter), and anti-human CD56 Ab (clone 2ST8.5H7; Beckman Coulter). Staining was also performed simultaneously with the Mamu-A*01 tetrameric complex, refolded in the presence of the Gag181–189 CM9 (p11C) peptide and the Mamu-A*01 molecule (13), and conjugated to allophycocyanin-labeled streptavidin (Molecular Probes). The Gag181–189 CM9 (p11C, CTPYDINGM)-specific tetramer was reacted with cells at room temperature in the dark for 30 min. One hundred thousand events were collected in the lymphocyte population (R1) and on CD8β expression (R2). The gated R2 events were identified as CD28+/CD95+ (R3; TCM) and CD28−/CD95+ (R4; TEM), respectively. Data are presented as percentages of TCM and TEM positive for the tetramer or as percentages of

*Animal Models and Retroviral Vaccines Section and National Cancer Institute, Bethesda, MD 20892; and †Department of Medicine and Cancer Center, University of California-San Diego, La Jolla, CA 92039

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1 Address correspondence and reprint requests to Dr. Genoveffa Franchini, Animal Models and Retroviral Vaccines Section, National Cancer Institute, 41/D804, Bethesda, MD 20892. E-mail address: franchi@mail.nih.gov

2 Abbreviations used in this paper: TCM, central memory T cell; ART, antiretroviral therapy; gpe, gag-pol-env; ICS, intracellular cytokine staining; TEM, effector memory T cell; TNC, naive T cell.
tetramer-positive cells in the CD28^+/CD95^+ (TCM) and CD28^-/CD95^- populations (TEM).

**Intracellular cytokine staining (ICS)**

For ICS, a total of 1 x 10^6 PBMCs were incubated in RPMI 1640 medium (containing 10% FCS and antibiotics) for 6 h in the presence of specific peptide pool at 2 µg/ml or in the presence of the superantigen staphylococcal enterotoxin B at 1 mg/ml final concentration as a positive control or with no Ag as a negative control. The costimulatory mAb CD49d (0.5 µg/ml; BD Pharmingen) was added to all the samples to maximize the detection of T cells with higher activation thresholds (15). CD28 was not used as a costimulator molecule as one of the mix of Abs used for the staining for the detection of different subsets. Brefeldin A (Sigma-Aldrich) at a final concentration of 10 mg/ml was added after 1 h. The cells were washed, stained for the surface Abs, permeabilized by incubation in FACSPerm solution (BD Pharmingen), and stained with anti-TNF-α and anti-IFN-γ.

**Statistical analysis**

Statistical analysis involved two-group comparisons of log-transformed frequencies performed using repeated measures ANOVA and corrected for multiple tests. Correlations were assessed using the Spearman rank-correlation method.

**Results**

TCM but not TEM elicited by vaccination before SIVmac251 challenge exposure inversely correlate with viremia containment

We performed a retrospective analysis to establish the ability of the two vaccination regimens to generate TCM on cryopreserved PBMC samples (7, 8). At first TCM and TEM were characterized (R3 and R4, respectively; Fig. 1a) using Abs to CD28 and CD95, as previously described, in rhesus macaques (16). In parallel, we used other markers that define TCM and TEM in humans and mice, such as CD45RA, CD11a, and CCR7 (Fig. 1b). We observed that the TCM population defined as CD28^+/CD95^- in macaques was also CCR7^high, as in humans (1). Also, the TEM population defined as CD28^-/CD95^- was CCR7^low or CCR7^-.

We focused at first on a dominant Gag response induced by vaccination or subsequent viral exposure in Mamu-A*01-positive animals that recognize the immune-dominant Gag peptide Gag181–189 CM9 (p11C), because this T cell response can be accurately measured using the Mamu-A*01 Gag181–189 CM9 (p11C) tetramer (Fig. 1a). This Gag response has been correlated to viremia containment in several studies (17–20).

Four Mamu-A*01-positive macaques previously vaccinated with four inoculations of 10^8 PFU of NYVAC-SIV, and five with three intradermal and i.m. inoculations of DNA-SIV and boosted with NYVAC-SIV-gag-pol-env (gpe; Fig. 2a) were studied. Two Mamu-A*01-positive macaques were mock-vaccinated with the parental NYVAC. Mucosal challenge exposure to SIVmac251 was performed 6 mo from the last immunization (Fig. 2a).

The frequency of tetramer-positive CD8^+ T cells was evaluated within the CD28^-/CD95^- and CD28^-/CD95^- PBMCs, because the coordinated assessment of these two markers is sufficient for discrimination, in this macaque species, of naive T cells (TEM), TCM (CD28^-/CD95^-), and TEM (CD28^-/CD95^-) (16). One DNA immunization did not elicit detectable Gag181–189 CM9 (p11C) responses (7, 8). In contrast, Gag181–189 CM9 (p11C) tetramer-positive CD8^+ T cells were induced by a single administration of NYVAC-SIV (Fig. 2b; wk 2). Two immunizations with NYVAC-SIV or three immunizations with DNA-SIV induced comparable frequencies of Gag181–189 CM9 (p11C) tetramer-positive CD8^+ T cells (see the continuous line in Fig. 2b). The frequencies of TEM and TCM were comparable within the wk 0–24 interval, but after the last immunization with NYVAC-SIV (wk 52–60 interval), macaques primed with DNA-SIV (group C) developed significantly more TCM (p = 0.020) and significantly fewer TEM (p = 0.047) specific for Gag181–189 CM9 (p11C) than macaques primed with NYVAC-SIV (group B).

After challenge exposure, all macaques experienced an acute phase of viremia with virus level ranging from 10^6 to 10^7 viral RNA copies/ml that subsided to lower levels at the set point (7, 8), particularly in macaques immunized with the DNA-SIV, NYVAC-SIV vaccine combination (8). Comparison of the medians of the log-transformed tetramer-positive TCM percentages over wk 53–60 (memory phase) before viral challenge to the plasma virus level after the set point (wk 6–24) demonstrated a significant negative correlation (r = −0.77; Fig. 3), suggesting that TCM play a role in the containment of viral replication. Conversely, we did not find a significant correlation between the levels of TEM and the level of viremia (not shown).

To test whether the response to the single Gag-dominant epitope measured was representative of the quality of the collective virus-specific immune responses elicited by vaccination, we also assessed the quality of the CD8^+ T cell response to the Env protein using the ICS assay after in vitro stimulation with overlapping peptides encompassing the C-terminal half of the SIV Env protein.
Both vaccination and SIVmac251 infection of rhesus macaques elicit sizeable responses to these Ags, enabling quantitation of the relative frequencies of TCM and TEM. After the last immunization with NYVAC-SIV, the Env-specific responses were highest in the animals in group C, which had received the DNA prime, NYVAC-SIV boost (Fig. 4a). Importantly, when CD28 and CD95 expressions were used to dissect the relative contributions of TCM and TEM to the CD8\(^+\) SIV Env response, we found that macaques immunized with NYVAC-SIV only developed equivalent frequencies of Env-specific TCM and TEM (Fig. 4b). In contrast, macaques immunized with the DNA prime, NYVAC-SIV boost had a higher frequency of Env-specific TCM than TEM (Fig. 4c), although the difference did not reach statistical significance. Because in the ICS assay we did not use the Ab to CD28 to costimulate cells, it is possible that we understated the ICS response. Nevertheless, it appears that the ability of the DNA prime, NYVAC-SIV boost vaccine regimen to increase virus-specific TCM may not be restricted to the dominant Gag\(_{181-189}\) CM9 (p11C), supporting the hypothesis that DNA priming influences the overall quality of CD8\(^+\) T cells in favor of TCM expansion.

SIV-specific TCM inversely correlate with the level of plasma viremia in macaques infected with SIVmac251

In a previous study we demonstrated that ART, alone or in combination with therapeutic vaccination with NYVAC-SIV, of SIVmac251-infected macaques results in better containment of viral replication (9). This previous study had been designed to investigate whether intervention with ART and therapeutic vaccination during primary infection could confer a virological benefit and included eight macaques per group; three animals in each group were Mamu-A*01-positive (Fig. 5a). Macaques in group A were treated with ART and mock-vaccinated with NYVAC, whereas the remaining macaques were vaccinated with the NYVAC-SIV construct in the presence (group B) or the absence (group C) of ART after the last immunization with NYVAC-SIV in animals of groups B and C.
Gag-specific TCM at wk 25 (2 wk after the last vaccination and ART (data not shown). Correlative analysis between the level of caques and analyzed the relative frequency of Gag181–189 CM9 retrieved cryopreserved PBMCs from Mamu-A*01-positive ma-
in the containment of viremia. This significant negative correlation

FIGURE 4. Env-specific TCM. a, Mean percentages (±SE) of CD8+ T cells producing IFN-γ and TNF-α after stimulation with an Env peptide pool derived from the SIVk6w DNA sequence (32). The data were ob-
tained from cryopreserved PBMCs collected at wk 53, 1 wk after the last immunization (see Fig. 1a) and represent the mean frequency values ob-
tained from animals in groups B and C. Mean percentages (with error bar) of cytokine-producing CD8+/CD95+/CD28− (TCM) and CD8+/CD95+/CD28− (TCM) cells from macaques in groups B (b) and C (c).

b

c

To investigate the role of TCM in this setting, we retrieved cryopreserved PBMCs from Mamu-A*01-positive ma-
caques and analyzed the relative frequency of Gag181–189 CM9 (p11C) tetramer-positive TCM and TEm before, during, and after all treatments. The last immunization with NYVAC-SIV of ART-
treated macaques expanded ~8-fold the mean frequency of the
Gag181–189 CM9 (p11C) tetramer-positive cells in macaques of group B (wk 25; Fig. 5b, middle panel) compared with unvaccinated controls (group A). In contrast, in the absence of ART, NYVAC-SIV was unable to expand these responses. Collectively, these results were in agreement with previous data obtained from ex vivo PBMCs (9). Qualitative analysis of Gag181–189 CM9 (p11C) tetramer-positive TCM and TEm demonstrated a significant increase in the frequency of TCM in macaques vaccinated while on ART (data not shown). Correlative analysis between the level of Gag-specific TCM at wk 25 (2 wk after the last vaccination and before ART suspension) and the level of viremia over subsequent weeks when virus rebounded after ART cessation revealed a Spearman rank correlation of either −0.85 or −0.87 with the same

Discussion

Immunological memory is an essential aspect of adaptive immune responses after natural infection or vaccination (21). Although B and T cell dynamics (22) and the role of memory Ab responses in protection against infection are well understood, less is known about the quality of memory T cells required for protection against disease. Evidence in murine systems of viral infection (4, 5) has recently suggested that CD8+ TCM may constitute the correlate of protection from disease. Along the same lines, it was recently reported that CD4+ TCM mediate long-term immunity against the parasite Leishmania major in the mouse (23).

In this study, using a model of SIV infection in the rhesus macaque, we show that vaccine-induced protection correlates with the expansion of CD8+ TCM cells in the settings of both preventive and therapeutic vaccinations. We have demonstrated that DNA priming induced higher levels of CD8+ TCM responses than priming with the NYVAC-SIV vaccine. These findings may relate to the ability of DNA to expand both CD8+ and CD4+ T cells, limiting activation-induced cell death (24) and exhaustion (25), which occur when T cells respond to a high Ag dose and/or the response occurs in inflammatory conditions. Indeed, we have previously reported that DNA-SIV prime, NYVAC-SIV boost also induces higher CD4+ T cell responses (7), which inversely correlate with virus levels in SIVmac251-challenged macaques (8). This is consistent with the idea that CD4+ T cell help is required to enhance the survival, maintenance, and further expansion of CD8+ T cells after Ag re-encounter (26–28). Possibly, the low level of Ags expressed by DNA-SIV favor the synchronous activation and expansion of CD8+ and CD4+ T cells. In contrast, the expression of higher levels of Ags by live vector-based vaccination (e.g., NYVAC-SIV) that also induces local inflammatory conditions is less dependent on cognate CD4 help and hinders the expansion of CD8+ TCM cells. Interestingly, NYVAC-SIV boost fully expands CD8+ TCM induced by and maintained through DNA priming. Because the dose of Ag at the time of priming does not dictate the rate of expansion of CD8+ memory T cells (5, 29), one could argue that the contribution of the NYVAC-SIV boost could be either to provide a different or richer cytokine milieu or to recruit different cells to the site of immune expansion. Consistent with this overall interpretation of the data are recent findings in the mouse, where complete protection from lethal virus challenge was obtained by immunization with a low dose of Ag (5).

The data also imply that therapeutic vaccination combined with maneuvers that reduce Ag load in vivo may reach the same objec-
tive. We speculate that ART resets the Ag load and concomi-
antly reduces the inflammatory conditions sustained by high lev-
els of virus replication, hence mimicking the starting conditions of DNA priming in naïve macaques. This provides evidence for the plasticity of the immune response and is consistent with the idea that although high doses of Ag may induce unresponsiveness, low doses of Ag are immunogenic (30, 31).

The findings presented in this study suggest that CD8+ TCM can be regarded as an important correlate of protection against disease in the SIV model of infection and provide a rational explanation for why the DNA prime, live vector boost immunization confers greater protection than immunization with live vector alone. They also point to new rules for the induction of protective T cell re-
sponses by vaccination, which, based on the present observations, should be focused on programming and maintaining CD8+ TCM
by appropriate vaccination regimens. Thus, efforts to develop effective therapeutic and preventive vaccines for HIV should promote the generation of long-lived Ag-specific CD8^+ TCM in conjunction with helper responses able to sustain the ability of CD8^+ TCM to expand adequately upon virus encounter.

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Disclosures
The authors have no financial conflict of interest.

References


**FIGURE 5.** Relative frequencies of Gag-specific T_CM and T_EM in ART-treated and/or vaccinated SIVmac251-infected macaques. *a*, Study design. The overall study results can be found in Ref. 9. *b*, Mean frequencies of total Gag_{181–189} CM9 (p11C) tetramer-specific CD8^+ T cells (solid line) or relative mean percentages (with error bar) of tetramer-positive T_CM and T_EM in macaques after immunization with NYVAC-SIV in the presence of the absence of ART (groups B and C, respectively) or mock immunization (NYVAC parental) in the presence of ART (group A). *c*, Inverse correlation of T_CM at wk 25 (2 wk after the last immunization) and viremia levels at wk 28–35 (after ART suspension) in all nine macaques studied.


