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Prolonged Antigen Expression following DNA Vaccination Impairs Effector CD8+ T Cell Function and Memory Development

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After priming, naive T cells undergo a program of expansion, contraction, and memory formation. Numerous studies have indicated that only a brief period of antigenic stimulation is required to fully commit CD8+ T cells to this program. Nonetheless, the persistence of Ag may modulate the eventual fate of CD8+ T cells. Using DNA delivery, we showed previously that direct presentation primes high levels of effector CD8+ T cells as compared with cross-presentation. One explanation now revealed is that prolonged cross-presentation limits effector cell expansion and function. To analyze this, we used a drug-responsive system to regulate Ag expression after DNA injection. Reducing expression to a single burst expanded greater numbers of peptide-specific effector CD8+ T cells than sustained Ag. Consequences for memory development were assessed after boosting and showed that, although persistent Ag maintained higher numbers of tetramer-positive CD8+ T cells, these expanded less (~4-fold) than those induced by transient Ag expression (~35-fold). Transient expression at priming therefore led to a net higher secondary response. In terms of vaccine design, we propose that the most effective DNA-based CD8+ T cell vaccines will be those that deliver a short burst of Ag. The Journal of Immunology, 2007, 179: 8313–8321.

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aive CD8+ T cells specific for a particular peptide-MHC class I complex exist at very low frequencies in the Ag-inexperienced host (1–3). Following activation, these rare T cells expand massively in number, increasing as much as 10,000-fold. During this phase of the response, CD8+ T cells acquire their effector function, disseminate to peripheral sites, and mediate Ag clearance. After expansion, the majority of the effector T cells die through apoptosis, leaving a small percentage of cells (5–10%) that survive as long-lived memory cells. It is the higher frequency of Ag-specific memory T cells compared with their naive counterparts, along with their more rapid and aggressive response, that confers superior protective immunity against intracellular pathogens or tumors. For both, there is great interest in developing vaccines that induce long-lasting T cell memory, requiring detailed knowledge of their induction.

Several groups have demonstrated that naive CD8+ T cells become committed to proliferate and differentiate into long-lived memory cells after a short period of antigenic stimulation (4–10). Thus, an interaction of <7 h between a CD8+ T cell and an Ag-pulsed dendritic cell is sufficient to enable naive T cells to develop effector function and produce memory cells in vivo (10). These studies have led to a model where memory development potential is a cell-intrinsic property that is programmed shortly after antigenic stimulation. However, this “autopilot” model does not exclude the potential impact that extrinsic factors might have on the magnitude and quality of ensuing responses. For example, chronic Ag exposure during persistent infection often erodes the ability of effector CD8+ T cells to secrete cytokines and leads to their eventual deletion (11, 12). In the postvaccination setting, factors that modulate the instructional program are poorly defined.

DNA vaccines have emerged as a fast and flexible platform for taking immunological principles into vaccine design (13). Our laboratory has been investigating how the molecular nature of the expressed Ag influences the mechanism of priming and the subsequent profile of the T cell response. The DNA vectors we have used contain MHC class I- and class II-restricted T cell determinants in two different configurations (see Fig. 1). In the first design, the CD8+ T cell epitope is expressed as a minimal peptide specifically targeted to the endoplasmic reticulum by an N-terminal leader sequence (14). To provide critical “help” for the minigene product, a separate expression cassette is incorporated within the plasmid backbone encoding a hybrid invariant chain molecule, with the CLIP sequence replaced by a Th determinant from tetanus toxin (p30, Ref. 15). By separating the minimal epitope from a “protective polypeptide,” this vaccine fails to cross-present Ag because of the short half-life of minigene products (16) and induces Th-dependent CD8+ T cell responses via directly transfected APC (14). In the second design, the CD8+ T cell epitope is fused immediately 3′ of the N-terminal domain of fragment C from tetanus toxin (p30, Ref. 15). For this vaccine, effective CD8+ T cell responses are highly dependent on CD4+ T cell help generated following uptake of exogenous Ag; therefore, the design operates only via cross-presentation (14).

We had observed previously that immunization with the cross-priming vector elicited a CD8+ T cell response, which, in comparison to that induced by the direct-priming vaccine, was faster in onset but less sustained, resulting in a net lower peak burst size (14). In the current study, we have further investigated the quality of the CD8+ T cell response. By using a drug-responsive gene regulation system to control Ag expression, we have shown that...
persistence of cross-presented Ag limits effector and memory responses to a DNA vaccine. Conversely, reducing Ag expression to a short burst results in a significant amplification of the primary response and to the development of a memory CD8\(^+\) T cell population with the capacity to expand dramatically on boosting.

**Materials and Methods**

**Abs and reagents**

Directly conjugated Abs were purchased from BD Biosciences. PE-labeled H-2K\(^b\)/SIINFEKL tetramers were obtained from Proimmune. Peptides were custom synthesized by Peptide Protein Research.

**Plasmids**

The vaccines pDUO and pDOM-peptide encoding the SIINFEKL peptide were constructed as previously described (14). To construct the GeneSwitch vectors, open reading frames flanked by HindIII and NotI restriction sites were amplified by PCR using pDOM-peptide as template and sub-cloned into pGene/V5 (Invitrogen Life Technologies). Plasmid DNA was purified for immunization using a QIAfilter Giga Kit (Qiagen). All constructs were sequenced and checked for expression in vitro using the TNT T7 Coupled Reticulocyte Lysate System (Promega).

**Mice and in vivo experiments**

C57BL/6 mice, bred in house, were vaccinated at 8–10 wk of age with a total of 50 \(\mu\)g of plasmid DNA in normal saline injected into two sites in the quadriceps. For the GeneSwitch experiments, mice were injected i.m. with 50 \(\mu\)g of pGene/DOM-SL8 and 25 \(\mu\)g of pSwitch (Invitrogen Life Technologies). Plasmid DNA was purified for immunization using a QIAfilter Giga Kit (Qiagen). All constructs were sequenced and checked for expression in vitro using the U.K. Home Office license guidelines and approved by the University of Southampton’s ethical committee.

**Evaluation of peptide-specific T cell responses**

ELISPOT analysis was performed using the BD ELISPOT Set for murine IFN-\(\gamma\). Spots were developed using 5-bromo-4-chloro-3-indolyl phosphate (Zymed Laboratories) and counted with a Transtec 1300 ELISPOT reader.

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**FIGURE 1.** Representation of plasmids used for immunization.

**FIGURE 2.** A and B. The dynamics of T cell recruitment differ for pDOM-SL8 and pDUO. Naive CFSE-labeled OT-1 T cells were transferred into Thy1.1\(^{+}\) congenic C57BL/6 mice. Two days later, recipients were injected i.m. with empty vector, pDOM-SL8, or pDUO. Splenocytes were harvested 4 days after vaccination, and CFSE staining of Thy1.1\(^{+}\) tetramer\(^{+}\) CD8\(^+\) T cells were measured by flow cytometry. Results are shown as representative dot plots gated on Thy1.1\(^{+}\)tetramer\(^{+}\) CD8\(^+\) T cells for an individual animal and as group means \(\pm SEM (n = 4)\). These experiments were performed twice with similar findings. In the absence of Ag, we observed one to two divisions in \(\sim 30\%\) of adoptively transferred naive OT-1 T cells. However, the percentage of cells that go through more than two divisions is very low \((2.9 \pm 0.45)\).
ular nature of the expressed Ag influences the quality of the ensuing response. To investigate the early expansion phase, we turned to an adoptive transfer model using T cells from OT-1 mice that express a transgenic TCR specific for Kb/ SIINFEKL (19). OT-1 T cells, labeled with CFSE, were transferred into Thy1.1<sup>+</sup> congenic mice and the recipients were immunized with either vaccine 2 days later. Splenocytes were harvested 4–8 days after vaccination, and CFSE staining of Thy1.1-negative, tetramer-positive CD8<sup>+</sup> T cells was measured by flow cytometry.

Marked differences in OT-1 proliferation were observed in mice vaccinated with pDUO or pDOM-SLS (Fig. 2). In the latter, ~60% of the tetramer-positive cells were CFSE negative by day 4, indicating that they had divided at least seven times. In contrast, following pDUO vaccination, the percentage of cells that had been recruited into the proliferative pool was very low, with few cells present in the population representing seven or more rounds of division (Fig. 2). Yet when we measured the magnitude of an endogenous T cell response to pDOM-SLS, the peak effector burst size was smaller than that generated by pDUO (Fig. 3A), confirming previous data (14). It appears, therefore, that although more naive Ag-specific precursors are recruited into the proliferative pool following vaccination with pDOM-SLS, their expansion/survival is not as extensive as that triggered by pDUO (Fig. 3A). We speculated that the level and kinetics of Ag expressed by the two vaccine routes was a factor.

Vaccination with pDOM-SLS is associated with prolonged Ag presentation

We had observed that CD8<sup>+</sup> T cells induced by pDUO stained more brightly with Kb/SIINFEKL tetramers than those elicited by pDOM-SLS vaccination (for representative staining, see Fig. 3A). Costaining with an anti-TCR<sup>αβ</sup> mAb suggested that the increased tetramer binding was due to higher TCR expression (Fig. 3B). A comparison of the TCR mean fluorescence intensity (MFI)<sup>2</sup> ratios between the tetramer-positive and tetramer-negative CD8<sup>+</sup> T cells within the same mouse population representing seven or more rounds of division (Fig. 2). Yet when we measured the magnitude of an endogenous T cell response to pDOM-SLS, the peak effector burst size was smaller than that generated by pDUO (Fig. 3A), confirming previous data (14). It appears, therefore, that although more naive Ag-specific precursors are recruited into the proliferative pool following vaccination with pDOM-SLS, their expansion/survival is not as extensive as that triggered by pDUO (Fig. 3A). We speculated that the level and kinetics of Ag expressed by the two vaccine routes was a factor.

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indicated this was due to TCR down-modulation in the pDOM-SL8-primed population (Fig. 3C). Expression levels of CD8α or CD8β did not differ (data not shown).

Several investigators have reported that T cell activation is associated with TCR down-modulation that persists for up to 2–3 days (20, 21). For pDOM-SL8, it was possible that TCRlowCD8+ T cells were the result of continuous re-exposure to Ag, while TCRhigh cells in pDUO-vaccinated mice had emerged from TCRlow cells that had re-expressed their TCR because they had not re-encountered Ag. To examine how long Ag presentation to CD8+ T cells occurs following vaccination, we injected Thy1.1/CD8+ T cells into these mice 5, 10, or 20 days after vaccination. After 8 days posttransfer, spleens from the recipient mice were analyzed for donor Thy1.1null CD8+ T cells and their CFSE intensity.

Vaccination of recipient mice with pDUO caused a reduction in CFSE fluorescence on donor cells transferred on day 5 but not on days 10 or 20 (Fig. 4). In contrast, when OT-1 cells were transferred into pDOM-SL8-vaccinated mice, proliferation was observed at all of the time points tested (Fig. 4). Thus, Ag presentation is of short duration following injection of pDUO, being undetectable by day 10. In contrast, Ag presentation persists for a surprisingly long period, out to day 20, following vaccination with pDOM-SL8, possibly accounting for the observed TCR down-modulation.

Effector T cells induced by pDOM-SL8 vaccination are functionally less competent than those elicited by pDUO

Upon observing that Ag persists following pDOM-SL8 vs pDUO immunization, we examined whether SIINFEKL-specific, IFN-γ secreting cells were able to coproduce IL-2. IL-2 production has previously been demonstrated to be highly sensitive to down-regulation if Ag persists (12). For both vaccines, only a fraction of the IFN-γ-producing cells also secreted IL-2 (Fig. 5A); however, immunization with pDUO resulted in a higher percentage of IFN-γ+IL-2+ CD8+ T cells (>3-fold over three independent experiments, n = 6/group). More notable was that pDUO-primed T cells produced significantly more IFN-γ on a per cell basis as shown by higher MFI (Fig. 5B), suggesting greater functional competence. No statistical differences were seen in IL-2 MFI (data not shown). We also stimulated splenocytes from pDUO- and pDOM-SL8-immunized mice with a range of SIINFEKL peptide concentrations. As shown in Fig. 6A, the amount of peptide required to induce half-maximal IFN-γ production from the T cell population primed

![FIGURE 4. Ag expression is prolonged following vaccination with pDOM-peptide. Groups of Thy1.1+/+ congenic C57BL/6 mice (n = 3) were vaccinated with pDUO or pDOM-SL8. CFSE-labeled OT-1 T cells were injected i.v. into these animals on the days indicated. After 8 days posttransfer, spleens were harvested and CFSE staining of Thy1.1nulltetramer+CD8+ T cells was measured by flow cytometry. Results are shown as representative dot plots. These studies were performed twice with similar findings.](http://www.jimmunol.org/)

![FIGURE 5. Production of IFN-γ and IL-2 in mice immunized with pDUO or pDOM-SL8. Splenocytes from mice (six per group) immunized with pDUO or pDOM-SL8 were harvested at the peak of the respective responses, pooled, and restimulated with SIINFEKL peptide (10^{-8} M). The capacity to coproduce IFN-γ and IL-2 was determined by cytokine capture analysis. A, Dot plots are gated on CD8α+ lymphocytes and are representative of three experiments. B, Histograms are gated on CD8α+ IFN-γ+ cells and numbers indicate IFN-γ MFI plus SEM (p < 0.01).](http://www.jimmunol.org/)
CFSE-labeled splenocytes from naïve syngeneic mice were pulsed with varying concentrations of SIINFEKL peptide (100, 10, or 1 nM) and i.v. injected into immunized animals. An equal number of peptide-unpulsed targets labeled with PKH26 were also transferred. After 5 h, CTL responses were quantified by flow cytometric analysis. Mice vaccinated with pDUO showed lysis of both 10 and 1 nM peptide-pulsed targets (illustrated by representative histogram profiles in Fig. 6B and as group means in Fig. 6C). In contrast, CTL generated by pDOM-SL8 were unable to lyse targets pulsed with the lowest peptide concentration. Taken together, these data suggest that prolonged Ag expression following DNA vaccination compromises effector CD8+ T cell function.

Assessment of GeneSwitch as a means of quantitatively and temporally controlling Ag presentation in vivo

To investigate whether prolonged Ag presentation could be a factor limiting the expansion of CD8+ T cells to pDOM-SL8 vaccination, we used a strategy to control Ag expression in vivo. This involved the drug-responsive gene regulation system GeneSwitch (22–24). We initially determined whether this system could be used in a quantitative manner. Naïve CFSE-labeled OT-1 cells were transferred into congenic C57BL/6 mice and the recipients were coinjected 2 days later with the GeneSwitch plasmids, incorporating the DOM-SL8 sequence (Fig. 1). After 4 h, single doses of 0.5, 1, or 5 mg/kg MFP were administered i.p. Increasing the dose of the inducer led to a stepwise increase in the percentage of OT-1 T cells that had undergone seven or more divisions (Fig. 7A), indicating a correlation between MFP dose and peptide presentation. The highest dose used (5 mg/kg) induced levels approaching those of the uncontrolled, CMV promoter-driven pDOM-SL8 vaccine. In the absence of MFP, very few tetramer-positive cells had divided.

We next asked whether the dosage of MFP would influence the persistence of Ag. Groups of Thy.1+/− congenic C57BL/6 mice were coinjected with the GeneSwitch plasmids and transgene expression was induced with single 0.5- or 5-mg/kg doses of MFP. In one group, MFP (5 mg/kg) was administered on alternate days throughout the experimental period. Naïve CFSE-labeled OT-1 T cells were then injected into these mice 5 or 10 days after transgene induction. On day 6 postransfer, spleens were removed and analyzed for donor tetramer-positive CD8+ T cells and their CFSE intensity. Maintenance of gene expression using repeated (five times) injections of 5 mg/kg MFP led to proliferative responses of OT-1 T cells injected at both 5 and 10 days after induction (Fig. 7B). In contrast, a single dose of 5 mg/kg MFP led to a proliferative response on day 5 which was not detectable on day 10 (Fig. 7B), indicating transient Ag expression. A single injection of 0.5 mg/kg had no detectable effect. Thus, the GeneSwitch system provides a means of quantitatively and temporally controlling Ag presentation via pDOM-peptide to T cells in vivo. For pDUO, which appears to prime T cell responses following direct transfection of professional APCs, the GeneSwitch strategy did not control Ag expression. The reason for this is likely to be that, after i.m. injection, the frequency of cotransfected professional APC is very low, with the GeneSwitch plasmids primarily taken up by muscle cells. We therefore focused on controlling gene expression only from pDOM-peptide. This has the advantage that the only variable is Ag expression rather than the nature of the priming APC which could vary with the two vaccine designs.

Prolonged Ag expression limits the burst size and avidity of a primary CD8+ T cell response to a DNA vaccine

The effect of transient or prolonged Ag expression on the magnitude of an endogenous CD8+ T cell response (i.e., in the absence by pDUO vaccination was ~50-fold lower than the amount of peptide required by pDOM-SL8-primed cells.

To evaluate the functional avidities of the effector populations further, we performed an in vivo CTL assay (18). Differentially

FIGURE 6. Effector T cells induced by pDOM-SL8 vaccination are functionally less avid than those elicited by pDUO. A. Splenocytes from mice (six per group) immunized with pDUO (○) or pDOM-SL8 (●) were harvested, pooled, and restimulated with the indicated doses of SIINFEKL peptide. IFN-γ production was detected after 18 h by ELISPOT. Results are expressed as a percentage of the maximum response attained with saturating peptide concentrations (10−9 M). B and C. In vivo killing of targets pulsed with different molar concentrations of peptides, as described in Materials and Methods. Results are shown as representative histogram profiles (B) and as group means ± SEM (B; n = 6). These studies were performed twice with similar findings.
of OT-1s) induced by the pDOM-SL8 vaccine was then investigated. C57BL/6 mice were coinjected with the GeneSwitch plasmids and Ag-expressing cell line that had received CFSE-labeled K\(^+\)/SIINFEKL-specific TCR-transgenic T cells (OT-1) 2 days previously were vaccinated with pDOM-SL8 or pSwitch and pGene/DOM-SL8. Transgene expression was induced 4 h later with the concentrations of MFP indicated. OT-1 proliferation was assessed by flow cytometry after 4 days. After 12 days, the number of SIINFEKL-specific T cells was determined by IFN-\(\gamma\) ELISPOT. These studies were performed twice with similar findings.

To examine whether Ag persistence would influence the peptide level required to stimulate CD8\(^+\) T cells induced in a primary response, splenocytes from GeneSwitch-immunized mice were restimulated with a range of SIINFEKL peptide concentrations in vitro. IFN-\(\gamma\) production was detected after 18 h by ELISPOT. Results are expressed as a percentage of the maximum IFN-\(\gamma\) response. These studies were performed twice with similar findings.

FIGURE 8. Ag persistence limits the CD8\(^+\) T cell response to pDOM-SL8. A, C57BL/6 mice were vaccinated with pDOM-SL8 (\(\triangle\)) or pGene/DOM-SL8 and pSwitch (\(\bullet\), \(\circ\)). MFP (5 mg/kg) was administered as single or multiple doses (on alternate days throughout the experimental period). After 12 days, the number of SIINFEKL-specific T cells was determined by IFN-\(\gamma\) ELISPOT. B, Mice (six per group) were immunized with pGene/DOM-SL8 and pSwitch, and Ag expression was induced with single (\(\bullet\)) or multiple (\(\circ\)) doses of 5 mg/kg MFP. On day 12, splenocytes were harvested, pooled, and restimulated with a range of SIINFEKL peptide concentrations in vitro. IFN-\(\gamma\) production was detected after 18 h by ELISPOT. Results are expressed as a percentage of the maximum IFN-\(\gamma\) response. These studies were performed twice with similar findings.

Specific response that was significantly (\(p = 0.002\)) higher compared with uncontrolled pDOM-SL8 immunization (Fig. 8A). Because this effect was not observed with single doses of MFP, but was dependent upon sustained MFP delivery, these data suggest that prolonged Ag expression following DNA vaccination limits the effector peak burst size. It should be stated that MFP at a concentration of 5 mg/kg, administered over 10 days, does not affect the magnitude of an endogenous response to pDOM-SL8 vaccination (data not shown).

To examine whether Ag persistence would influence the peptide level required to stimulate CD8\(^+\) T cells induced in a primary response, splenocytes from GeneSwitch-immunized mice were restimulated with a range of peptide concentrations in vitro and the production of IFN-\(\gamma\) measured by ELISPOT. The amount of peptide required to induce half-maximal IFN-\(\gamma\) production from the T cell population primed under conditions of sustained Ag presentation was 10-fold higher than the amount of peptide required by cells from mice where Ag expression had been induced by a single dose of MFP (Fig. 8B). These data demonstrate that, for DNA vaccination, transient Ag presentation during priming results in an effector CD8\(^+\) T cell population with higher functional avidity.
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Effect of transient or prolonged Ag expression on secondary responses

We finally asked whether limiting Ag expression during priming would impact on the ability of memory T cells to respond to rechallenge. GeneSwitch-immunized mice, in which Ag expression had been induced with single or multiple doses of 5 mg/kg MFP. On day 50 postimmunization, groups of mice were boosted with pDOM-SL8 and ex vivo K\(^+\)SIINFEKL tetramer (A) or IFN-\(\gamma\) ELISPOT (B) analysis was performed 10 days later at the peak of expansion. The results shown are combined from two separate experiments, with each data point representing an individual mouse.

By day 60 after immunization and induction with a single dose of MFP (5 mg/kg), \(\sim 0.2\%\) of the resting CD8\(^+\) T cell population was tetramer positive (Fig. 9A). In contrast, the size of the memory population was \(\sim 5\)-fold higher \((p = 0.0043)\), when Ag expression had been sustained by multiple administrations of MFP during priming. It appears therefore that persistent Ag maintains higher numbers of tetramer-positive CD8\(^+\) T cells able to survive for 60 days, as compared with transient Ag expression. Boosting with pDOM-SL8 expanded both memory T cell pools (Fig. 9A). However, the CD8\(^+\) T cells that had been primed under conditions of transient Ag expression showed greater expansion \((\sim 35\text{-fold})\) than those primed under conditions of sustained Ag expression \((\sim 4\text{-fold})\). Similarly, when functional IFN-\(\gamma\) ELISPOT analysis was performed, boosting with pDOM-SL8 elicited substantially higher numbers of IFN-\(\gamma\)-producing cells \((\sim 3\text{-fold})\) when memory CD8\(^+\) T cells had been primed under conditions of transient Ag expression (Fig. 9B). These results demonstrate that limiting the duration of Ag expression following DNA vaccination does not impair the ability of primed T cells to respond to a secondary Ag challenge. In fact, they suggest that memory CD8\(^+\) T cells elicited under such conditions have the greater proliferative capacity.

Discussion

In this study, we show that duration of Ag expression is a critical variable in the development of T cell memory to DNA-encoded Ags. The question of the effect of the level and kinetics of Ag expression was raised initially by the marked contrast between the performances of DNA vaccines acting via cross-presentation (pDOM-peptide) vs direct presentation (pDUO) (14). We had speculated that one possible explanation for the superior ability of pDUO to prime CD8\(^+\) T cells was that the dwell time of T cell-APC interactions differed between the vaccines. Several reports have shown that the duration of TCR stimulation can determine whether subsequent clonal expansion will be abortive or extensive (25, 26). Because cross-loaded Ag represents a finite source of peptides (27), sustained T cell-APC interactions might be best achieved by direct priming, where biosynthesis constantly supplies peptides. Arguing against this hypothesis, however, were the observations that immunization with cross-presented pDOM-peptide-encoded Ag is associated with TCR down-modulation and peptide insensitivity, indicating sustained antigenic stimulation (20). The persistence of Ag following pDOM-peptide vaccination was perplexing because we would expect that activated effector cells would rapidly clear transfected cells (28, 29). The strategy of using the GeneSwitch system allowed the investigation of this specific point and has shown that cross-presentation of expressed Ag persists in the face of CTLs. However, extended presentation of Ag does not lead to amplified responses.

By using a drug-responsive transcription factor to regulate Ag expression in vivo, we have demonstrated that sustained delivery of Ag effectively reduces a primary CD8\(^+\) T cell response to a DNA vaccine. These findings mirror those observed in chronic lymphocytic choriomeningitis virus infection, where persistence of virus leads to impaired CD8\(^+\) T cell responses and eventual depletion in an epitope-dependent manner (11, 12, 30–32). The mechanism behind this functional exhaustion is thought to be due to sustained, high epitope presentation to T cells, leading to near continuous TCR triggering and apoptosis by activation-induced cell death (12). Our system reveals an epitope-driven initial down-regulation of TCR expression, dependent on sustained Ag expression, which precedes a relative loss of the CD8\(^+\) T cell response at priming. In contrast, transient expression of the same epitope generates a higher level response.

The fact that the level and timing of expression of a peptide epitope from the same vaccine construct can be controlled by GeneSwitch removes any variables that might derive from different routes of peptide presentation or variability in the T cell repertoire. It allows the speculation that the susceptibility of CD8\(^+\) T cells to depletion might be influenced by the activation status of the APC. Thus, an important variable may be the inflammatory milieu at the site of Ag expression and its effect on the APC. It is possible that the proinflammatory signals associated with injection of naked plasmid DNA are short-lived and that persistent Ag is then expressed without these signals. Successive waves of APC cross-loaded with Ag under different conditions may provide distinct stimuli to responding T cells (33, 34), with second wave cells...
of a quiescent phenotype limiting expansion/survival of daughter cells (32). Interventions that sustain APC activation may thus promote T cell responses to DNA vaccines (35); however, an important variable will be the timing between DNA vaccination and adjuvant administration. Wilson et al. (36) have shown that APC activation is associated with diminished cross-presentation. Consequently, systemic agonists will have to be given after a suitable interval after DNA vaccination, allowing APC cross-loading before activation.

An important goal of vaccination is to generate memory T cells with the capacity to undergo vigorous expansion in response to secondary Ag challenge. Compatible with this aim, memory CD8⁺ T cells elicited via transient rather than sustained expression of a DNA-encoded Ag exhibited a greater proliferative potential. Why this should be remains to be determined but may reflect the poor recall response potential of exhausted T cells (37, 38). Alternatively, limiting the duration of Ag expression may speed the conversion of effector to central memory T cells (39, 40). The latter subset, originally described by Sallusto et al. (41), has greater proliferative capacity upon Ag re-encounter compared with effector-memory T cells (39). To distinguish between these possibilities, additional experiments will be performed to characterize the memory phenotype, activation status (42), and effector functions of the T cell populations generated by the GeneSwitch plasmids. We also intend to determine whether shortening Ag expression after DNA vaccination impacts on memory CD4⁺ T cell development (43).

In terms of vaccine development, our data provide information and relevant tools required for the design of T cell-based vaccines. By taking into account the dose and temporal delivery of Ag and by targeting the Ag-processing pathway, we have shown that it is possible to modulate the intensity of SIINFEKL-specific CD8⁺ T cell responses. Although it remains to be seen whether these principles apply to full-length Ags, we have data that the SIINFEKL peptide-epitope fused to fragment C requires N-terminal processing and is dependent to be seen whether these principles apply to full-length SIINFEKL-specific CD8⁺ T cell differentiation: initial antigen encounter triggers a developmental program in naive cells. Nat. Immunol. 2: 415–422.


Prlc, M., G. Hernandez-Hoyos, and M. J. Bevan. 2007. Duration of the initial TCR stimulus controls the magnitude but not functionality of the CD8⁺ T cell response. J. Exp. Med. 204: 2133–2143.


