OncoImmunology 2:7, e25032; July 2013; © 2013 Landes Bioscience

Boosting anticancer vaccines
Too much of a good thing?

Alessia Ricupito[1], Matteo Grioni[1], Arianna Calcinotto[1,2], and Matteo Bellone[1,*]

[1]Cellular Immunology Unit; San Raffaele Scientific Institute; Milan, Italy; [2]Università Vita-Salute San Raffaele; Milan, Italy

Keywords: cancer, vaccines, cytotoxic T lymphocytes, TRAMP, B16, melanoma, memory, T cell, prostate cancer, adjuvant

Using both transplantable and oncogene-driven autochthonous tumor models challenged with dendritic cell-based vaccines, we have recently found that boosting provides a clear advantage in prophylactic settings, unless performed on an excessively tight schedule, which causes the loss of central memory T cells. In therapeutic settings, boosting turned out to be always detrimental.

Therapeutic anticancer vaccines have eventually reached the bedside, but their clinical effects are limited. This might (at least in part) reflect our limited knowledge on the behavior of tumor-specific T lymphocytes in cancer patients. Indeed, it can be argued that T cells are stimulated by the vaccine on one hand, while—on the other hand—sensing the endogenous antigen either directly on tumor cells or via antigen-presenting cells. Such a continuous antigen stimulation in a microenvironment that is often immunosuppressive may favor T-cell exhaustion. Thus, whether, how and how frequently a cancer patient should be boosted upon vaccination remains an open conundrum.

The therapeutic potential of anticancer vaccines stems from their ability to stimulate a strong and long-lasting memory T-cell response against tumor-associated antigens (TAAs). Memory T cells can be distinguished in central memory (T_{CM}) and effector memory (T_{EM}) cells, which have different functional and phenotypic characteristics.[1] In particular, a greater antitumor function has been attributed to T_{CM} cells compared with T_{EM} cells.[2]

On the basis of these clues, we have recently investigated the impact of dendritic cell (DC)-based vaccines and different vaccination schedules on the persistence and antitumor activity of T_{CM} cells, in both prophylactic and therapeutic settings. Assuming that fully activated T_{EM} cells immediately respond to an antigenic challenge whereas quiescent T_{CM} cells must get activated first,[3] we have set up a long (24 h) ex vivo intracellular interferon γ (IFNγ)-specific assay to better detect the latter population.[4] Adopting this strategy, we have been able to demonstrate that, in healthy mice, a single DC-based vaccination elicits an antigen-specific immune response that lasts for at least 5 mo in the absence of subsequent antigen stimulation, confirming what has been reported for healthy humans[5] and extending this concept to T_{CM} cells.[4]

We have also found that boosting has a considerable impact on the pool of IFNγ-producing cytotoxic $CD8^+$ T_{CM} cells, which exceeds by more than 2-fold the pool detected in non-boosted mice.[4] This holds true for both exogenous and endogenous antigens, which are recognized by T cells bearing high- and low-affinity TCR, respectively.[4]

However, the timing of boosting is critical. Indeed, a lag of at least 4 weeks was required to obtain the most potent T_{CM} response, correlating with the ability of vaccinated mice to reject a challenge with B16F1 melanoma cells.[4] When mice received booster injections at earlier time points (i.e., after a 2-week interval; tight boosting), a reduced amount of T_{CM} cells was found in the spleen and the survival curve of these mice resembled that of mice that received only the priming injection.[4] Unexpectedly, also boosting with complete and incomplete Freund's adjuvants (CFA and IFA, respectively), even when performed at 4-week intervals, was detrimental for the pool of T_{CM} cells.[4] These findings are in line with a recent report showing that IFA leads to the trapping of tumor-specific $CD8^+$ T cells at the vaccination site, where they become dysfunctional and undergo apoptosis.[6]

The effect of boosting was totally unexpected in the context of minimal residual disease, which most likely benefits of vaccination. Indeed, when mice were challenged with B16F1 cells and the first dose of vaccine was given one day later, when a well-defined mass of viable melanoma cells is clearly visible at the inoculation site, no difference was found in the overall survival of mice primed and either boosted (at 2- or 4-week intervals) or not.[4] Strikingly, a very tight (i.e., weekly) boosting schedule reduced the survival of vaccinated melanoma-bearing mice (**Fig. 1**). Even more surprisingly, while priming was indispensable, a 4-week boosting schedule was detrimental for the treatment of transgenic adenocarcinoma of the mouse prostate (TRAMP) mice bearing advanced autochthonous prostate cancers.[4] Along similar lines, a tight boosting regimen has been shown

*Correspondence to: Matteo Bellone; Email: bellone.matteo@hsr.it
Submitted: 05/13/13; Accepted: 05/13/13
Citation: Ricupito A, Grioni M, Calcinotto A, Bellone M. Boosting anticancer vaccines: Too much of a good thing? OncoImmunology 2013; 2:e25032; http://dx.doi.org/10.4161/onci.25032

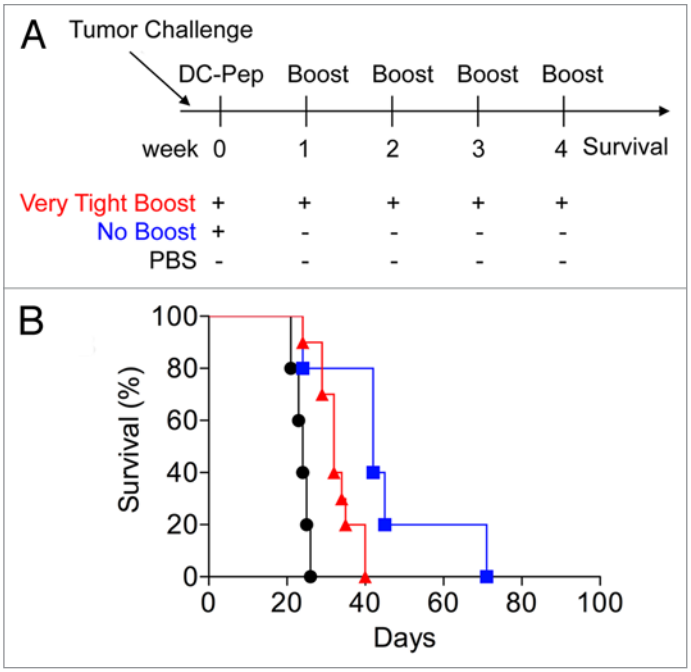

Figure 1. A very tight boosting schedule has a negative impact on the therapeutic efficacy of a dendritic cell-based anticancer vaccine. (**A**) Mice were challenged s.c. with 5×10^4 B16F1 melanoma cells and, one day later, either given PBS (n = 5) or primed with dendritic cells (DCs) pulsed with the cytotoxic T lymphocyte epitope $TRP2_{180–188}$ (DC-pep, n = 15). A fraction of vaccinated mice (n = 10) was thereafter boosted every week with the same vaccine (Very Tight Boost). Animals were followed for disease progression, and they were euthanatized when tumors reached a diameter of 10 mm or became ulcerated. (**B**) Kaplan–Meier survival curves representative of 2 independent experiments are reported. Long-Rank test: No Boost vs. PBS, p = 0.01; Really Tight Boost vs. PBS, p = 0.0003; Really Tight Boost vs. No Boost, p = 0.01.

to negatively influence the therapeutic potential of adoptively transferred cytotoxic T lymphocytes when compared with a single inoculation.[7] Thus boosting is either dispensable or detrimental in these preclinical scenarios.

We are not aware of any study in humans that has directly compared different vaccination schedules. However, the results of two subsequent studies on the efficacy of a bacillus Calmette-Guérin (BCG)-adjuvanted vaccine in advanced melanoma patients upon surgical resection of the primary lesion suggest that an increase in the frequency of booster vaccinations is associated with a reduced median survival (36 vs. 32 mo), reinforcing the results obtained by us[4] and others.[6,7] Thus, the activation of a TAA-specific immune response with prime vaccine injections appears to be essential to promote tumor eradication. Conversely, boosting strategies in subjects with residual disease or undergoing tumor recurrence should be carefully revisited.

Why is boosting so detrimental in therapeutic settings? We speculate that the release of antigens from neoplastic lesions naturally boosts vaccine-induced immune responses, while exogenous boosts may expose T cells to excessive antigen stimulation. Indeed, the chronic exposure of T cells to TAAs may drive exhaustion.[3]

Altogether, our results should warn against including excessively tight boosting schedules in the design of preventive vaccines and should also prompt clinical trials that specifically address the impact of boosting on tumor-specific T_{CM} cells in cancer patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Sallusto F, Lenig D, Förster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. Nature 1999; 401:708-12; PMID:10537110; http://dx.doi.org/10.1038/44385
2. Klebanoff CA, Gattinoni L, Restifo NP. CD8+ T-cell memory in tumor immunology and immunotherapy. Immunol Rev 2006; 211:214-24; PMID:16824130; http://dx.doi.org/10.1111/j.0105-2896.2006.00391.x
3. Sallusto F, Lanzavecchia A, Araki K, Ahmed R. From vaccines to memory and back. Immunity 2010; 33:451-63; PMID:21029957; http://dx.doi.org/10.1016/j.immuni.2010.10.008
4. Ricupito A, Grioni M, Calcinotto A, Hess Michelini R, Longhi R, Mondino A, et al. Booster vaccinations against cancer are critical in prophylactic but detrimental in therapeutic settings. Cancer Res 2013; In press; PMID:23539449; http://dx.doi.org/10.1158/0008-5472.CAN-12-2449
5. Dhodapkar MV, Krasovsky J, Steinman RM, Bhardwaj N. Mature dendritic cells boost functionally superior CD8(+) T-cell in humans without foreign helper epitopes. J Clin Invest 2000; 105:R9-14; PMID:10727452; http://dx.doi.org/10.1172/JCI9051
6. Hailemichael Y, Dai Z, Jaffarzad N, Ye Y, Medina MA, Huang XF, et al. Persistent antigen at vaccination sites induces tumor-specific CD8+ T cell sequestration, dysfunction and deletion. Nat Med 2013; 19:465-72; PMID:23455713; http://dx.doi.org/10.1038/nm.3105
7. LaCelle MG, Jensen SM, Fox BA. Partial CD4 depletion reduces regulatory T cells induced by multiple vaccinations and restores therapeutic efficacy. Clin Cancer Res 2009; 15:6881-90; PMID:19903784; http://dx.doi.org/10.1158/1078-0432.CCR-09-1113