Antigen depots: T cell traps?

Sacha Gnjatic & Nina Bhardwaj

Although cancer vaccines can induce tumor-specific cytotoxic CD8+ T cells, tumors treated by vaccination often fail to regress. A study in mice provides a potential explanation for this phenomenon by showing that a peptide vaccine in a water-oil adjuvant leads to the trapping of tumor-specific CD8+ T cells at the vaccination site, instead of promoting an effective T cell response at the tumor site (pages 465–472).

Three years ago, a young patient with synovial sarcoma received an experimental cancer vaccine containing antigen in a water-in-oil emulsion in one of our clinical trials. Today, her tumor may not have completely disappeared, but neither did her vaccine, which remains as hard bumps in her skin at the original injection sites. She now wonders whether these persistent 'antigen depots' help keep her cancer in check.

Using oil to emulsify antigens for human vaccines dates back to 1915 when C.C. Warden, an American physician, reported the results of a therapeutic vaccine against gonorrhea prepared with gonococcus fats[1]. A year later in France, Le Moignic and Pinoy demonstrated that a petroleum jelly oil and water emulsion, aided by lanolin, improved the efficacy of antityphoid vaccines in mice and humans by protecting antigens and slowing down vaccine resorption[2]. It took another quarter of a century for Jules Freund to rediscover the effectiveness of water-in-oil emulsion for inoculations, which led to his formulation of complete (with mycobacterial lysates) and incomplete (water in oil only) adjuvants based on paraffin oil. To this day, incomplete Freund's adjuvant (IFA) is the most common adjuvant used for experimental vaccine procedures in animals[3].

The success of Freund's adjuvants led to the development of a more refined commercial version of light mineral oil and surfactant for human injection, Montanide ISA 51 (made by Seppic)[3]. Widely used as an experimental vaccine adjuvant, Montanide elicits immunity in humans toward infectious agents and tumor antigens[3]. In general, adjuvants are vaccine constituents intended to efficiently prime B and T cells by activating antigen-presenting cells (APCs), such as dendritic cells (DCs), and enhancing their capacity to process and present antigens to lymphocytes. This results in the generation of durable immune responses that can be rapidly deployed when antigens are reencountered in the form of pathogens or tumor cells. The adjuvanticity of oil-water emulsions is superior to those of water-soluble compounds as they protect antigen from dilution, degradation and rapid elimination. Given that oil-water emulsion adjuvants act as antigen depots that can persist for several months (evidenced by flare-ups of previous injection sites upon recall immunizations), they may lead to higher immunogenicity and protection due to sustained antigen release. Moreover, the presence of antigen in the oil phase may facilitate its uptake by APCs. Lipoid structures may also have immunomodulatory activity of their own, through inciting local tissue inflammation and necrosis and thereby enhancing APC activation and/or through facilitating antigen uptake by APCs[4].

In this issue of *Nature Medicine*, Hailemichael *et al.*[5] challenge the use of antigen in IFA emulsions for vaccination. They report that although they are capable of priming tumor-specific T cells, the emulsions trap primed T cells at vaccination sites rather than tumor sites, and the T cells ultimately die at the vaccination sites (**Fig. 1**). Similar to the case in a prior study[6], the outcome is a reduced response to subsequent booster vaccinations and a failure to control tumor growth.

Using an established mouse model of melanoma, the authors first confirmed that sequestration of killer CD8+ T cells requires persistence of antigen in IFA, as vaccines consisting of antigen and water failed to trap T cells at inoculation sites (in addition to being less immunogenic)[5]. Antigen persistence has many important consequences. Locally produced chemokines and cytokines drive T cells into the vaccination site where they develop an 'exhausted' phenotype, similar to that of T cells circulating in high-antigen environments such as advanced cancers and chronic viral infections[7], and lose their ability to eliminate tumor cells. Furthermore, the vaccine site fills with macrophages and neutrophils expressing ligands that reinforce T cell dysfunction and ultimately induce their death[5]. These findings help to explain why several Montanide-based vaccines that successfully prime blood tumor–specific responses in humans fail to promote tumor regression[8].

How can the more desirable adjuvant properties of IFA (strong priming) be applied for successful therapeutic vaccination? Hailemichael *et al.*[5] first tried to boost the adjuvanticity of IFA by adding a combination of strong proimmunogenic signals (an antibody to CD40 and a Toll-like receptor (TLR) agonist, both DC-activating agents, along with the T cell growth factor interleukin-2; 'COVAX'). Although COVAX improved the immunogenicity of short-lived vaccine formulations (peptide antigen and water), it only partially reversed local antigen-driven chemokine induction, T cell sequestration and dysfunction at vaccination sites. When the authors substituted the short nine-amino-acid melanoma peptide antigen that is conventionally used in IFA vaccines for a longer extended 20-amino-acid peptide encompassing the nonamer epitope, they found this resulted in reduced vaccine-site T cell sequestration and deletion and superior antitumor activity compared with the short peptide (**Fig. 1**). This result mimics recent studies in humans in which CD8+ T cell priming was preferentially sustained when long peptides were emulsified in Montanide, and the improved priming correlated with clinical outcome[9,10].

Sacha Gnjatic and Nina Bhardwaj are at the Tisch Cancer Institute at the Icahn School of Medicine at Mt. Sinai, New York, New York, USA.
e-mail: nina.bhardwaj@mssm.edu

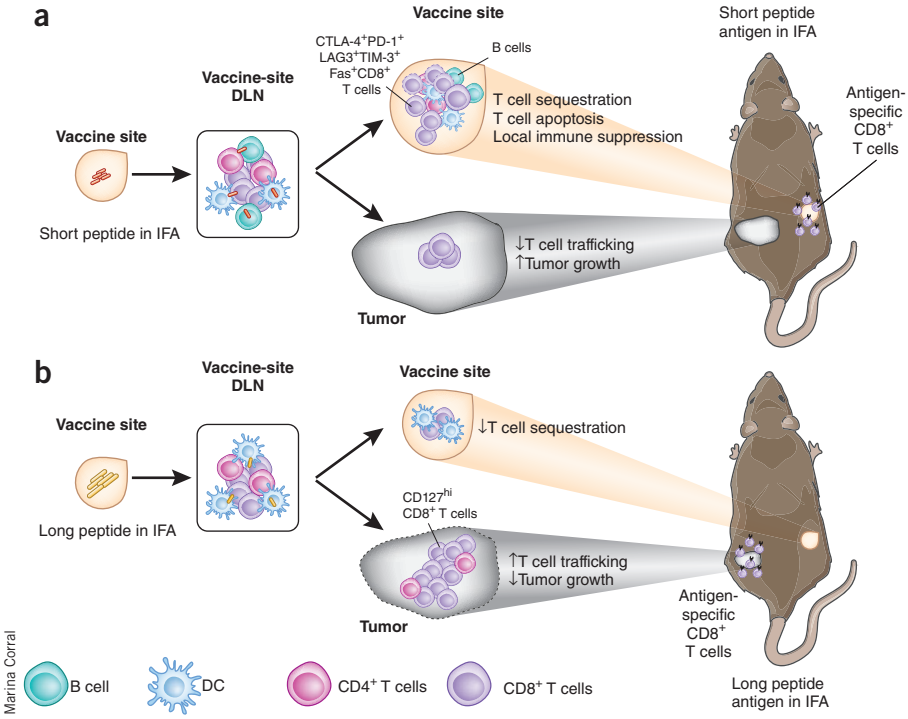

Figure 1 Antigen-driven T cell trapping in vaccine sites. (**a**) Short tumor peptide antigen emulsified in IFA primes $CD8^+$ T cells that preferentially traffic to the original vaccine site. Short peptide antigen can be presented to T cells by many types of APCs in the vaccine-site DLN, including DCs and B cells. Hailemichael *et al.*[5] now show that, once primed, antigen-specific T cells accumulate at the antigen-rich vaccine site, where they become dysfunctional, upregulating markers of exhaustion (CTLA-4, PD-1, LAG-3 and TIM-3) and ultimately undergoing apoptosis via Fas–FasL interactions. Tumor growth can proceed, as there is little infiltration of tumor-specific T cells. (**b**) Long tumor peptide antigen in IFA relocates T cells toward tumors and enhances antitumor activity. Long peptides are preferentially processed and presented to T cells by DCs in the DLNs and in the vaccine site, leading to more efficient T cell priming. Tumor-specific $CD8^+$ T cells ($CD127^{hi}$) preferentially traffic to tumor sites where they eliminate tumor cells. There is reduced trafficking of tumor-specific T cells to the vaccine site, probably because only DCs, but not other types of APCs, can present the minimal antigenic epitope to T cells.

Why are longer peptides more efficient antigens? Efficient T cell priming requires that DCs capture complex antigens from peripheral sites (in this case the vaccine site) and traffic to draining lymph nodes (DLNs) where they present processed, short antigenic epitopes on their human leukocyte antigen (HLA) molecules to T cells. The minimal epitopes used in many vaccines bypass the requirement for preprocessing; therefore, they can be directly acquired by all APCs, including those that lack the capacity to process long antigens (such as B cells and macrophages). Because they lack the inherent adjuvanticity of DCs, these nonprofessional APCs may block optimal T cell priming. In this study, although the short melanoma peptide was found on B cells in vaccine-site DLNs, the elimination of B cells did not affect the induction of T cell dysfunction[5]. In contrast, the longer peptides were processed and presented as minimal epitopes only by DCs in DLNs, and possibly also at the vaccine site where DCs can accumulate and present antigens[11]. This could account for the induction of strong antigen-specific $CD8^+$ T cells and effective antitumor immunity by long vaccine peptides.

It is unclear why long peptides do not lead to the sequestration of effector responses in the way short peptides do. A likely explanation is that as long peptides must be taken up and processed by DCs, there is less chance for locally accumulated antigens at the injection site to be efficiently presented by nonprofessional APCs[9]. Another hypothesis is that long peptides may inherently activate DCs differently than short peptides do and may facilitate the migration of DCs to DLNs, leading to systemic immunization. Moreover, T cell priming may heavily rely on the cross-presentation of antigen-bearing migrating DCs by DLN-resident DCs[12]. Long peptides have the additional advantage of containing minimal epitopes for $CD4^+$ T cells, which, when simultaneously activated, help $CD8^+$ T cells to acquire their full potential. Unlike short peptides, which must be matched to the HLA type of the recipient, long peptides can be administered to patients who express virtually any HLA alleles, as their processing occurs *in vivo*.

The substitution of short peptides for long ones mitigates many of the undesirable qualities of IFA. However, the local necrosis and inflammation that accompanies IFA injection has precluded its use in healthy humans. Either lower does of IFA (the authors used one-fifth of the dose used in humans) or, preferably, nonpersistent and rapidly biodegradable adjuvants may be better choices for future vaccine development. Indeed, the authors showed that peptide-saline vaccines combined with COVAX were superior to IFA-based vaccines plus COVAX. Thus, combining long peptides with short-lived formulations may confer superior adjuvanticity and reduce local toxicity. The good news is that IFA for humans comes in many varieties. For example, MF59, a squalene oil compound, is an oil-in-water adjuvant that has been safely used in seasonal influenza vaccines and is partially dependent upon TLR and inflammasome pathway adaptors for optimal adjuvanticity[4,13]. By taking advantage of nontoxic oil-in-water emulsions that induce endogenous TLR and inflammasome agonists, it may be possible to safely incorporate long peptides into therapeutic vaccines to achieve effective immunity. These may eventually be helpful for other antigen formulations, including full-length proteins, which are currently being tested by our group for immunogenicity when combined with TLR ligand in the presence or absence of Montanide (ClinicalTrials.gov registry NCT01079741).

Fortunately for the patient with synovial sarcoma, her vaccine was long peptides emulsified in IFA and a TLR agonist, so it may be that, in her case, some bumps are OK.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

1. Warden, C.C. *J. Am. Med. Assoc.* **65**, 2080–2084 (1915).
2. Le Moignic, E. & Pinoy, E. *C. R. Hebd. Seances Mem. Soc. Biol.* **79**, 201–203 (1916).
3. Stills, H.F. Jr. *ILAR J.* **46**, 280–293 (2005).
4. Seubert, A. *et al. Proc. Natl. Acad. Sci. USA* **108**, 11169–11174 (2011).
5. Hailemichael, Y. *et al. Nat. Med.* **19**, 465–472 (2013).
6. Muraoka, D. *et al. J. Immunol.* **185**, 3768–3776 (2010).
7. Wherry, E.J. *Nat. Immunol.* **12**, 492–499 (2011).
8. Rosenberg, S.A., Yang, J.C. & Restifo, N.P. *Nat. Med.* **10**, 909–915 (2004).
9. Kenter, G.G. *et al. N. Engl. J. Med.* **361**, 1838–1847 (2009).
10. Sabbatini, P. *et al. Clin. Cancer Res.* **18**, 6497–6508 (2012).
11. McLachlan, J.B., Catron, D.M., Moon, J.J. & Jenkins, M.K. *Immunity* **30**, 277–288 (2009).
12. Yewdall, A.W., Drutman, S.B., Jinwala, F., Bahjat, K.S. & Bhardwaj, N. *PLoS ONE* **5**, e11144 (2010).
13. Ellebedy, A.H. *et al. Proc. Natl. Acad. Sci. USA* **108**, 2927–2932 (2011).