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Chemokine-enhanced DNA vaccination in cancer immunotherapy

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We have demonstrated that priming of intratumoral and intradermal vaccination sites with chemokines enhances cytotoxic immune response against established neoplasms. Additional insights into the molecular mechanisms that underlie these findings and the optimization of such an approach may lead to the development of cost-effective and generic immunotherapeutic regimens against cancer.

During the past decade, immunotherapy has emerged as a powerful modality to treat aggressive neoplasmsfor which a few (if any) curative treatment modalities are currently available. Cell-based immunotherapies, including the adoptive transfer of tumor-infiltrating lymphocytes, dendritic cell (DC)-based vaccines and T cells expressing recombinant tumor-specific T-cell receptors (TCRs) or chimeric antigen receptors (CARs), showed promising results in both pre-clinical and clinical settings. Nevertheless, the widespread application of these strategies to treat cancer is hindered by several factors.[1] Along similar lines, the administration of cytotoxic T lymphocyte-associated protein 4 (CTLA4)-targeting antibodies (such as Ipilimumab) to patients with advanced melanoma appears to be particularly effective in the elicitation of tumor-specific immune responses. However, severe adverse events related to the unspecific activation of T cells at the systemic level (and hence to the elicitation of autoimmune reactions) remain a barrier to the widespread application of this immunotherapeutic regimen.[2] Active vaccination protocols based on tumor-associated antigens, including peptide- and DNA-based vaccines, have also been tested in different pre-clinical and clinical settings.[3] Although these approaches offer multiple advantages and have been associated with antineoplastic activity in pre-clinical settings, the lack of efficacy in clinical scenarios offsets the advantages of peptide- and DNA-based anticancer vaccines. Hence, there is an unmet need to improve these approaches to achieve adequate activation of tumor-specific immunity and clinically significant outcomes.

Multiple studies demonstrated that recruitment of different subsets of leukocytes to neoplastic lesions facilitates recognition and elimination of malignant lesions. As we had previously observed that elevated intratumoral levels of a secondary lymphoid chemokine, CCL21effectively recruit T cells to the deep recesses of melanoma mass, hence boosting tumor elimination,[4] we hypothesized that altering chemotactic gradients within established tumors would improve the therapeutic efficacy of anticancer vaccines. Consistently, we recently reported a significantly greater tumor growth inhibition in CCL21-primed tumors following DNA vaccination (**Fig. 1A**).[5] This vaccination protocol resulted in the regression of the pre-established melanomas associated with generation and expansion of the intratumoral antigen (Ag)-specific cytotoxic T cells (CTL) (**Fig. 1A**).

Two distinct components of this immunotherapeutic regimen favorably influenced its outcome: the composition of the DNA-based vaccine and the priming of neoplastic lesions with chemokines. In our hands, administration of a fusion protein containing the pan DR epitope (PADRE), which enhances activation of helper, effector and memory $CD8^+$ T cells in mice and humans,[6,7] was critical for the activation of tumor-specific immune response. On the other hand, priming of neoplastic lesions with selected chemokines, namely, CCL20 and CCL21 (alone or in combination), led to somehow contrasting results. Indeed, while the expression of CCL20 was expected to recruit immature DCs and promote both antigen uptake and activation of tumor-specific T cells, elevated levels of this chemokine led to the recruitment of regulatory T cells, which inhibited vaccine-elicited immune response. On the contrary, priming of melanoma lesions with CCL21 alone resulted in the activation of superior melanoma-specific immune response. Although we and others have previously demonstrated that a permanent intratumoral gradient of CCL21 facilitates recognition of malignant cells by the immune system,[4] our recent findings demonstrated that even a transient gradient of this chemokine

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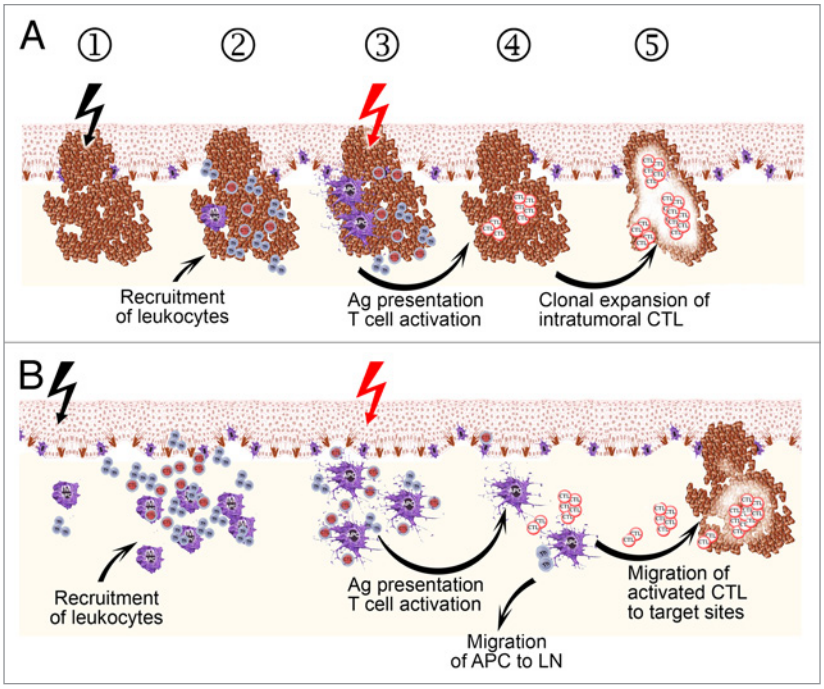

Figure 1. Intratumoral and intradermal chemokine-enhanced DNA-based anticancer vaccination. (1) Priming of intratumoral (**A**) and intradermal (**B**) vaccination sites with chemokines. (2) Chemokine-mediated recruitment of T cells alone (**A**) or accompanied by immature antigen-presenting cells (APCs) (**B**). (3) Administration of DNA-based vaccines into chemokine-primed tissues, leading to enforced antigen expression by immature APCs, APC maturation, extranodal antigen presentation via MHC class I mechanism , and T-cell activation. (4) Immediate recognition of target (cancer) cells by activated antigen-specific cytotoxic T lymphocytes (CTLs) and CTL expansion (**A**), or emigration of activated CTLs from the skin to antigen-expressing neoplastic lesions and relocation of vaccinated APCs to draining lymph nodes (LNs) and intranodal T-cell activation (**B**). (5) Killing of antigen-expressing target cells by activated CTLs.

established prior to DNA vaccination favors the accumulation, activation and expansion of tumor-specific T cells.[5] This is consistent with previous data demonstrating that CCL21 can promote the clonal expansion of T cells.[8] Thus, our data clearly demonstrate the efficacy of priming malignant lesions with specific chemokines prior to the administration of a therapeutic DNAanticancer vaccine. The optimization of this approach is likely to result in the development of a generic and cost-effective immunotherapeutic regimen for the treatment of unresectable (metastatic) tumors.

Currently, DNA-based anticancer vaccines are most commonly administered via the intramuscular route, a setting in which multinucleated, elongated muscle cells express target antigens to high levels, favoring antigen uptake and cross-presentation by intramuscular DCs. On the contrary, the intradermal route allows for direct antigen presentation via MHC class I mechanism by DNA-transfected cutaneous antigen-presenting cells (APCs), including Langerhans cells and DCs.[3] We therefore hypothesized that chemokine-mediated recruitment of immature DCs to the vaccine administration site may favor direct antigen presentation to T cells by vaccinated DCs. We werealso convinced that the concurrent recruitment of T cells may further promote antigen presentation at the vaccination site. In support of these notions, we have recently demonstrated that the simultaneous recruitment of DCs and T cells to the skin prior to DNA vaccination, allows for extranodal antigen presentation via MHC class I molecules, T-cell activation, expansion, and antigen-specific cytotoxic immune response in both prophylactic and therapeutic settings (**Fig. 1B**).

In summary, further optimization of the priming procedures is likely to allow for the efficient recruitment of immature DCs and naïve T cells to the cutaneous tissue, possibly resulting in the formation of transient lymph node-like structures similar to those observed in mucosal tissues,[9,10] or for the intratumoral accumulation of T cells, 2 are expected to improve the efficacy of therapeutic DNA-based anticancer vaccines. It is also plausible that optimizing the composition of DNA-based vaccines, for instance by including various immunostimulatory epitopes or interferon-responsive factors, and/or administering them in the context of CTLA4 inhibition would promote the DC-mediated activation of tumor-specific CTLs and hence the elicitation of therapeutically relevant tumor-specific immune response.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were to be disclosed.

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