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Of microspheres and microbes
A double-hit strategy for cancer immunotherapy

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Efficient immunotherapy relies on the rapid generation of elevated amounts of cancer-specific T lymphocytes. We have recently demonstrated that both rapid and potent tumor-targeting immune responses can be induced with a heterologous prime-boost regimen consisting of poly-lactic co-glycolic acid (PLGA) microsphere-based immunization followed by *Listeria monocytogenes* infection.

To avoid elimination by the immune system, malignant cells establish a number of immunosuppressive mechanisms that inhibit cancer-specific immune responses. Owing to such a tumor antigen-specific immunosuppression, current anticancer vaccines induce immune responses that generally remain below the limit of detection. Ideally, vaccination should allow for the induction of T-cell responses that are as robust and rapid as possible. Functional studies of T lymphocytes, however, have demonstrated that these 2 features of T-cell responses are nearly mutually exclusive, as strong inflammatory signals drive the rapid replication of T cells but prevent the acquisition of the memory features that are required for boosting.[1,2] A solution to this problem may be provided by heterologous prime-boost vaccination regimens, involving antigen carriers that induce different types of systemic inflammation. In this setting, T cells are primed under conditions of low systemic inflammation followed by booster immunizations with highly inflammatory viral or bacterial vectors. Ideal priming conditions of $CD8^+$ T cells in the absence of inflammation have been achieved by means of autologous dendritic cell (DC)-based immunizations, which generate T cells with early memory characteristics that can be boosted productively less than 1 wk after priming.[3] The isolation and expansion of autologous human DCs, however, is costly and time-consuming. Synthetic polymers such as poly-lactic co-glycolic acid (PLGA) represent a promising "off-the-shelf" alternative to autologous DCs. PLGA microspheres are components of multiple FDA-approved long-acting release (LAR) formulations, in which they are coupled to hormones to form biodegradable, long-lasting drug depots.[4] When conjugated to PLGA and injected into mice, whole proteins or polypeptides are taken up by DCs and cross-presented to $CD8^+$ T cells on MHC class I molecules. Similar to DC-based immunizations, this approach promotes the proliferation of $CD8^+$ T cells with early memory characteristics that are able to expand rapidly upon antigenic re-challenge. As previously shown, the expansion of T cells rechallenged with an antigenic stimulus requires a strong systemic inflammation state that is best generated by live pathogens that express the same antigen.[5,6] *Listeria monocytogenes* (LM), a facultative intracellular pathogen that can secrete antigens into the cytosol of DCs upon infection, represents an ideal tool to promote the acquisition of an early memory phenotype by effector $CD8^+$ T lymphocytes. Based on these premises, we recently set out to test a combinatorial approach involving PLGA-based immunization and LM-based vaccination in a model of hepatic cancer immunotherapy.[7] Our results demonstrate that neither repeated immunizations with PLGA microspheres (resulting in weak inflammation) nor repeated administration of LM (which promote a strong inflammation) leads to optimal T-cell expansion, but only a sequential immunization with PLGA microspheres followed by LM does so. Compared with DC-based immunization, PLGA microspheres induced primary $CD8^+$ T-cell responses of limited magnitude, but exhibiting a superior proliferative potential. Indeed, the magnitude of the secondary immune responses induced by the sequential administration of PLGA microspheres and LM exceeded that of a DC- and LM-based vaccination performed according to the same schedule. Surprisingly, PLGA/LM-elicited immune responses could be further potentiated by the co-administration of PLGA microspheres with the Toll-like receptor 3 (TLR3) agonist polyinosinic:polycytidylic acid (polyI:C), but not other TLR agonists.

The therapeutic efficacy of the optimized prime-boost protocol involving PLGA microspheres plus polyI:C followed by LM was tested in a model of transplatable

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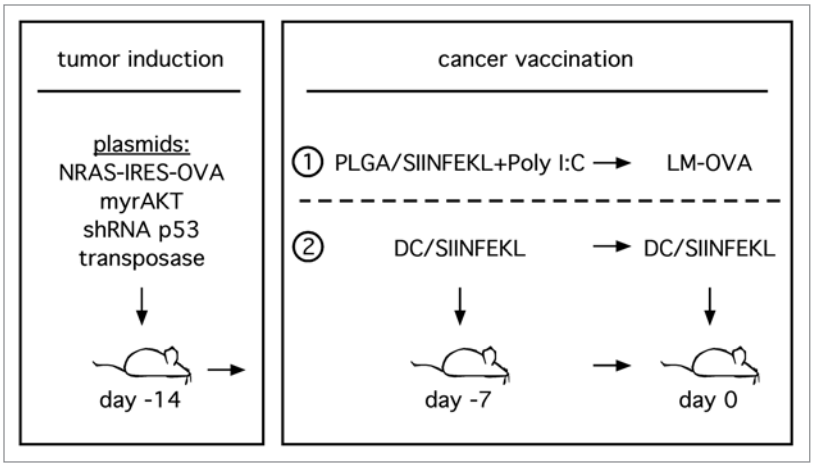

Figure 1. Experimental setup employed in our study. Autochthonous liver tumors were induced by the hydrodynamic injection (via the tail vein) of transposon-flanked plasmids encoding $NRAS^{G12V}$, ovalbumin (OVA), myristoylated AKT1 (myrAKT), a short hairpin RNA (shRNA) specific for p53 and a transiently expressed transposase (day 14). Subsequently, mice were either vaccinated with SIINFEKL-coupled poly-lactic co-glycolic acid (PLGA) plus polyinosinic:polycytidylic acid (polyI:C; day -7) and OVA-expressing *Listeria monocytogenes* (LM-OVA; day 0) or 2 injections of SIINFEKL-loaded dendritic cells (DC- SIINFEKL; day 7 and day 0).

hepatoblastoma, in which it promoted the complete regression of established tumors. Additionally, we generated autochthonous liver cancers by the hydrodynamic injection into the tail vein of plasmids encoding transposon-flanked oncogenes, microRNA targeting tumor suppressor genes, a model antigen and a transiently expressed transposase (**Fig. 1**). In this experimental setup, the therapeutic activity of PLGA/LM-based vaccination exceeded that of repeated DC-based immunizations, eradicated established neoplasms, and prolonged the survival of cancer-bearing mice.

Our findings highlight the need for studies that carefully examine the kinetics of vaccine-induced T-cell responses as well as the phenotype and function of the resulting T lymphocytes in the context of cancer immunotherapy. Our data also suggest that current anticancer vaccines do not exploit the full therapeutic potential of $CD8^+$ T cells. The low frequency of cancer-specific T cells represents an important obstacle to current and future immunotherapeutic interventions. Even more importantly, it precludes a better understanding of the physiology of $CD8^+$ T-cells in humans, since weak immune responses can neither be sufficiently monitored nor studied with regard to phenotype and function. Thus, the generation of potent T-cell immune responses may represent an important step toward a more rational design of anticancer vaccines.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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