Low-Frequency Sonophoresis Assisted Cancer Immunotherapy

by

Diviya Sinha

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Abstract

Inducing and maintaining a long-lived potent cellular CTL (Cytotoxic T Lymphocyte) response is of significant interest to the global scientific community. Protection against most intracellular pathogens (e.g., HIV) and tumors require functional CTL immunity and are ineffectively treated by antibodies or humoral immunity. Traditional needle-based vaccination strategies primarily induce humoral immunity and are inefficient at eliciting robust CTL responses using subunit or whole protein formulations. Viral adjuvant vectors capable of generating strong CTL responses face limitations of associated toxicity and anti-vector immunity limiting their boosting potential. Hence, the alternative availability of adjuvant-free vaccination strategies capable of inducing a decades long or lifetime potent CTL response using simple vaccine formulations would be very desirable.

In this thesis, it is demonstrated that a short 30 second pretreatment of the skin with low-frequency ultrasound, also known as low-frequency sonophoresis (LFS), followed by topical ovalbumin antigen application, results in the induction of a potent CTL response in the absence of any co-administered adjuvants. LFS is a minimally invasive, FDA approved technique for the transdermal delivery of lidocaine. Significant research has been carried out on its applicability for transdermal drug delivery. However, there are only a few reports on its use for cutaneous immunization (CI), and these have focused on induction of humoral immunity. Here, we fill this gap in knowledge by investigating the cellular arm of the immune response upon LFS CI.

LFS pretreatment of the skin caused rapid dispersion of topically applied antigen into the skin and draining lymph nodes, targeting both skin and lymph node resident dendritic cells, which are known to be potent activators of the CTL response. Additionally, only antigen specific T cells were activated and proliferated upon LFS immunization, eliminating the possibility of general inflammation in the absence of antigen causing non-specific T cell activation. Physical perturbation of the skin by LFS, resulting in the secretion of inflammatory cytokines by skin immune cells, is hypothesized to induce the observed polyfunctional Th1 CD4 and CTL immune responses. These resulting attributes of the T cells have been correlated with strong efficacy for

protection and treatment with regards to cancer vaccines and viral infections, including HIV. The potency of resting CTLs in LFS immunized mice was investigated with a viral LCMV challenge months after a single immunization. Following the viral challenge, LFS-immunized mice had a much faster recall response of more than 24 hours ahead of, and an order of magnitude higher expansion of CTLs than, both the priming phase and the naïve control groups. This observation of long-lived potent CTLs was subsequently followed-up in the functional investigation of CTLs in tumor therapy. Functional potency of induced CTL cells for effective therapy was investigated against two subcutaneous tumor challenge models, the B16-OVA melanoma and EG7-OVA thymoma, wherein significant prolonged survival and tumor rejection through LFS CI was attained in the respective tumor challenges.

In summary, the findings of this thesis demonstrate the potential for LFS skin immunization as an adjuvant-free vaccination strategy for protection against viral infections and tumors via induction of robust CTL responses using simple vaccine formulations.

Thesis Supervisor: Daniel Blankschtein
Title: Herman P. Meissner (1929) Professor of Chemical Engineering

Thesis Supervisor: Robert S. Langer
Title: David H. Koch (1962) Institute Professor