Spotlight

By Anne Forde

Safe and Effective Immunization Regimes

Oosterhuis *et al*.
http://doi.wiley.com/10.1002/ijc.25894

Mueller *et al.*
http://doi.wiley.com/10.1002/ijc.25914

Ongoing expression of HPV16 proteins E6 and E7 is required for cervical carcinogenesis, making them ideal targets for immunological interventions. E6 and E7 DNA vaccines offered promising results in mice models but performed poorly in clinical trials. Therefore, the authors took a different tack by using DNA tattoo vaccination – an intradermal approach – and compared a range of E7 and E6 DNA vaccines encoding an immunodominant T-cell epitope, full-length E7 or E6 to allow for MHC polymorphism; point-mutated E7 and E6 where the oncogenic potential of the proteins was disrupted; and, finally, a 'gene shuffled' vaccine where the oncogenic potential of the protein is completely removed.

Immunogenicity to these vaccine constructs varied greatly – only the epitope-specific vaccine performed well. But the authors wanted to make an effective vaccine that encompassed the full epitope-encoding potential of E7 or E6 and used the nononcogenic forms of E6 or E7. They fused Tetanus toxin fragment 1 — which is known to enhance specific $CD8^+$ T cells when fused to the antigenic peptides — to wild-type or point-mutated versions of the E7 vaccine. Vaccination of mice with this Tetanus toxin fragment fusion vaccine significantly increased the $CD8^+$ T cell response and these cells produced IFN-γ upon E7 peptide stimulation, reacted strongly to a secondary antigen encounter and could specifically kill nearly 60% of target cells. Gene-shuffled E7 and E6 fused with the Tetanus fragment also showed enhanced immunogenicity. *In vivo* mice inoculated with E7 expressing tumors, and then vaccinated with Tetanus fused to gene-shuffled E7 showed tumor regression and prolonged survival.

These encouraging results show that using the Tetanus fragment means the DNA vaccine can be effective as a full-length gene with the oncogenic potential removed. The authors intend to test this approach starting with a phase 1 clinical trial.

In the second paper, Mueller *et al.*, also generate a strong anti-tumor immune response using vaccination but in this case they investigate an alternative to incomplete Freund's adjuvant (IFA). IFA illicts a potent immune response but has adverse side effects and is not approved for routine immunotherapy in humans. The

authors tested biodegradable PLGA microspheres -- which are approved for human use -- as an antigen carrier system. They loaded these microspheres with ovalbumin (OVA) and the pattern molecule CpG (a member of the pathogen-associated molecular pattern, PAMP) and in some experiments they added microspheres with polyI:C.

BL/6 mice immunized with OVA and CpG in microspheres or OVA and CpG in IFA showed a similarly robust IgG1, IgG2 but low IgE response. The authors increased the potency of the vaccines by administering polyI:C with both regimes. Mice vaccinated with either regime and then given ovalbumin specific $CD8^+$ and $CD4^+$ T cells from OT-1 and OT-2 mice stimulated *in vivo* proliferation of the transferred cells. Furthermore, a cytotoxic *in vivo* response was observed even when target cells were injected at 21 days after vaccination.

The ultimate test was whether microsphere based immunotherapy could eradicate tumors. When BL/6 mice were injected with OVA expressing thymoma or melanoma cells and immunized with the microsphere or IFA based regime as soon as there were palpable tumors, both regimes could cure the thymomas but had similar, if much poorer, effects against melanoma. When the authors induced lung metastatis with melanoma cells both formulations could markedly reduce the number of metastases formed.

The study clearly shows that the PLGA-microsphere administration route is equal or superior to IFA-based vaccination and, as it's safer than IFA ,warrants clinical investigation.