

## **Improved immunogenicity of adenoviral vaccines: Impact of tethering the vaccine antigen to MHC-class II associated invariant chain**

The ideal vaccine induces a potent protective immune response, which, should be rapidly induced, long-standing and of broad specificity. Recombinant adenoviral vectors induce potent antibody and CD8+ T-cell responses against transgenic antigens within weeks of administration, and they are among the most potent and versatile antigen delivery vehicles available. Here we show that the protective immune response to an adenovirus encoded vaccine antigen can be accelerated, enhanced, broadened, and prolonged by tethering of the recombinant antigen to the major histocompatibility complex class II-associated invariant chain. Thus, adenovirus vectored vaccines expressing lymphocytic choriomeningitis virus (LCMV) derived glycoprotein linked to invariant chain increased the CD4+ and CD8+ T-cell stimulatory capacity *in vitro* and *in vivo*. Antibody responses were unaltered by addition of Ii, and vesicular stomatitis virus glycoprotein tethered to the C-terminus of invariant chain efficiently induced neutralizing antibodies and protected against lethal intranasal infection with live virus. Furthermore, mice vaccinated with a single dose of adenovirus expressing lymphocytic choriomeningitis virus derived glycoprotein linked to invariant chain were protected against lethal virus-induced choriomeningitis, lethal challenge with strains mutated in immunodominant T-cell epitopes, and systemic infection with a highly invasive strain. In therapeutic tumour vaccination against tumour cells expressing a viral neo-antigen, the vaccine was as efficient as live virus. In comparison animals vaccinated with a conventional adenovirus vaccine expressing unmodified GP were protected against systemic infection, but only temporarily against lethal choriomeningitis, and this vaccine was less efficient in tumour therapy. Finally, while a significant protective effect of the unlinked vaccine against a lethal virus challenge was restricted to a few H-2 haplotypes, complete or nearly complete protection was observed in seven murine haplotypes tested. Taken together, tethering of the vaccine antigen to the invariant chain seem to represent a very marked improvement to the best of existing vaccine technologies.