VACCINES

Nano-adjuvant: double TLR stimulation is the key

Successful vaccines activate antibody responses that last a lifetime. However, the molecular underpinnings for the induction of such long-lived responses have remained enigmatic. Now, reporting in *Nature*, Pulendran and colleagues present a synthetic nanoparticle adjuvant that stimulates Toll-like receptor 4 (TLR4) and TLR7, thus synergistically enhancing the production of antigen-specific neutralizing antibodies. This conferred protection to lethal viral challenges in mice and induced robust immunity against the pandemic H1N1 influenza strain in *Rhesus macaques*.

The nanoparticle-based adjuvants that were designed for this study were similar to viral particles in size and composition, and were composed of the biodegradable polymer poly(D,L-lactic-co-glycolic acid) (PGLA). They were loaded with either the TLR4 ligand MPL (PGLA(MPL)) or the TLR7 ligand R837 (PGLA(R837)), or with both (PLGA(MPL + R837)), and administered together with nanoparticles that were loaded with antigen. Compared to the stimulation of either TLR4 or TLR7 alone, the double TLR stimulation significantly enhanced a high-affinity neutralizing antibody response to experimental vaccination. This effect was still evident after secondary immunization.

Further dissection of the immune response revealed that double TLR stimulation enhanced the secretion of pro-inflammatory cytokines by dendritic cells. It was also shown that simultaneous engagement of the two TLRs on the same B cells was required for the enhanced antibody response, as B cell-deficient mice that were reconstituted with a mixture of TLR4- and TLR7-signalling-deficient B cells did not show this effect. Interestingly, double TLR stimulation did not enhance the antibody responses of the early short-lived plasma cells, but enhanced the persistence of germinal centres and induced early programming towards memory B cell development. Similarly, it was found that there was a preferential effect on memory T cell generation.

The efficacy of the nano-adjuvant was demonstrated in mice that were challenged with lethal doses of the 2009 pandemic H1N1 influenza A virus or the H5N1 avian influenza virus. Compared to vaccination with whole inactivated virus (WIV) or WIV plus the widely used adjuvant alum, mice that were treated with WIV and PLGA(MPL+R837) showed enhanced antigen-specific humoral immunity and survival. The vaccination strategy was also tested in non-human primates, which — like humans — show a different TLR7 expression pattern on dendritic cells compared to mice. For double stimulation of all subsets of dendritic cells, the authors chose the combined TLR7–TLR8 ligand R848 instead of R837. Vaccination with H1N1 WIV and PLGA(MPL+R848) resulted in a greater than fivefold dose-sparing effect compared to vaccination with WIF alone, and the magnitude of antibody titres 28 days after a single immunization was much higher than what is considered the correlate of protection against influenza in humans.

The authors conclude that the nanoparticle-based vaccine recapitulates the immunogenicity of live viral vaccines and induces long-lived protection, possibly as a consequence of persistent antigen–adjuvant complexes in germinal centres. Besides providing fundamental insights into the factors that shape innate immunity, such nanoparticle-based adjuvants hold promise as a universal platform for vaccine design against pandemics and emerging infections.

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