DNA DELIVERY

Timing is everything

A new polymer for delivering DNA in synchrony with the life cycle of white blood cells stimulates a cell-killing immune response and makes DNA vaccines much more potent.

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Vaccination is the best and most cost-effective defence against many diseases, and has been known, in various forms, for hundreds of years. For example, in the Middle Ages the Chinese found that inhaling a powder made from smallpox scabs could protect from future infection. Modern vaccines are somewhat more palatable, comprising purified, inactivated microorganisms typically administered by a sterile — if painful — injection. Today's vaccines — perhaps the greatest medical advance of the twentieth century — generally introduce a weakened version of an antigen that stimulates the production of specific antibodies. These subsequently flag an invader for destruction before infection can take hold. In a new and promising approach, DNA vaccination, genes encoding an antigen are delivered to cells that then produce the antigen and display it on their surface[1]. In this way, the vaccine fools the immune system into thinking that a foreigner has already infected the body, prompting killer blood cells (cytotoxic T lymphocytes) to seek out and destroy cells that display the vaccine protein. In this issue of *Nature Materials*, Wang and co-workers — a group of academic and industrial researchers — present a new polymer material specifically designed to deliver DNA vaccines to dendritic cells, the sentries of our immune systems, and most importantly to release the DNA at a rate that is synchronized to the natural timing of the immune system[2] (Fig. 1).

Such new DNA-delivery systems are needed to fulfil the promise of DNA vaccination, which can treat infections such as AIDS, malaria and hepatitis B, entice the body to attack cancers, or alleviate so-called autoimmune diseases — such as rheumatoid arthritis, multiple sclerosis, and insulin-dependent diabetes — in which our immune system mistakenly attacks perfectly healthy cells. Although several clinical trials have demonstrated that DNA vaccines are safe and can generate good immune responses, vaccine potency has in general been disappointing[1]. Microparticle-based DNA delivery, in which the genes are encapsulated within[3] or immobilized on[4] a spherical polymer particle, can improve potency by targeting the genes to appropriate cells of the immune system. Indeed, some companies already have microparticle-based DNA vaccines in clinical trials.

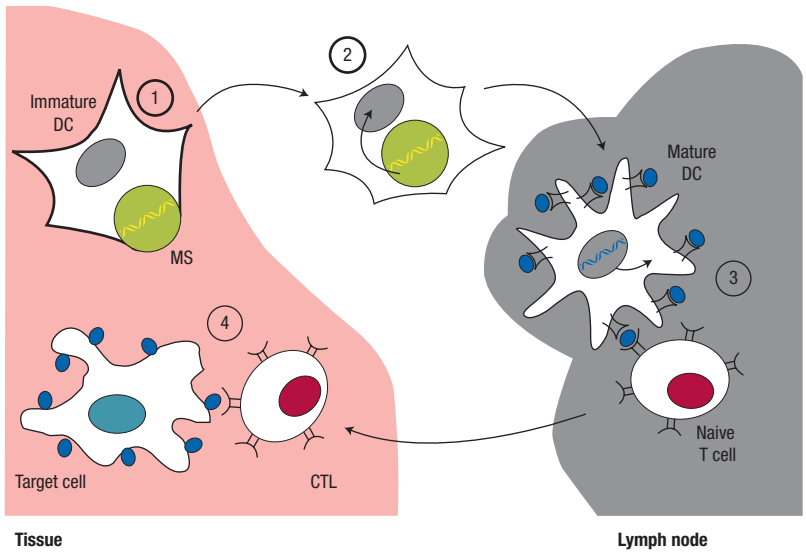

Figure 1 Microspheres faking an attack. The mechanism by which the DNA delivery system devised by Wang and colleagues works as a vaccine[2]. Immature dendritic cells (DCs) (1) are present in virtually any tissue where eventually adverse organisms may be found. They have the ability to take in polymeric microspheres containing DNA (MS; the yellow circles containing a blue double helix). Once the microspheres have entered them, the dendritic cells migrate through the lymphatic system (2). By the time the dendritic cells have reached the lymph node, DNA has been released from the microspheres and expressed to produce antigens. In the lymph nodes, the mature dendritic cells present the antigens on their surface for recognition by naive T cells (3), which then become cytotoxic T lymphocytes (CTL) or killer cells. These migrate back into the tissue (4) where they recognize and attack any target cell expressing the antigen.

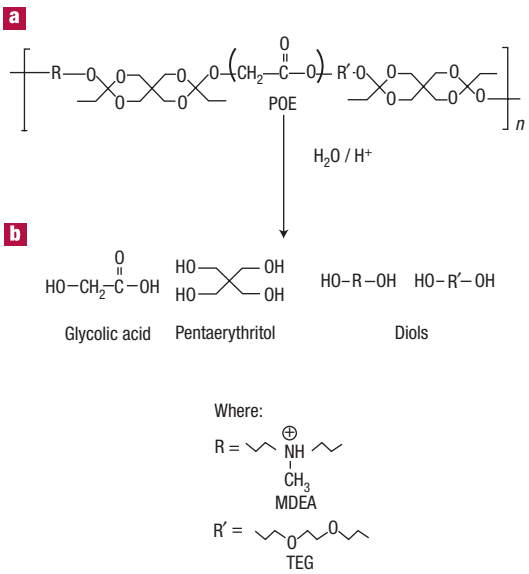

Figure 2 Degradation under control. **a**, Fourth-generation poly(ortho esters) (POEs) are synthesized by the reaction of a diketene acetal with diols. In this case, one of the diols is triethylene glycol glycolide, which helps control the degradation rate of the polymer. **b**, Under acidic conditions, found inside dendritic cells, POEs hydrolyse in several steps to produce the monomer diols, glycolic acid and pentaerythritol. To delay the release of DNA (which is negatively charged) *N*-methyldiethanolamine (MDEA) was used as one of the diols to provide a positive charge in the polymer backbone.

To date, reliance on off-the-shelf materials has limited the development of DNA delivery systems. Wang *et al.* have now addressed this need by identifying a set of design criteria for DNA-vaccine delivery materials that includes safety (for the patient), compatibility with and protection of encapsulated DNA, and most importantly the ability to tune the rate of DNA release. Following these principles, they chose to use biodegradable polymers known as poly(ortho esters) (POEs) originally developed by Heller[5] in 1970. POEs such as those used by Wang *et al.* are stable at near-neutral pH, but at pH 5 — which is close to the pH encountered inside the dendritic cells after they have internalized the particles — they degrade rapidly into relatively inert, non-toxic materials (Fig. 2). To control the rate of DNA release from the polymeric microspheres, the authors chose to incorporate a monomer with a positive charge that holds onto the negatively charged DNA. In fact, their polymer delayed release of DNA by at least 24 hours in comparison with a control polymer lacking the positive charges.

Why is timing of DNA release so important? Once a sentry cell has detected and gobbled up an invader, it will need one to two days to migrate to the lymph nodes and activate the killer cells (Fig. 1). Early display of the protein on the sentry-cell surface can actually induce immune tolerance — exactly the opposite of the desired effect. The new polymers delay DNA release and, as a result, slow the expression of the genes and antigen display on the sentry cells. Thus, the new microspheres activated killer cells at least threefold more effectively than control microspheres, which released DNA either more quickly or much more slowly. Furthermore, when the POE microspheres contained a gene encoding a protein expressed on experimental tumours in mice, the cancer grew very slowly. Treatment of the mice with the same gene in microspheres made from other materials allowed tumours to grow three to four times larger.

This is the first attempt to tune the rate of DNA delivery through materials design. However, there are certainly other means to control drug release from biodegradable polymer microspheres, including varying the particle size or surrounding the particles with a second polymer shell to form a microcapsule[6]. Such precise control of the particle structure, when combined with improved materials, may provide even more efficient targeting to specific sentry cells and timing of DNA release.

The next advance in DNA vaccine development is likely to be a system that provides pulsatile delivery of the genes at predefined times over the course of weeks or even months. As with traditional vaccines, a truly protective and long-lasting immunity often requires multiple booster shots. For example, the current anthrax vaccine requires five boosters after 2 and 4 weeks, and 6, 12 and 18 months, and a previously reported DNA vaccine against HIV infection required injections at 0, 4, 8 and 40 weeks[7]. Booster injections are expensive, amplify the risks of unsafe injection practices, and require patients to return to the doctor over and over. All these problems are exacerbated in developing countries where new vaccines are most desperately needed. Because of their specificity, safety and cost advantages, DNA vaccination offers new hope for those afflicted either with modern diseases, such as HIV/AIDS, or ancient scourges, such as malaria, cancer and diabetes. Ultimately, advances in delivery technology, including new materials and methods, will help make DNA vaccines a clinical reality.

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