VACCINES

Imaging the early fate of mRNA vaccines

The biodistribution of the components of a messenger RNA vaccine following its administration in non-human primates can be non-invasively monitored by labelling the vaccine with a dual radionuclide–near-infrared probe.

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Many millions of vaccine doses are administered each year. Yet the fundamental understanding of the biodistribution of vaccine components, and of the quality of the early immune responses that dictate the degree of protection elicited by vaccines, remains limited. It has been proposed that, after vaccine administration, a depot of vaccine antigen and adjuvant forms at the site of injection, and that the gradual antigen release then triggers the immune system to mount a response to the vaccine. However, studies in mice have shown that vaccine antigen is rapidly disseminated from the site of injection to the draining lymph nodes, as removal of the injection site (typically, in a mouse's ear) a few hours after immunization did not reduce the vaccine response[1]. This observation is supported by other studies that used radioactive labelling of vaccine antigens to show that the majority of those antigens leave the injection site within a day after immunization[2]. Although vaccine development has historically been empirical, the rational design of new vaccines requires a deeper understanding of the interplay between the vaccine and the immune system. Reporting in *Nature Biomedical Engineering*, Philip Santangelo and colleagues now describe a method of monitoring, via positron emission tomography–computed tomography (PET–CT) and near-infrared imaging, the biodistribution of a messenger (m)RNA vaccine labelled with a radionuclide–near-infrared probe[3] after immunization.

Santangelo and co-authors labelled a model vaccine (yellow-fever *prME* mRNA) with a probe consisting of the radionuclide ^{64}Cu, for PET imaging, and DyLight 680, for near-infrared imaging. Following longitudinal assessment of the vaccine's biodistribution after administration to cynomolgus macaques, they found that the vaccine exclusively targeted the injection site and the lymph nodes that drained the tissue of the injection site (Fig. 1a). The vaccine was administered by intramuscular injection in the quadricep muscle of the leg, and consequently ended up in inguinal, iliac and paraaortic lymph nodes (Fig. 1b). Within four hours after vaccine injection, the intensity of the radioisotope signal in lymph nodes decreased in proportion to the distance from the injection site, suggesting that the vaccine would either be distributed from the injection site to various lymph nodes, or that it initially traffics to the closest lymph node and then leaves and travels to the next. In this context, a previous report of cannulation experiments in sheep showed that egress of vaccine and cells from a lymph node happens at a very slow pace (in the range of 48 hours), if at all[4], therefore indicating that vaccines probably disseminate directly to different nodes, and preferentially to the nodes with the most efficient drainage or to those that are closest to the injection site. The authors also observed that the mRNA vaccine continued to accumulate in draining lymph nodes for at least 28 hours post-vaccination, and that in most cases only a single node from each anatomical lymph-node cluster would display a signal. However, because of the limited number of animals analysed in these experiments, it is hard to conclude whether one anatomical cluster in the chain of lymph nodes was preferentially targeted over others. Nevertheless, it is clear that the vaccine has a very restricted localization to these sets of lymph nodes, as no systemic spread to other organs was observed. This is in line with previous studies that used flow-cytometry-based detection of fluorescently labelled vaccines after administration in non-human primates[5,6] and in mice[7–9]. In fact, essential processes in the context of vaccination, such as antigen presentation and stimulation of antigen-specific T-cell responses, seem to exclusively take place in the lymph nodes draining the vaccine injection site after both prime and boost immunizations[6].

The high degree of similarity between humans and outbred non-human primates, in anatomy, genetic diversity and immune system (including specific immune cell subsets and receptor expression), makes primates more representative models of vaccine responses in humans than rodents or other smaller mammals frequently used in vaccination studies. When using animal models that better approximate human

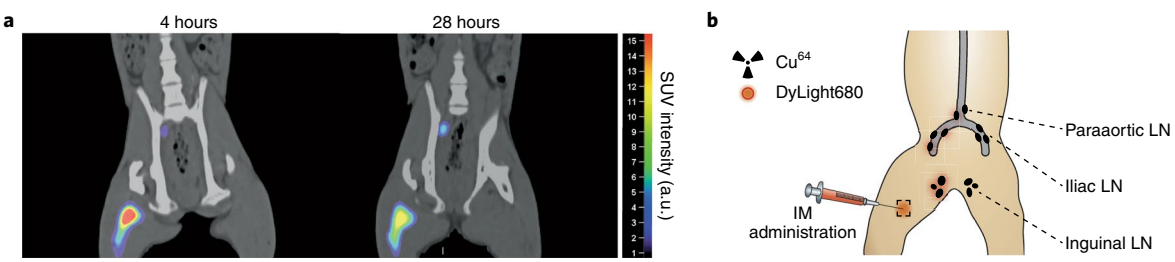

Fig. 1 | The biodistribution of an mRNA vaccine, viewed via PET–CT and infrared imaging. a, Vaccine-specific signal in an injected macaque at 4 and 28 hours post-vaccination. SUV, standardized uptake values. **b**, Anatomical positions of draining lymph nodes (LN) in relation to the injection site in the macaque. IM, intramuscular. Figure 1a reproduced from ref. [3], Springer Nature Ltd.

physiology, the doses, injection routes and biodistribution of vaccines can be evaluated more accurately in preclinical studies for vaccine development. Consequently, the ability to image early vaccine dynamics in a closely related animal model can be a valuable tool for the optimization of vaccine formulations, and for the testing of new materials and delivery regimens for human vaccines.

In contrast to the flow-cytometry methods typically used to study vaccine trafficking, the use of PET–CT imaging obviates the need for biopsies or euthanasia, and enables the non-invasive longitudinal analysis of vaccine dissemination in the same animal. Notably, in another example of PET–CT being used for non-invasive immune monitoring, similar approaches have been explored to study the distribution of labelled antiviral therapeutic antibodies in macaques infected with the Simian immunodeficiency virus[10]. The fact that vaccine antigen load at the different sites can be quantified over time also opens the possibility to correlate such data with information about the vaccine-specific responses that develop in the same animals. A variety of parameters can therefore be evaluated to obtain a more comprehensive picture of the development of immune responses to vaccines, to help understand whether the quality and quantity of vaccine responses can be predicted from the very early innate responses directly after administration. Nevertheless, the characterization of the specific cell populations that take up the vaccine, including their activation and function, may still require alternative or complementary methods. In fact, Santangelo and colleagues also performed flow-cytometry analyses and microscopy imaging of the tissues identified by PET–CT, finding that professional antigen-presenting cells in the lymph nodes take up the mRNA vaccine and translate the encoded antigen, in line with earlier studies[5].

Vaccine development has come a long way since 1796, when Edward Jenner inoculated an eight-year-old boy with cowpox material collected from pustules on the hand of a milkmaid to induce protection against smallpox. Today's rational design of vaccines is based on modern recombinant technology, high-throughput sequencing of the full genomes of pathogens, and structure-based design of antigens. Pathogens for which no ideal vaccines have been developed typically pose tighter constraints on the type, breadth and sustainability of the adaptive immune responses in order for the vaccine to be protective. A much better mechanistic understanding of vaccine dynamics after administration is therefore needed to select safe formulations with the capacity to elicit stronger immunity. ❐

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