

## VACCINES

# Engineering immune evasion

John R. Mascola

**One obstacle to realizing the promise of viral vectors for vaccine delivery is pre-existing immunity to such vectors. An adroit application of structure-based design points to a way around that problem.**

There are still no vaccines against such devastating and widespread diseases as malaria, tuberculosis and AIDS. Because the traditional approach of live-attenuated vaccination is not feasible for most diseases, scientists have turned to molecularly engineered viruses that contain pathogen-specific gene inserts. Such viral vectors direct host cells to produce the foreign protein of interest, thus prompting a pre-emptive immune response. Among the most promising viral vectors is a form of common-cold virus known as adenovirus serotype 5. The recombinant adenovirus vectors (rAd5) cannot replicate and can be safely administered, and they elicit both of the two main types of immune response — secreted antibodies and disease-fighting T cells.

There is a problem, however. The existing immunity to rAd5 in many adults means that the vector could be neutralized before it can have an effect. Hence the work of Roberts *et al.*<sup>1</sup>, described on page 239 of this issue. They have taken a fresh approach to the molecular engineering of rAd5, one that has the potential to circumvent anti-vector immunity and expand the applicability of such vectors for human vaccination.

Numerous viral vectors are being studied for use in gene-based vaccine strategies. The most commonly used vectors are derived from poxviruses, alphaviruses and adenoviruses. Among these, rAd5 is the best characterized and is perhaps the most attractive for vaccine development. As a stand-alone vaccine, rAd5 can elicit different types of T-cell immunity (those due to CD4 and CD8 cells), and more potent immune responses can be achieved with a 'prime-boost' approach. For example, use of vehicles known as DNA plasmids followed by boosting with rAd5 can generate durable antibody and T-cell immune responses<sup>2,3</sup>. Preclinical studies of rAd5 vaccines include vaccines against Ebola, SARS, HIV-1 and anthrax<sup>4-7</sup>, and phase II human clinical studies of rAd5 HIV-1 vectors are in progress.

But anti-vector immunity may be a serious limitation. Adenovirus serotype 5 is common — depending on the geographical region of the world, most adults are exposed to it and

develop some level of immunity. This may lessen the effectiveness of rAd5 as a vaccine vector. Potential ways around this problem include the use of adenoviruses derived from other human serotypes or from non-human animal species. Indeed, there are more than 50 known human adenoviral serotypes, some of which are quite rare in the human population. The genetic manipulation required to engineer alternative serotypes is not trivial, however. The rAd5 vectors contain specific genetic deletions that render them unable to replicate. This contributes to their safety, but also means that specially engineered cells must be used to produce them. The advantages of rAd5 are that the necessary groundwork has been laid, in terms

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of basic molecular engineering and production of the vector, and that it has been through the regulatory approval process for use in humans. Adaptation of other serotypes will require a methodical process of research and development, and safety testing. Furthermore, preliminary data<sup>5,8</sup> from other serotypes, such as rAd35 and rAd11, suggest that they may be less immunogenic — that is, less effective in producing immunity — than rAd5.

With this as background, Roberts and colleagues<sup>1</sup> took advantage of our improved understanding of anti-vector immunity, coupled with structural data about viral proteins, to derive a rational approach to re-engineering the rAd5 vector. In adenoviruses, the viral DNA is surrounded by a protein shell called a capsid that contains hexon and penton subunits. Because host antibodies that neutralize rAd5 are directed against the hexon subunit, Roberts *et al.* studied the atomic structure of

this protein to understand where antibodies would probably bind. Molecular modelling revealed that the seven hypervariable regions (HVRs) of the hexon form the outer surface of the protein, making the HVRs a likely target for antibody binding.

By exchanging all seven HVRs of rAd5 with those of the rare adenovirus serotype 48 (Ad48), the authors constructed a chimaeric adenovirus that could potentially evade the neutralizing antibody response against rAd5. The core structure of the hexon protein was not altered, so the resulting HVR-chimaeric rAd5 vectors retained their ability to grow well in culture and, importantly, the immunogenicity of the chimaeras was comparable to that of rAd5. As Roberts *et al.* hoped, when the HVR chimaeras were administered to mice or monkeys that had antibody immunity to rAd5, there was no decrease in the immunogenicity of the vector.

These data provide a proof-of-concept that viral vaccine vectors can be engineered to evade pre-existing immunity. The results are a tribute to the application of modern immunology and structural biology to vaccine design. The potential of this technology is considerable. One can envisage the construction of numerous HVR chimaeras that could be used to vaccinate against various pathogens. Thus, if rAd5 itself were used to vaccinate children against malaria, a chimaeric vector could still be used as an HIV vaccine. Furthermore, the use of multiple chimaeric serotypes could allow booster vaccinations to sustain the long-term immune memory response needed for durable immunity.

Yet we are still some time away from studies in humans. Vaccine developers will have to show that these new HVR-chimaeric rAd5 vectors can be manufactured, and that they have stable gene inserts, can pass regulatory review and, finally, are immunogenic in humans with pre-existing immunity. Current rAd5 vectors for HIV-1 are being evaluated in phase II human trials that will more precisely define the extent and effect of pre-existing anti-vector immunity. As we await these data, chimaeric vectors can be manufactured

and tested in humans, so that we can further assess the potential effects of anti-vector immunity. ■

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## SOLAR SYSTEM

# Interplanetary kidnap

Alessandro Morbidelli

**Triton, Neptune's largest moon, was probably part of a two-body object similar to the Pluto–Charon system. This tandem might have been ripped apart when it strayed too close to the planet that Triton is now orbiting.**

The neptunian moon Triton weighs in at 1.4 times the mass of Pluto, making it the largest irregularly orbiting satellite in the Solar System. So how did this kept giant come to be where it is? On page 192 of this issue<sup>1</sup>, Agnor and Hamilton advance a capture mechanism that, if correct, could have repercussions for the life stories of other, similar moons.

A cohort of satellites surrounds all four giant planets in the Solar System — Jupiter, Saturn, Uranus and Neptune. These satellites are divided into two distinct groups, regular and irregular, according to their orbital characteristics. Regular satellites are closer to their parent planet, with orbits that are essentially circular and that lie on the planet's equatorial plane. These satellites thus constitute miniature solar systems around their planet. And just as the planets of the Solar System are thought to have formed from a disk of gas and dust (the protoplanetary disk) orbiting the Sun, so the regular satellites are assumed to have formed from a 'planetesimal disk' orbiting their planet.

Irregular satellites, in contrast, are more distant from their planet and typically have orbits with larger eccentricities (a measure of deviation from a perfect circle) and/or inclinations. About half the irregular satellites orbit their planet in the retrograde direction; that is, in the opposite direction to the rotation of their planet. Because of these strange orbital characteristics, the general assumption is that these satellites formed on heliocentric orbits, and only later were captured on elliptic orbits around giant planets.

Several mechanisms have been proposed for this capture process. Some invoke the effect of gas-drag exerted by the atmospheres of the planets, which were more extensive when the planets formed some 4.5 billion years ago than they are now, owing to the heat generated by the accretion process. Others posit the abrupt growth of a planet's mass (the 'pull-down

mechanism') as the culprit. Still others employ gravitational interactions or collisions with the system of regular satellites already established around the planet, or fortuitous encounters with other planetesimals on heliocentric orbits as they passed through the sphere of influence of the planet.

But none of these mechanisms seem appropriate for Triton, Neptune's huge retrograde companion. Its mass — in another word, its inertia — means it was unlikely to have been captured by interactions with the existing satellites or other passing planetesimals. Additionally, Neptune is assumed to have undergone slow growth, and never to have had an extended atmosphere; both the pull-down and gas-drag mechanisms would therefore have been inefficient.

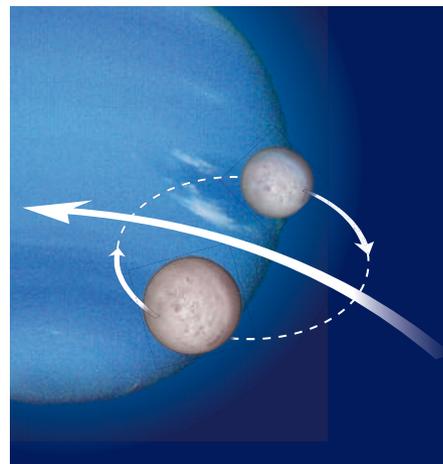
Agnor and Hamilton postulate<sup>1</sup> that Triton was originally part of a binary object, for instance similar to the Pluto–Charon system. They show that if this binary had passed sufficiently close to Neptune at low velocity, the different forces acting on its two constituent bodies would have ripped them apart. Each of the constituents would have, relative to Neptune, a velocity that was essentially the vector sum of the velocity of the binary's barycentre (its centre of gravity) and its own orbital velocity relative to this barycentre (Fig. 1). Most of the time, the orbital motion of one of the bodies is opposed to the barycentre's motion, so the net velocity of this body relative to Neptune could easily have been smaller than the escape velocity from Neptune's gravitational field. Thus, it would have become captured in a bound, planetocentric trajectory.

To be a likely explanation of Triton's capture, this model requires that two conditions be met. First, the protoplanetary disk in which Neptune evolved must have contained a very large number of Pluto-sized objects<sup>2</sup>. This condition cannot be checked directly, but is plausible: the protoplanetary disk is presumed to have had

a total mass of about 50 Earth masses<sup>3,4</sup>, or 5,000 times greater than that of the Kuiper belt<sup>5</sup>. (This relic of the protoplanetary disk<sup>6</sup>, where Pluto resides, now orbits the Sun beyond Neptune.) Second, a substantial fraction of the large objects in the protoplanetary disk must have been binary. The likelihood of this is increased by the observation that between 10 and 15% of the objects in the Kuiper belt are two-bodied<sup>7</sup>. In addition, three of the four largest Kuiper-belt objects — in decreasing order of size, 2003 UB<sub>313</sub>, Pluto and 2003 EL<sub>61</sub> — have satellites<sup>8</sup>. (The third largest, 2005 FY<sub>9</sub>, is the odd one out.)

Although Agnor and Hamilton focus exclusively on Triton, it is tempting to conjecture that this mechanism applies to the capture of most of the irregular satellites. It has been pointed out that for all four giant planets, the number of irregular satellites larger than a specific size<sup>9</sup> is about the same. This fact argues against the gas-drag and pull-down mechanisms for their capture: because the flux of planetesimals through the giant planets' orbits was about the same<sup>7</sup>, both mechanisms should have been much more effective for Jupiter and Saturn, which grew rapidly as gas giants, than for Uranus and Neptune, which formed more slowly in a gas-starved environment<sup>10</sup>.

The only thing that the giant planets have in common is the size of their sphere of gravitational influence, or Hill sphere. This fact, together with the giant planets' similar number of irregular satellites, suggests that some sort of two-body interaction inside the Hill sphere played the dominant role in the capture of such satellites. Additional support for this picture



**Figure 1 | Imminent capture.** A two-body object approaches Neptune, the scenario envisaged by Agnor and Hamilton<sup>1</sup> for the capture of Triton. Both constituents of the binary rotate around the trajectory of their mutual centre of mass (barycentre), such that their own motion is almost half of the time with, and almost half of the time against, the motion of the binary as a whole. The net velocity of the constituents relative to the planet is accordingly increased or reduced; if the net velocity of a constituent drops below the escape velocity from a planet's gravitational field, it is captured, entering a new life as an irregular satellite of the planet.