Box 1. Other gene therapy advances

Three other potential advances in gene therapy have been reported recently.

(1) Introgen Therapeutics (Austin, TX, USA) immunized tumor-bearing mice with mouse dendritic cells treated with Advexin®, which incorporates the p53 tumor suppressor gene in an adenoviral delivery system. This completely prevented tumor development when the cells were activated, with no evidence of toxicity or autoimmune complications.

(2) Researchers at Stanford University Medical Center (Stanford, CA, USA) used a genetically engineered live adenovirus to attack gastrointestinal cancers that had spread to the liver. The attenuated virus is engineered to infect only cells with an abnormality in the tumor suppressor gene, p53. It was found to be safe in a Phase I trial in patients with advanced cancer, and those receiving the highest dose showed increased median survival time. Phase II studies in combination with chemotherapy are due to start this year.

(3) DNA compaction technology developed by Copernicus Therapeutics (Cleveland, OH, USA) is being tested in cystic fibrosis (CF) patients. Compacted genes are small enough to pass through the cell membrane and then into the nucleus. A compacted version of the normal CF gene is administered to the nasal passages via a saline drip. Gene uptake and expression in the nasal tissue will be monitored to see if the approach is effective.

ultrasound at 0.5 W cm^{-2} [5] (Fig. 1). The ultrasound increases DNA release from the liposomes at much lower power than is required to produce the same effects with standard liposomes. The frequency used is similar to that used in diagnostic ultrasound, so cell damage is not expected to occur. However, safety has not yet been evaluated *in vivo*.

Acoustic liposomes conjugated to antibodies have been shown to target sites of vascular disease. 'Using this technology it becomes feasible to both identify a disease site and to activate a therapeutic agent *in situ*,' says Shaoling Huang, a postdoctoral research fellow at Northwestern University.

'Gene transfer is still very much an open field,' says Ijeoma Uchegbu, Senior Lecturer in Drug Delivery at the University of Strathclyde (Glasgow, UK). 'No technology has yet emerged as a problem-free winner, so anything that works has merit. Electroporation has produced impressive data and is promising for *ex vivo* work or superficial tissues, but it would be difficult to apply to tissues that are not accessible. The same applies to other physical techniques – you need to get the tissue close to the device being used.'

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Vaccinating against ticks

Martina Habeck, freelance writer

Anti-tick vaccines would provide a much needed alternative to current methods for the control of these blood-feeding parasites. Recent molecular approaches are now raising fascinating possibilities.

Ticks feed several times during their life cycle and can become infected with many pathogens. These can be transmitted to their host – humans and a wide range of animals, including pets and livestock – and can cause potentially serious diseases (Fig. 1). Sarah Randolph, a tick expert at the University of Oxford (Oxford, UK), says that in Africa, most farmers will identify ticks as the single biggest problem they are presented with in terms of livestock pests (Table 1). There are ~850 tick species and 30 major tick-borne diseases. These diseases generally affect the blood and/or lymphatic system and cause symptoms

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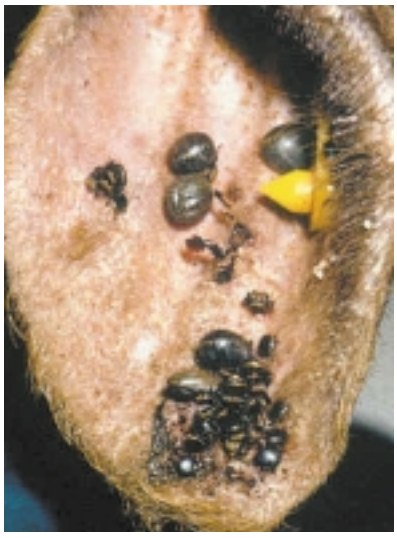

Figure 1. Normal tick load on the ear of dairy cattle in Italy. The eartag is shown in yellow. Major tick-borne diseases in Mediterranean countries are: Tropical theileriosis (or Mediterranean theileriosis), Babesiosis and Anaplasmosis. Figure kindly provided by Olivier Sparagano, Department of Agriculture, University of Newcastle, (Newcastle, UK).

of fever, anaemia, weight loss, milk-drop, lymphnode swelling, abortions and death.

Key strategies

Traditional approaches to prevent disease among livestock include immersing animals in toxic pesticides called acaricides. However, this method causes environmental pollution and sooner or later results in the evolution of resistance to these chemicals. Another strategy is to vaccinate against the pathogens. 'But ticks carry more than one pathogen, so if you have a vaccine killing only the bacteria, then the tick could still transmit the virus and the protozoa,' warns Olivier Sparagano, a parasitologist at the University of Newcastle (UK). 'The idea came up to vaccinate against the vector, not the pathogens,' he told delegates at the spring meeting of the Society for General Microbiology (University of Warwick, Coventry, UK; 8–12 April 2002; see p. 30 of http://www.socgenmicrobiol.org.uk/MTGPAGES/previous.htm).

This idea is around 60 years old, but progress to produce effective vaccines has been slow. Research concentrates on two different approaches:

- Vaccinating with exposed antigens. To obtain a blood meal, ticks secrete a plethora of compounds with anticoagulant and immunosuppressant activity. These antigens typically stem from the salivary gland and induce innate and naturally acquired immune responses.
- Vaccinating with concealed antigens. Antigens located in the midgut or other tick tissues are hidden from the host's immune system. Using these 'concealed' antigens, researchers try to induce an immune response artificially.

The second strategy has led to the commercialization of a recombinant vaccine in Australia (TickGard Plus™; Intervet, Bendigo, Victoria, Australia) and Cuba (Gavac™; Heber Biotec, Havana, Cuba). The vaccine is based on the Bm86 antigen, a membrane-bound glycoprotein derived from the surface of midgut cells in the tick *Boophilus microplus*. Immunizing with Bm86 causes the host to produce antibodies against the midgut protein [1]. When ticks feed on blood from a vaccinated host, they will also take up the antibodies, which will cause damage and prevent the ticks from digesting their food; however, this mechanism is poorly understood. This approach enables the elimination of up to 90% of the current generation of susceptible species. Because the strategy also reduces egg production, future generations are also affected.

But Sparagano says, 'There is still a lot of research that needs to be done. The vaccines on the market are all targeting a single tick species, *B. microplus*, which is mainly a problem in Australia and in the Caribbean. Other countries will have to develop their own vaccine against local ticks.' Sparagano is collaborating with scientists in Palermo (Italy) to find a

Table 1. The major tick species and tick-borne diseases

Vector tick genera	Hosts	Main tick-borne diseases and their agents	Distribution
Boophilus	Cattle	Babesiosis (*Babesia* ssp.), anaplasmosis (*Anaplasma marginale*)	Tropical and subtropical areas of the world
Ixodes	Humans, pets, livestock, wild animals	Lyme disease (*Borrelia burgdorferi*), tick-borne encephalitis (TBE virus), babesiosis (*Babesia* ssp.), ehrlichiosis (*Ehrlichia* ssp.), anaplasmosis (*Anaplasma* ssp.)	Worldwide
Hyalomma	Domestic ruminants, horse, camel and occasionally humans	Tropical theilerisosis (*Theileria annulata*)	Africa, south-eastern Europe, Asia
Rhipicephalus	Cattle and wild ruminants	East Coast Fever (*Theileria parva*)	Africa
Amblyomma	Cattle, small and wild ruminants	Heartwater (*Cowdria ruminantium*)	Africa and Caribbean

vaccine against *Hyalomma lusitanicum* and *H. marginatum*, tick species that are a major problem in Mediterranean countries. 'At the moment, we are collecting the ticks. We will then extract the guts and try to identify the proteins that we think are the most immunogenic.'

Dual-action vaccine shows promise in laboratory tests

The problem with the concealed-antigen approach is that it is difficult to maintain adequate immunity, cautions Patricia Nuttall of the Centre for Ecology and Hydrology (Oxford, UK). 'Since they are targeting a hidden antigen, there is no natural boost of the immune system when the ticks feed. Therefore, immunity is just going to wane.' However, this might not be an issue with the vaccine candidate developed by Nuttall's colleague Adama Trimnell. The vaccine is based on truncated versions (64TRPs) of an antigen that was isolated from the salivary glands. Antibodies to 64TRPs cross-react with concealed tick antigens, including midgut antigens. 'In theory, the natural feeding of the tick on immunized animals should stimulate the immune response and maintain the immunity [to the antigen from the salivary gland],' says Nuttall. 'In addition, antibodies that are taken up [during the blood meal] recognize the cross-reacting epitopes in the midgut and cause some damage to the midgut and a certain degree of mortality, which is what you can see in the concealed-vaccine approach. So it is a double whammy.'

Trimnell tested several 64TRPs in laboratory animals and found raised antibody titres that provided immunity against several tick species, including *Rhipicephalus* and *Ixodes*. A paper describing the results of this research has recently been submitted to *Vaccine* for publication. Trimnell is now collaborating with the International Livestock Research Institute in Nairobi (Kenya) to see whether the vaccine is effective in cattle.

This is going to be the real test, as Randolph points out: 'I feel quite positive about vaccines, but there is a huge difference in getting something to work in the laboratory and transferring it to the field to achieve effective, sustainable control.'

Towards a vaccine for humans

The rapid development in molecular biology also facilitates the search for anti-tick vaccines for humans. Thomas Mather and colleagues at the University of Rhode Island (Kingston, RI, USA) and Jesus Valenzuela at the National Institutes of Health (Bethesda, MD, USA) have made extensive cDNA libraries of *Ixodes scapularis* genes encoding pharmacologically active substances in the tick saliva. The group has recently received a grant from the NIH to begin screening their library for a candidate vaccine. Mather said, 'We are immunizing [laboratory] animals with DNA-based vaccines and challenging them with ticks that are infected with Lyme disease bacteria to see whether we raise an immune reaction to the salivary molecule and whether we block pathogen transmission.'

How long the search will take is hard to tell. Mather points out that some similar types of work have progressed much more rapidly. Scientists at the NIH managed to develop a vaccine against the sand fly that transmits Leishmaniasis in approximately 18 months, but they were only dealing with a library of 15–20 saliva molecules [2]. Mather says, 'In the tick project, we already have 500 molecules identified, and full-length sequences of 80 of them, so it is a little bit more complex.'

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A vaccine against atherosclerosis

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The development of a vaccine to prevent the build-up of atherosclerotic plaque has come a step closer. Researchers at the Cedars-Sinai Medical Center (Los Angeles, CA, USA) and Lund University (Malmo, Sweden) have tested a peptide-based vaccine that reduces arterial plaque formation in mice by up to 70% [1]. Developing effective therapies for atherosclerosis is important because the disease is the root cause of more than half of all deaths in the developed world (http://www.atherosclerosis-drug.com).

A prolific killer

Atherosclerosis is a chronic disease that causes artery walls to thicken and become less elastic. The resulting restriction of blood flow can lead to heart attacks, kidney failure, stroke and other serious cardiovascular illnesses. People's susceptibility to atherosclerosis varies with their genetic make-up and lifestyles. Those most at risk include smokers and those with high blood pressure, diabetes or high cholesterol levels. Other risk factors could include obesity, advancing age and lack of exercise.

Atherosclerosis occurs when monocytes, a type of white blood cell, migrate

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