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

 1359-6446/02/$ – see front matter PII: S1359-6446(02)02319-X

from the bloodstream and into the artery wall. They are then transformed into cells that can accumulate fatty materials and eventually form a plaque, usually at arterial branch points, forming areas of thickening called atheromas. As the arteries thicken and become narrower, the atheromas can rupture causing the formation of blood clots, which can then lead to an embolism.

Current therapies

At present, there are several treatments for the disease. Much can be done through lifestyle changes, for example, giving up smoking or switching to a low fat–low cholesterol diet, but the effectiveness of these approaches is limited. Statins are currently the most powerful cholesterol-lowering drugs available. They lower levels of low-density lipoprotein (LDL), the so-called 'bad' type of cholesterol, while raising levels of high-density lipoprotein (HDL), which is beneficial because it transports cholesterol to the liver for degradation. However, there are risks associated with statins, including liver toxicity and muscle inflammation.

Other drug therapies, such as acetylsalicylic acid drugs (e.g. aspirin), limit the aggregation of platelets and thereby inhibit the formation of blood clots. However, this can lead to uncontrolled bleeding or haemorrhage. Other drugs include the ADP-receptor antagonists ticlopodine and clopidogrel, although these have similar side-effect profiles to aspirin.

The new vaccine

Cholesterol is one of the main constituents of atherosclerotic plaques, and thus provides an obvious target for anti-plaque therapy. When LDL is oxidated, it becomes 'sticky' and can attach to blood vessel walls, thus contributing to atherosclerosis (Fig. 1). It was previously known that immunization with homologous LDL reduces atherosclerosis in animals [2,3]. To develop a clinically testable vaccine, the researchers from Cedars-Sinai, led by Prediman Shah, and those from Lund University, headed by Jan Nillson, concentrated on the major protein component of LDL, Apo B100. Because this is one of the largest proteins known, having ~4500 amino acids, the researchers decided to test small pieces of the protein for an immunological response.

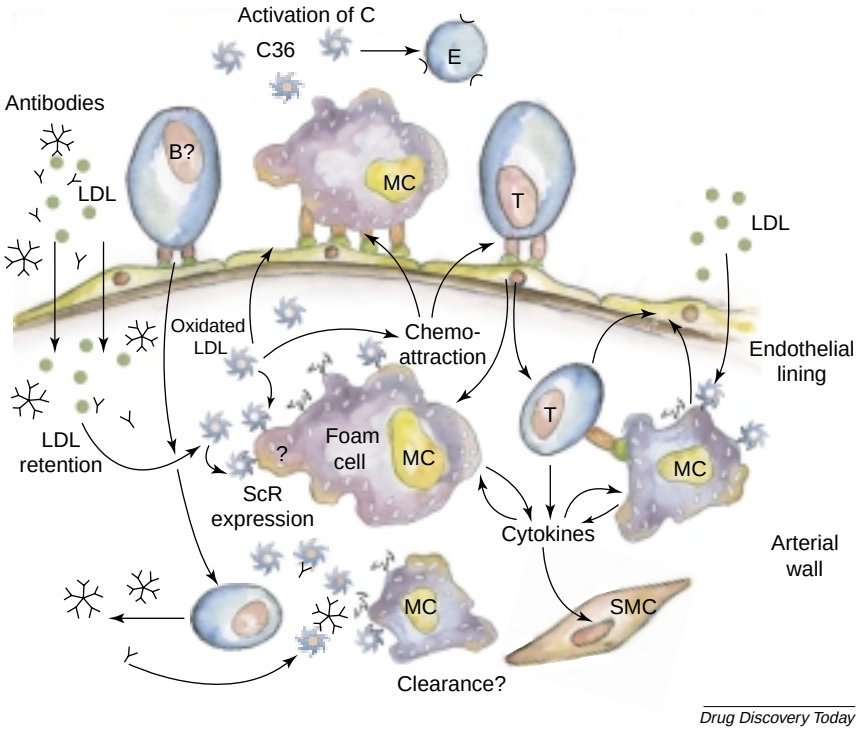

Figure 1. Antibodies to oxidized low-density lipoprotein (LDL) and atherosclerosis. LDL particles penetrate the endothelial lining and become trapped in the extracellular matrix of the arterial wall. Some of these LDL particles become aggregated and oxidized resulting in the release of several proinflammatory and toxic substances that activate endothelial expression of adhesion molecules. Monocytes (MC), T cells (T) and, occasionally, B cells (B) attach to the endothelial surface and infiltrate the arterial intima. Monocytes differentiate into scavenger receptors (ScR) expressing macrophages that ingest oxidized LDL and become foam cells. Activation of macrophages and T cells results in the release of cytokines that further enhance inflammation and stimulate smooth muscle cell (SMC) proliferation. Antibodies produced against oxidized LDL released from activated B cells could have anti-atherogenic effects by removing oxidized LDL from the extracellular space and the circulation. Figure kindly provided by Jan Nilsson of Lund University (Malmo, Sweden). Abbreviations: C, complement; E, erythrocyte.

They designed a library of peptides, each of 20 amino acids, that together covered the human Apo 100 sequence. After screening the library, ~100 structures were identified that induce an immune response in humans. The most effective 25 peptides were tested further in genetically engineered mice with high cholesterol levels. The mice were immunized with the different aldehyde-modified peptides and an adjuvant, with a booster immunization after three weeks, while control mice were given only the adjuvant. All mice were fed a high-cholesterol diet for 14 weeks. After this time, five peptide sequences that reduced the amounts of atherosclerotic plaque by at least 50% were identified.

Nilsson and Shah have been collaborating closely for the past 10 years to study the connection between LDL and the immune system. In ongoing experiments, they have set out to identify the autoantibodies associated with oxidated LDL and, therefore, atherosclerosis. 'The conclusion at the moment is that there

is a strong correlation between the concentration of certain antibodies and the extent of atherosclerosis,' said Nilsson. 'The vaccine would be unlikely to work like familiar vaccines, such as those for measles. It is more probable that the vaccine would be used in a combined therapy with statins to combat atherosclerosis.'

Future developments

Nilsson expects the vaccine to enter Phase I clinical trials in the next two years. 'It's always difficult to predict at this stage whether there will be any side effects when the vaccine is used in humans,' said Nilsson, although he remains optimistic about side effects from the results with the mouse model. 'The vaccine could be administered by the traditional subcutaneous injection, although with the rate that delivery technology is progressing, an alternative method, a nasal spray, for example, could be used.'

Wulf Palinski of the University of San Diego California (USDC; CA, USA), one of the first investigators to study this area, believes that such a vaccine is still a long way off. 'The efficacy of immunizations in various animal models of atherosclerosis has now been confirmed by at least six published papers, but we are a long way from proposing this as a preventive approach in humans,' he commented. 'From a scientific point, the main unresolved question is that the mechanism responsible is not known. Both humoral and cellular immune responses have been implicated. The nature of the immunogen and the frequency of immunization will also need much more work.'

The teams from Lund and Cedars-Sinai will now focus on increasing the efficacy of the vaccine and investigating the regulatory factors involved in the immune response to LDL.

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News in brief

Targets and mechanisms

Could exercise be a thing of the past?

Researchers have discovered a biochemical pathway in muscle cells that can generate the beneficial effects of exercise, without the hard work [1]. The research was conducted at Duke University Medical Center (Durham, NC, USA) and the University of Texas Southwestern Medical Center (Dallas, TX, USA). R. Sanders Williams, the Dean of Duke, said: 'We think this discovery could lead to the synthesis of new drugs that will allow individuals to acquire the health benefits of regular exercise, even if they cannot exercise. It has the potential to improve the lives of patients with heart failure, pulmonary disease, renal failure, diabetes and other chronic diseases,' says Williams.

In this study, the scientists discovered a cellular signalling pathway involving calmodulin-dependent protein kinases (CaMKs); these control genes that are responsible for the physiological and metabolic properties of muscle cells. The researchers generated transgenic mice that express a constitutively active form of CaMK IV in skeletal muscle, which subsequently showed increased levels of mitochondrial biogenesis, as well as the upregulation of enzymes involved in fatty acid metabolism and electron transport, and reduced susceptibility of the muscle cells to fatigue.

Skeletal muscle is made up of two types: muscle that handles long-term low-level loads and muscle that responds to sudden heavy loads. Exercise such as weightlifting makes muscles larger, while sustained exercise, such as long-distance running, increases resistance to fatigue and thus reduces the risk of disorders such as cardiovascular disease and diabetes.

Rhonda Bassel-Duby, Associate Professor of Internal Medicine and co-author of the study, said: 'The muscles of individuals who are on bed-rest resemble type II muscle fibres; they fatigue quickly and the muscles are tired.'

CaMK also induced expression of peroxisome proliferator-activated receptor γ co-activator (PGC1), a regulator of *in vivo* mitochondrial biogenesis. 'Activation of CaMK recapitulated the effects of exercise indicating that this is a central pathway by which exercise modifies the metabolic properties of skeletal muscles,' said Williams. 'Until now, scientists did not suspect that this particular enzyme was involved in that control.'

Hai Wu, postdoctoral research fellow and lead author of the study commented that, because CaMK is also responsible for neuron plasticity and is involved in learning and memory: 'Both neurons and muscle cells are excitable, and they share a lot of

1359-6446/02/$ – see front matter PII: S1359-6446(02)02314-0