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Tackling the most difficult diseases

Genetics and genomics open new strategies to fight vector-borne diseases

The 1990s witnessed one of the most successful programmes to eradicate malaria by tackling its vector, the *Anopheles* mosquito. Its main component was the release of sterilised male mosquitoes into the environment, which competed with wild ones for the available females. By repeating this every year with ever-increasing numbers of sterilised flies, health officials from the World Health Organization (WHO) and various countries hoped to eradicate the mosquito and thus malaria. In some parts of the world, this method indeed worked; southern Europe and large areas of Asia, North and Central America and Africa became virtually malaria free. But while Europe has remained so, the mosquitoes and the diseases they transmit have come back with a vengeance in many areas around the world.

The latest data released in August 2001 show that there are now between 700 000 and 2.7 million deaths each year from malaria, 75% of which are African children. According to the Multilateral Initiative on Malaria, this is a higher mortality rate than the yearly toll of AIDS in Africa. Besides the fatalities, between 400 and 900 million acute episodes of fever occur in children under the age of five which can slow brain development and retard cognitive abilities. Anthony James, a geneticist at the University of California in Irvine, refers to malaria as 'the most difficult vector-borne disease to control'.

Controversy abounds in the scientific community about the causes of the resurgence of these and other vector-borne infectious diseases such as leishmaniasis, yellow fever, encephalitis and dengue fever. Theories vary from global warming to the discontinuation of the use of DDT, but as Paul Reiter from the Centers for Disease Control in Atlanta, Georgia,

remarked 'Public concern should focus on ways to deal with the realities of malaria transmission, rather than on the weather'.

A number of scientists are responding to Reiter's challenge by focusing on genetics in order to analyse and ultimately modify the parasites or their vectors. 'Genetic engineering complements the other modalities of improved public health care and vaccine, drug and pesticide development, and is not aimed at replacing them', said Bruce Christensen, chair of the Department of Animal Health and Biomedical Sciences at the University of Wisconsin in Madison. 'It is unlikely that any single strategy will be successful for the complete control of malaria', James agrees. 'We anticipate that an integrated approach that includes bed nets,

to vary the peptide sequence in order to make the vaccine effective against several strains of the organism.

Another group is investigating the ability of *P. falciparum* to suppress an immune response, also with the goal of developing a vaccine against malaria. Magdalena Plebanski of the Austin Research Institute in Melbourne, Australia discovered that *Plasmodium* uses an 'altered peptide ligand' (APL) antagonism to dampen the cell-mediated immune response. It specifically disarms the protective T cells that ordinarily would kill infected cells. Plebanski's team has produced synthetic APLs that are agonists for T-cell activation, counteracting the antagonistic APL peptide variants found in *Plasmodium*. Mice infected with a mouse version of malaria that had been stimulated with two

Genetic engineering complements other modalities of improved public health care and vaccine, drug and pesticide development

parasite-resistant mosquitoes and vaccines will ultimately control or eliminate the disease if the role of each component intervention method is optimised for a specific region of transmission', he said.

Indeed, developing vaccines against the protozoan causing malaria and leishmaniasis has been extremely challenging, because the pathogens have developed various ways to evade the patient's immune system. But a number of groups are finally making headway. Giampetro Corradin of the University of Lausanne in Switzerland recently stated that his synthetic malaria vaccine, derived from a surface protein of the *Plasmodium falciparum* parasite, was the first to produce both strong B- and T-cell responses in early-stage human testing. Corradin plans

antagonistic APLs responded to an agonistic APL and had an immune reaction against all three. The team now plans to move into human trials within a year using agonistic peptides that they hope will reactivate the T-cell response in humans.

Researchers at the National Institute of Allergy and Infectious Disease (NIAID) achieved success in finding a vaccine against *Leishmania*, a protozoa affecting 12 million people in South and Central America, Africa and the Middle East—albeit a somewhat unusual one. 'Rather than targeting the parasite, as is typical, our researchers produced a vaccine to the saliva of the insect that transmits the parasite. This approach could potentially be used to develop vaccines for other insect- or tick-borne diseases', NIAID director

Anthony Fauci said. Early in August 2001, NIAID announced that a vaccine against a component in the saliva of sand flies—the insects transmitting leishmaniasis—protects against the pathogen. Head researcher Jose Ribeiro has focused on the chemistry of blood-feeding insects' saliva for the past three decades because of its effects on immunomodulation of the host, and demonstrated that animals immunised with sand fly saliva often resist infection when challenged with parasites or when bitten by a *Leishmania*-carrying bug.

The new study, published in the *Journal of Experimental Medicine*, used that information to produce a DNA vaccine encoding a saliva protein found in sand flies infected with *Leishmania*. Mice immunised with the vaccine contracted a much milder version of the disease than those that were unvaccinated. The researchers will next test dogs and monkeys, as well as examine other strains of *Leishmania* and sand flies in order to produce a vaccine for other types of the disease. They are also studying humans exposed to the disease to determine which particular components of saliva could be protective for them. 'Different sand fly species transmit different *Leishmania* species. If anti-saliva vaccines are to work in humans, they will have to be specifically engineered for the problem insects of each particular region', Ribeiro said.

Other researchers think that it could be worthwhile to use genetics and target the vector rather than the pathogen. '[The malaria mosquito] is an exquisitely complex host that supports a complicated life-cycle', Frank Collins, Professor of biological sciences at the University of Notre Dame in South Bend, Indiana, said, 'There are many points where it may be possible to genetically interfere with this life-cycle'.

Indeed, in August 2001 NIAID announced a US\$ 9 million award to Celera Genomics to expand the efforts to map the genome of *Anopheles gambia*, the malaria-transmitting mosquito, and to make the results freely available to the scientific community. Celera and an international consortium established two years ago, including the WHO, the Pasteur Institute, the European Molecular Biology Laboratory and others, will then analyse the *Anopheles* genome together with that of the human and the nearly completed sequence of *P. falciparum*.

This is good news because, so far, difficulties in raising malaria mosquitoes

under laboratory conditions and the small amount of biological material obtainable from mosquito organs have been stumbling blocks for identifying genes, Fotis Kafatos from the European Molecular Biology Laboratory in Heidelberg, Germany, said. His group and colleagues at the University of Iowa recently published first results from their *A. gambiae* pilot gene discovery project, which focuses on the mosquito's immune system's ability to block *Plasmodium*. They identified 30 new candidate genes, based on sequence similarity that may be implicated in immune reactions including antimalarial defence. Of these, 19 were shown experimentally to be inducible by bacterial challenge, 'lending support to their proposed involvement in mosquito immunity', as Kafatos commented. Only eight such genes had been previously identified; all these together should help unfold the nature of *A. gambiae*'s innate immunity and their role in the mosquito's defence system against the parasite.

The potential of genetically engineering mosquitoes has already been demonstrated by Marcelo Jacobs-Lorena of the Department of Genetics at Case Western Reserve University in Cleveland, who was the first scientist to make a mosquito resistant to *Plasmodium*. He looked at epithelial cells in the midgut and the salivary glands of the mosquito—the surfaces that *Plasmodium* must cross to complete its lifecycle—and identified a ligand that the parasite uses to infect these cells. Jacobs-Lorena then found a 12-amino acid peptide, SM1, that binds to these

disease transmission from vector to host by engineering mosquitoes that kill the pathogen before they bite and infect the host. Engineering a transgenic mosquito proved to be difficult, however, but James succeeded where others had failed. In 1998, James used a transposon called Hermes to be the first to transfer new genetic material into the mosquito genome, and produced a bug with red eyes. 'One needed to have the appropriate promoters so that the gene of interest could be expressed in the right time and at the right place', explained University of Wisconsin's Christensen, referring to the initial problems.

In 1999, James and his colleagues described the development of a malaria-resistant transgenic mosquito using the avian malaria parasite, *Plasmodium gallinaceum* and its vector *A. aegypti* as a test case for *P. falciparum* and *A. gambia*. The resistance gene James used consisted of a mosquito promoter controlling the expression of a mouse monoclonal antibody directed against a *P. gallinaceum* protein that interferes with parasite development and infectivity. Last year, James further described the use of another mouse monoclonal antibody that blocks infection of *A. aegypti* salivary glands when expressed from a Sindbis virus and which reduces infection by as much as 99.9%. At Michigan State University, Alexander Raikhel and Vladimir Kokoza are building on James' work by aiming to boost the mosquito's immune response to kill the parasite. They are designing *Aedes* mosquitoes to contain a gene that churns

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sites and recognises the same surface receptor as the parasite. 'The peptide inhibits parasite development in the mosquito by competing with *Plasmodium* for an essential salivary gland and midgut receptor', he explained. Indeed, SMI strongly inhibits *Plasmodium* invasion of the mosquito's salivary gland and midgut.

Other scientists even went a step further and are looking at ways to modify the mosquito such that it actively kills infectious pathogens. Using *Aedes aegypti*, a carrier of yellow fever and dengue, UC Irvine's Anthony James sought to interrupt

out more defensin, an immune protein that recognises and destroys certain pathogens.

But others question the value of such sophisticated high-tech approaches. Andrew Spielman from Harvard University is sceptical of developing transgenic mosquitoes for disease control. He wonders whether any African village would allow such mosquitoes to be released in their environment. 'Do you suppose one could convince villagers not to use mosquito netting and allow themselves to be bitten in order to test whether the transgenic mosquitoes could lower transmission?' he

asked. 'Why would anyone consent to this, and how could a field trial be done without consent of the local population?' Spielman also noted that 30 years ago when the WHO tried to release sterilised mosquitoes in the Indian countryside, the local population were so suspicious of the project that it was ultimately called off. He is also somewhat cynical about the funding of genetic engineering; he believes that's where the money is currently for research, which has attracted

researchers, but that it is little more than a passing fad.

'My biggest concern in working with the genome of these vectors is that parasites have a genome, too; who's to say that the parasite won't be able to mutate in response to a changing host genome?' asked Bruce Christensen. Most researchers in the area are examining the response of the vector to the parasite, but fewer are looking at the parasite's response to the vector, which he believes is an area

worthy of attention. 'There's been a lot of co-evolution between the two organisms, with different strains developing in different areas', Christensen said. 'We are a long way from impacting vector-borne infections, but I'm the last to say it won't work', he added.

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Salt of the Earth

Genetic engineering may help to reclaim agricultural land lost due to salinisation

During medieval times, salt was a precious commodity that was traded weight for weight with gold. Arab traders with salt caravans of up to 300 camels crossed the desert for days to communities such as the mystical Timbuktu, which rose to glory because of this lucrative trans-Saharan salt trade.

But salt can be more of a threat than an asset, and it lies at the heart of one of today's major agricultural problems. Excess salinity in soil has devastating effects on plant growth, reducing crop yields worldwide and even leading to complete crop failure in the worst-affected areas. To overcome these limitations, plant breeders have long been trying to generate more salt-tolerant crops, largely without success. Now, researchers from the University of Toronto and the University of California in Davis have genetically engineered plants to withstand significantly higher salt concentrations than their wild-type counterparts. Through the manipulation of just one gene, they have shown that plants are able to thrive in saline conditions that would otherwise be sufficient to class a soil as useless.

Salinisation is indeed a growing problem worldwide. Christopher Leaver of the Department of Plant Sciences in Oxford, referring in particular to Western Australia, commented, 'I have seen the problems of dry land salinisation and it is frightening'. And it comes at a time when food production is under great pressure. Global food supply is at risk from farming

methods that have degraded soils, depleted aquifers, polluted waters and caused the loss of animal and plant species. With the projected increase in population of 1.5 billion people over the next two decades coupled to increased urbanisation in developing countries, world agriculture is faced with an enormous challenge simply to maintain, let alone increase, our present level of food production. Ways must be found to achieve this without resorting to unsustainable farming practices and without major increases in the amount of new land under cultivation, which would further threaten forests and biodiversity. It is estimated that productivity will need to increase by 20% in the developed world and by 60% in the developing world; therefore, the best possible use must be made of these once productive soils.

Salinisation can take one of two forms: primary salinity is due to 'natural' causes

often disturbed the delicately balanced salt cycle of the earth.

Secondary salinisation stems most often from the over-irrigation of land, which is ironic given that this commonly occurs in countries where water supply is a problem. In order to remove the uncertainty of an erratic climate, the standard practice in many arid and semi-arid zones of the world is to irrigate agricultural land with fresh water on a regular basis (see Figure 1). This water contains very low levels of ions such as sodium, calcium, magnesium, potassium, sulfate and chloride, and as the water evaporates, these are left behind. Poorly drained soils are a particular problem where the water rises to the surface by capillary action and the land is never well flushed.

The accumulation of salts wreaks havoc on most plants, causing dehydration due to osmotic stress and biochemical perturbations due to the influx of sodium ions.

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such as salt carried in the air, while secondary salinity is due to man's effect on the environment. The latter problem is larger and more widespread since changing land use and management in connection with industrialised agriculture has

Estimates vary, but approximately one-third of the world's irrigated land is now deemed useless due to its excess salinity and this area is constantly increasing. This translates to a complete loss of 10 million cultivatable hectares a year and crop