RESEARCH ANALYSIS

CANDIDATE MALARIA VACCINE SYNTHESIZED

WASHINGTON, D.C.—It is less than a year since groups headed by J. B. Dame and V. Enea independently announced they had cloned and sequenced the gene for the circumsporozoite (CS) antigen of the malaria parasite *Plasmodium falciparum*. In that short time a collaborative effort among scientists at Smith Kline & French Laboratories (Philadelphia, PA), the Walter Reed Army Hospital (Washington, D.C), and the National Institutes of Health (Bethesda, MD) has turned the sequence into a promising candidate vaccine.

In a recent issue of *Science*, James Young and colleagues on this unique team describe the high level expression of an immunodominant epitope of the CS antigen. The CS protein is the key antigen associated with the protective immunity induced by sporozoites.

The group first attempted to express the complete CS protein in *Escherichia coli*. Although the genetic manipulations were straightforward, the protein was unstable when synthesized in the bacterial host. The strategy which eventually led to the successful synthesis of the candidate vaccine molecules may prove of general use in designing parasite vaccines.

The CS protein contains a large central repeat domain composed of 37 asparagine-alanine-alanine-proline tetrapeptides interspersed with four asparagine-valine-aspartic acid-proline tetrapeptides. Synthetic peptides representing this region are effectively immunogenic, and antibodies directed to epitopes contained in the repeat domain have biological activity. Thus Young and co-workers decided to clone and express only the repeat region of the protein. To accomplish this, they excised a XhoII fragment from the CS gene encoding 16 repeats of the tetrapeptide and inserted one, two, or three copies into an expression vector. Expression of the cloned sequence is controlled by a thermolabile λ repressor, and high level expression can be induced in the culture following a temperature shift to 42°C.

When the repeat regions were fused to a downstream open reading frame encoding 297 amino acids of the tetracycline resistance region of the plasmid, the product once again broke down into a heterogeneous mixture of proteins. The scientists reasoned that this instability might be due to the length of the carboxy-terminal fusion, and that if the tail were shortened a more uniform class of protein might be produced. This proved to be the case: when a deletion was made which introduced a stop codon 33 amino acids downstream of the repeated sequences, the constructs synthesized a single protein species at levels that allowed purification of 50 mg from a liter of culture.

When injected into mice, each of these constructs—containing one to three copies of the XhoII fragment—provokes high-titer synthesis of antibodies which recognize authentic CS antigen. They also possess biological activities associated with protective immunity. Protection to malaria induced by sporozoites is correlated with circumsporozoite precipitin (CSP) antibodies. Even when administered without adjuvant, the constructs with two or three copies of the repeat region induce antibodies with strong CSP reactivity. Another correlate of sporozoite induced immunity is the ability of sera to block sporozoite invasion of cultured hepatoma cells. The two and three copy repeat proteins elicit antibodies with strong blocking power, and again adjuvant is not required.

Further animal studies are in progress, and the first clinical trials are scheduled for this month. An effective vaccine against malaria, which kills 2–4 million people a year, may at last be close to hand.—**Harvey Bialy**

MEETING REPORT

OPPORTUNITIES CROP UP IN PLANT GENETICS

BERLIN—The future for genetic engineering in plants should not be underestimated just because many desirable characteristic are controlled by multiple rather than single genes, according to James Peacock, chief of the division of plant industry at the Commonwealth Scientific and Industrial Research Organisation (CSIRO, Canberra).

During a Dahlem conference on "Biotechnology: Potentials and Limitations," Peacock drew his audience's attention to the many cases where resistance to disease or pests is attributable to a single dominant gene. This is precisely the situation, he suggested, where gene isolation and transfer would provide a target genotype with a new function without impairing existing favorable attributes. Australian wheat production, for example, was dependent on continued plant breeding to bestow resistance against stem and leaf rusts. "In recent years the new disease of striped rust entered the country and was soon limiting yields significantly in some areas. Now we face the need to find suitable sources of resistance genes and breed them into the different cultivars which we use in contrasting ecoclimatic zones—a task which has already diverted major segments of our wheat breeding expertise," Peacock said. "But sources of resistance are known and appear to be associated with a single locus. If we could isolate that locus or gene segment and transfer it to each of the specialized cultivars, we could solve the striped rust problem."

Genetic engineering might well have helped the Australian lucerne (alfalfa) industry too, had appropriate techniques been available earlier. "This major pasture legume has been selected for a series of specialized properties in our integrated animal and cropping production systems," said Peacock. "It proved to be highly susceptible to two aphid species which had not existed previously in Australia but were accidently introduced. We succeeded in generating aphid-resistant cultivars by limited back-cross breeding, using related American material containing appropriate genes. But we were not able, as quickly as we would have wished, to back-cross sufficiently to restore completely the highly-adapted genotype to the previous cultivar."

Here again, however, the problem was so simple in theory as to suggest that gene splicing could have provided the answer. For each of the two pests, resistance was encoded by just one or two dominant genes. Adding this capability to the existing cultivar by gene transfer would have been greatly preferable to the more tedious and time-consuming procedure of hybridization and back-crossing.

—**Bernard Dixon**