Human Malarial Gene Cloned

Cloning of the gene for the major surface protein of the Plasmodium falciparum *sporozoite opens the way to a malarial vaccine*

With the malaria threat increasing because of the growing resistance of the mosquito vector to insecticides and of the parasite itself to drugs, there is a growing need for an antimalarial vaccine. In this issue of *Science* two groups of investigators report a possible step toward achieving that goal.* They have cloned the gene coding for the major surface protein of the sporozoite of *Plasmodium falciparum*, the species that causes most of the severe malaria infections in humans.

The sporozoite is an especially attractive target for a vaccine. While in its human host, the malarial parasite passes through several antigenically distinct phases, the first of which is the sporozoite, the form injected into the bloodstream when the mosquito bites its victim. Sporozoites rapidly enter the liver where they multiply and develop into merozoites, which in turn invade and multiply in red blood cells. Finally, a small fraction of the parasites within the red blood cells form gametocytes, which may be picked up by another mosquito, thus initiating a new round of infection. An effective antisporozoite vaccine would prevent malaria in the vaccinated individual and block its spread.

Until now, attempts to develop an antisporozoite vaccine have been handicapped by the great difficulty in obtaining sufficient quantities of that parasitic form. Sporozoites have to be extracted from the salivary glands of mosquitoes, not exactly a plentiful source of antigens for vaccinating millions of people.

Sporozoite scarcity has also been a problem for investigators who wanted to clone genes and use them to synthesize antigenic proteins. "Classic" cloning methods require a source of the messenger RNA corresponding to the desired gene, which in this case would be the hard-to-obtain sporozoites. Still, the classic methods could be used, as investigators at New York University Medical Center have shown.

Last year, the gene coding for the major surface protein, called the circumsporozoite (CS) protein, of *P. knowlesi*, which causes malaria in monkeys, was cloned in that way by G. Nigel Godson and Joan Ellis. And now, Vincenzo Enea, Ruth Nussenzweig, and their colleagues have done the same for the analogous gene of *P. falciparum*. "We had to feed mosquitoes on the blood of patients in the infective stages, which was done by our collaborators in Bangkok, Thailand," Nussenzweig says of the *P. falciparum* work. "It took about 2 years to accumulate enough material to do one cloning experiment."

A second group, including Thomas McCutchan and Louis Miller of the National Institute of Allergy and Infectious Diseases (NIAID) and several investigators from the Walter Reed Army Institute of Research, also cloned a sporozoite CS gene, although from a different strain of *P. falciparum*. These researchers did not use the classic cloning methods but employed a new method that does not require messenger RNA's or sporozoites. They obtained the gene directly from the DNA of an erythrocytic form that can be grown in culture.

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The new method, which was developed by McCutchan and his NIAID colleagues, may prove to be generally applicable. It uses a nuclease enzyme from the mung bean plant to cut the DNA of the organism in question. At least for the plasmodial DNA, and possibly for DNA's from other organisms as well, the nuclease clips just before the beginnings and after the ends of genes. The DNA segments thus produced encompass intact genes, which can then be cloned in bacteria. The mung bean nuclease does not appear to recognize specific nucleotide sequences, incidentally, but some other, higher order of DNA organization and may also serve as a tool for probing gene punctuation structures.

Sequencing of the cloned gene has revealed some interesting features in the predicted structure of the CS protein. According to John Dame of the NIAID group, a major portion of the protein consists of 41 tandomly linked tetrapeptides, 37 of which are identical. These are composed of residues of the amino acids asparagine, alanine, asparagine, and proline, in that order. The remaining four repeats consist of asparagine, valine, aspartic acid, and proline residues. The gene cloned by Enea of the Nussenzweig group is incomplete and contains a tandem sequence of 23 tetrapeptides, all of them having the same amino acid sequence as the major tetrapeptide identified by the NIAID and Walter Reed workers.

Dame and his colleagues have not found any uninterrupted stretches of 23 repeats of the major tetrapeptide, and the lack of the minor tetrapeptide in the Enea sequence may represent an actual variation between the two genes. "That's the place where one would predict variation," Miller says. "I expect that the number of repeats or their composition would vary in different strains. The more interesting question is, does this matter for immunization?"

Antigenic variation among different parasitic strains of the same species is of great concern to investigators who are trying to develop vaccines. If the antigens of two strains are sufficiently different, then a vaccine against one may not protect against the other.

Vaccines against the sporozoite may have an advantage in this regard. For example, Fidel Zavala of the Nussenzweig group has found that a single monoclonal antibody recognizes the same repeating structure in sporozoites of more than a dozen *P. falciparum* isolates from diverse geographic areas. "The beauty of the sporozoite," says George Cross of Rockefeller University, "is that it does not appear to have significant interstrain variation. The only variation so far is between species."

The big issue—one that cannot be resolved yet—concerns whether a vaccine directed against the CS protein will protect humans against malaria. In previous work, Nussenzweig and her colleagues including Victor Nussenzweig, also of NYU, have shown that antibodies to the

*J. B. Dame *et al.*, p. 593; V. Enea *et al.*, p. 628.

CS protein can protect against malaria in animals such as mice. One monoclonal antibody, Ruth Nussenzweig says, "completely inhibited the invasion of the liver by interfering with recognition and penetration."

In the more recent work, both cloning groups have found that short synthetic peptides containing the repeating tetrapeptide block the binding of monoclonal antibodies to the sporozoite protein, results which show that the antibodies are specifically directed against the repeat. In addition, the NYU workers have shown that monoclonal antibodies against the repeating peptide of *P. knowlesi*, which is 12 amino acids long, bind to sporozoites of this species and abolish their infectivity.

The results suggest that it might be possible to immunize humans against malaria by using either synthetic peptides or the whole CS protein. But, Miller notes, a very high level of immunity will have to be maintained to inactivate 100 percent of the sporozoites. If even one escapes unscathed into a liver cell, the disease can still occur.

Work on an antisporozoite vaccine is only one aspect of current research on malaria prevention. A great deal of effort has been directed at identifying antigens from the merozoite and red blood cell stages of the parasite that might prove effective for vaccination. In general, these stages are more complex and variable in their antigenic composition than the sporozoite.

Nevertheless, several laboratories have been making progress in cloning genes that might be used to vaccinate against these forms of the parasite. One such gene appears to code for the protein by which the merozoite recognizes the red blood cell. This protein may be a good candidate for a vaccine. It is effective in evoking an immune response and, because it must interact specifically with the human red blood cell membrane, may be less subject to variation than other merozoite antigens.

An effective antimerozoite vaccine would prevent or ameliorate symptoms in an infected individual, but would not block further spread of the parasite. An antigametocyte vaccine, which is also in the works, would prevent the spread of malaria without affecting the course of the disease in the currently infected person. "It's the same as killing off the mosquito," notes Cross.

The different approaches to a malarial vaccine all have different strengths and weaknesses and it is currently difficult to say which will ultimately pay off. It is also possible that more than one type of malarial vaccine will be needed to give effective protection. As Cross sums up the current situation, "The science is in a very exciting stage, but whether it will lead to clinical results is unknown."

—JEAN L. MARX

Artificial Intelligence in Parallel

Working with many processors at once could accelerate computation enormously—and suggest new ways to think about thinking

It is a painful fact that modern computer intelligence is sharply constrained by the computers. "The bigger we make our programs, the 'smarter' they get and the slower they get," laments Thomas Knight, a researcher in artificial intelligence (AI) at the Massachusetts Institute of Technology (MIT). "We're in the embarrassing position that we give a program more information, and it gets worse."

Consider expert systems, for example, which are programs that try to encapsulate human expertise in a set of rules. The available processing power sets a practical limit of just a few thousand rules, which means that even the best artificial expert is nothing more than an idiot savant in one narrow area. Getting computers to exhibit "common sense" in real time means speeding them up and increasing their memory capacity by a factor of thousands or millions.

Or consider one recent experiment in which a computerized cart successfully used a vision system to pick its way through obstacles in a hallway: every time it advanced a meter it had to stop and reassess—for 15 minutes. Getting a system to walk and chew gum at the same time, so to speak, means speeding up this kind of performance by a factor of roughly a million.

The irony is that the brain is beating out the computers with neurons that operate about a million times slower than silicon. And the secret, of course, is in the wiring: the neurons are in there doing millions or billions of operations simultaneously. Whereas computers, with few exceptions, are still based on the serial, one-step-at-a-time architecture laid out by computer pioneer John von Neumann in the 1940's: one central processing unit, one memory bank, and one data channel connecting them. It is rather like having one bank teller on duty during the lunchtime rush.

Thus the current rage in the AI community, and for that matter in the computer science community generally, is "parallelism." The idea is to build machines with many independent processors doing many things at once—perhaps even several million things at once.

Actually, this is a new revival of an old idea. People were proposing and designing parallel computers as long ago as the 1940's. And starting in the 1970's, as advancing technology began to make it possible, high-performance supercomputers began to incorporate certain kinds of concurrency through the use of "array" processors and the "pipelining" of data. The current generation of supercomputers can handle as many as 16 data streams simultaneously.

But the striking thing about the current ground swell is its emphasis on massive, million-processor parallelism. A big part of the push comes from the ferment in AI applications. For example, the Defense Advanced Research Projects Agency (DARPA)'s Strategic Computing Initiative has given top priority to the development of very fast symbolic processors. So has Japan's Fifth Generation project (*Science*, 24 February, p. 802). Moreover, both military and industrial users of AI are putting a premium on real-time performance in such things as robot vision and natural language interfaces.

But a large part of the interest also comes from the possibilities being opened up by semiconductor technology. It happens that many of the parallel designs are well suited to very large scale integration (VLSI). And by no coincidence, it also happens that DARPA has had a VLSI program since 1979: simply send in a design to one of the DARPA-sponsored "silicon foundries" and back it comes as a chip. The result is