CATCHING THE FEVER, FASTER

by Bernard Dixon

> On Wednesday morning, with the news that Jordi Casals was going downhill more rapidly than ever, a major meeting was called in Room 608 of the library of the Yale arbovirus laboratory. Just before the meeting, Wil Downs put through a phone call to his colleague, Dr. Karl Johnson, a leading international virologist. Johnson was in Panama for the U.S. Public Health Service, working with the Machupo virus, which caused the deadly Bolivian hemorrhagic fever.
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> Downs knew that Johnson had run into a parallel crisis in Panama. People were dying like flies from the Machupo virus, and Johnson had made the painful decision to administer immune serum from patients who had recovered from the disease. It had worked in some cases; in others it did not work. But Johnson was certain of one thing: if you waited too long, the serum was useless...
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> from *Fever!*, by John G. Fuller

Far more compelling than any number of SF novels about killer viruses and baffling epidemics, Fuller's book portrays the emergence of Lassa fever in Nigeria in 1969 with atmospheric suspense. And no single incident in his absorbing narrative is more powerful than the moment when, after one victim had partially recovered following a devastating bout of Lassa fever, physicians had to decide whether to transfuse some of her serum into another, terribly ill patient suspected of having contracted the same disease. In theory, antibodies in the serum could help the second victim to fight the infection. But there was no certainty of this, and on previous experience the fever would be expected to prove fatal before the completion of tests to confirm the diagnosis.

Worse—the patient might even have some other disease altogether, in which case the precious serum would be wasted. There was also a chance that he could unwittingly be given Lassa fever, if virus particles had persisted in the serum. Then there were the routine risks associated with serum transfusion, including those of anaphylactic shock and the chance of transferring hepatitis B virus; plus dangers to the donor, who was far from entirely healthy. Another possibility was that passive immunization could adversely affect the patient's own immune system, if it was beginning to mount a defense against the Lassa virus.

In the event, and after much heart-searching, the team did decide to transfuse the serum—and it worked. But the situation they encountered continues to haunt communicable disease specialists, who still have an extremely limited armamentarium to deal with virus infections. Although active immunization with vaccines remains the main strategic approach to viral infections today, passive immunization with immune serum from recovered individuals is still an important technique to be considered at any time when facing a newly recognized infection with high morbidity and mortality. Added to the risks and hazards which taunted the Lassa fever researchers, a highly transmissable agent could pose horrendous problems of triage for health personnel in deciding exactly how to use an extremely limited supply of serum.

Now a solution may be at hand, as a result of work by Greg Winter and his colleagues at the Medical Research Council Laboratory of Molecular Biology in Cambridge, England. Winter was very much the man of the moment recently, when he addressed this year's BioTechnica meeting in Hannover, just five days after his group had published their paper (*Nature* **341**:544, 1989) on the manufacture of single domain antibodies by *Escherichia coli*. His audience, and the chatterers around Hannover's gigantic exhibition area, quickly latched onto the more immediate ways in which "DAbs" could supplant and improve on monoclonal antibodies. While expected to rival MAbs over the next few years in applications such as diagnosis, protein purification, the ridding of toxins from the body, and the tissue targeting of toxins as magic bullets, the much smaller DAbs molecules should be able to penetrate infected and malignant tissues more readily, and reach deep "canyon" sites on viruses. But this same technique could, surely, also provide a means of generating large quantities of pure, specific antibodies to help medicos in handling exactly the sort of dilemma that was precipitated by the emergence of Lassa fever.

The beauty of the Cambridge approach—immunizing animals against a particular antigen, using the polymerase chain reaction (PCR) to clone the genes encoding antibody specificities (rearranged immunoglobulin heavy chain variable genes) directly into vectors for expression in *E. coli*, and then screening the bacterial colonies for antigen-binding activities—is, of course, its disarming speed. Not only have Winter and his co-workers discovered that many VH domains bind antigen in the absence of their light chain domain partners; they have also found that these can be generated within two days following the harvesting of spleen cells, as compared with a month or so for conventional hybridoma technology. Another advantage, of great topical and social significance, is a considerable reduction in the number of animals that will be required for antibody production. Bacterial fermentation is a much cheaper and considerably more acceptable alternative.

True, as Winter pointed out in Hannover, several aspects of DAbs remain to be investigated. We need to know more about their affinities, their specificity, their cross-reactivity ("probably less than with MAbs because the overall footprint is smaller"), and their possible immunogenicity when injected (because surfaces normally concealed will be exposed to a host's own immune defenses). Another question concerns how rapidly DAbs are cleared from the bloodstream.

Will it be possible, then, faced with the next newly emergent Lassa fever virus, to harness PCR to clone the relevant genes from a convalescent patient's spleen cells (or indeed from lymphocytes in peripheral blood) and to splice them into *E. coli* as a workhorse for immunoglobulin production? Time will tell, but it's certainly an enticing scenario.