Chip-based diagnostics for meningitis and food poisoning

Julie Clayton, freelance writer

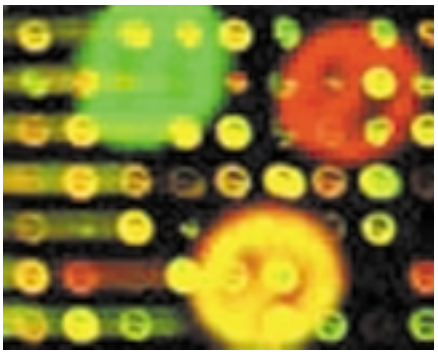
Scientists have revealed a novel technique that integrates DNA microarray technology with PCR, and can distinguish between the different organisms that cause meningitis and make a diagnosis within hours rather than days. This advance could lead to more appropriate antibiotic therapy, thus reducing the risk of infection with drug-resistant bacteria. A second group is using a similar technology to diagnose and monitor food poisoning.

Yuri Boriskin and colleagues from the Department of Medical Microbiology at St George's Hospital (London, UK) presented their technique at the *12th European Congress of Clinical Microbiology and Infectious Diseases* (24–27 April 2002, Milan, Italy). So far, the DNA chip can distinguish between 14 different viral causes of meningitis and encephalitis. Within six months, the researchers hope to have added the capacity to detect several different types of meningitis-causing bacteria and fungi to the test kit.

Meningitis and drug resistance

Infection with drug-resistant bacteria is reaching epidemic proportions in hospitals, particularly in intensive care units where patients with meningitis are treated. Meningitis is often fatal, and can be caused by bacteria, such as *Neisseria meningitides*, and viruses. A rapid diagnosis is essential for determining the best treatment option.

Although there is no treatment for viral meningitis, bacterial meningitis can be treated, if caught early enough, with antibiotics. Diagnosis often requires sequential tests for many different organisms, which currently can take several



days, and so hospitals usually start treatment immediately with broad-spectrum antibiotics while they await results. However, this approach has the disadvantage of leaving patients vulnerable to contracting drug-resistant bacterial infections.

For patients with viral meningitis, this new technique could mean that antibiotics could be avoided altogether, or at least stopped quickly. For those with a bacterial diagnosis, treatment could be changed quickly to alternative narrow-spectrum antibiotics, which is less likely to cause resistance or lead to infection with other opportunistic pathogens such as the yeast, *Candida albicans*.

Novel integrated technology

The test is the first to integrate DNA probes for distinguishing between multiple different viruses on a single chip, according to Boriskin and his colleague, Philip Rice, consultant virologist at St George's Hospital. DNA chips enable a potentially unlimited repertoire of specific pathogen probes to be spotted onto a glass microscope slide, which is then hybridized with multiplex-PCR-amplified DNA from patients' cerebrospinal fluid (CSF) samples. The presence of a specific pathogen is detectable 'at a glance' as a fluorescent signal.

To date, Boriskin's team has used the array to diagnose the viral cause of 39 cases of meningitis. Of those, 22 were caused by enteroviruses including herpes simplex, Varicella–Zoster, herpes viruses types 6 and 7, Epstein–Barr virus, cytomegalovirus and papovavirus.

The team will add other viral probes, such as mumps and measles, as well as probes for other pathogens, including bacteria, leptospira, fungi and protozoa. 'That is the ultimate aim, to maximize the [use of the] CSF sample that you have, to keep it in one lab, with one test,' declared Rice. He continued: 'In the UK, laboratories typically perform viral diagnoses with PCR for three or four viruses at a time, and often have to divide samples among many different labs.' The new test is as sensitive as PCR, but with the advantage that it would save time in diagnosis and call a halt to prophylactic treatment.

'Although the test is still a long way from routine clinical use, it is a potentially very exciting approach,' says Jonathan Cohen, microbiologist and Dean of the Brighton University Medical School (Brighton, UK). He added: 'If you could be sure it was *E. coli* rather than *Pneumococcus*, for example, that would be enormously helpful for focusing antibiotic therapy.'

Diagnostics for food poisoning

Meanwhile, Olivia Champion from the London School of Hygiene and Tropical Medicine (London, UK) is compiling a second-generation DNA chip containing the genes of different strains of

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Campylobacter jejuni, a bacterium that causes severe food poisoning. This will assist both accurate diagnosis of the pathogen, as well as monitoring its evolution over time.

Using the DNA of *C. jejuni* NCTC 11168, the first strain to be sequenced, Champion has already assembled a microarray for this strain, and is now adding individual genes of other *C. jejuni* strains, isolated and donated by research groups worldwide. Different *C. jejuni* strains can vary considerably (e.g. in their content of different virulence factors) according to analysis by Champion's colleague Nick Dorrell. The *C. jejuni* composite chip will better represent the whole species, Champion told delegates attending the *Ordinary Meeting of the Society for General Microbiology* (8–12 April 2002, Warwick, UK).

With approximately 60 microbial genome sequences now deciphered, and an additional 100 or more under way [1], including a second *C. jejuni* strain by The Institute for Genomic Research (TIGR; Rockville, MD, USA), the progress of DNA chip-based diagnostics looks extremely promising. 'In theory it could be used for all sorts of diagnostic applications,' Champion concluded.

Reference

1 Doolittle, R.F. (2002) Biodiversity: microbial genomes multiply. *Nature* 416, 697–700

Bacteria prove gutsy against inflammatory bowel disease

Julie Clayton, freelance writer

A gene responsible for the deadly effects of the bacterium *Yersinia pseudotuberculosis* – a relative of the plague-causing pathogen *Y. pestis* – has a beneficial side. The 'Jekyll and Hyde' gene, which is active in non-pathogenic strains of *Salmonella* bacteria, has been shown to block immune rejection of these non-pathogenic strains in *in vitro* studies. This finding promises to be the first of many new insights into the molecular signaling between gut-dwelling microbes and their hosts, and could lead to more rational treatments for diseases in which this signaling has broken down.

Salmonella strains

Salmonella spp. cause severe diarrhoea in humans by triggering a massive immune reaction in the gut lining. This involves the activation of signalling molecule nuclear factor-κB (NF-κB), which in turn triggers release of the cytokine interleukin-8 (IL-8) to attract neutrophils and other white blood cells to the site of infection.

Andrew Neish and his team at the Emory University School of Medicine's Epithelial Pathobiology Unit (Atlanta, GA, USA) have discovered two *Salmonella* strains that actively inhibit, rather than trigger, NF-κB activation and IL-8 secretion in cultured epithelial cells. These strains are *S. pullorum*, which is non-pathogenic in humans but causes disease in chickens, and a laboratory mutant of *S. typhimurium* NF-κB [1]. Both strains also promote apoptosis, which is normally inhibited by NF-κB.

It has now been shown that the inhibitory effect on NF-κB and IL-8 is caused by a gene called *AvrA*, the *S. typhimurium* homologue of a disease-promoting gene in *Y. pseudotuberculosis*, Neish told delegates at the *Ordinary Meeting of the Society for General Microbiology* (8–12 April 2002, Warwick, UK). The *AvrA* gene product is one of several signalling proteins known as type III secretion systems. These are highly conserved in nature and have homologues in the plant kingdom that trigger the appearance of a protective ring of apoptotic cells around sites of virus infection, according to Neish. He suspects that, by analogy, promoting apoptosis in the gut lining could be protective for other bacteria, particularly the commensal flora that normally inhabit the intestine. He stresses, however, that the *in vivo* relevance of his findings is speculative: 'How that translates to biology in a whole animal is open to interpretation – is that a pathogenic or a non-pathogenic mechanism?'

Martin Maiden, Senior Research Fellow at the Department of Zoology, University of Oxford (Oxford, UK) commented that: 'Neish's work suggests that a lot of these things that people have only studied from the point of view of disease, particularly type III secretion systems, actually are rather more complicated and interesting than that, in that the bacteria are modulating an interaction with the host.'

Other colonization inhibitors

Elizabeth Furrie, a postdoctoral researcher at the University of Dundee (Dundee,

 1359-6446/02/$ – see front matter PII: S1359-6446(02)02334-6