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A role for arrays in clinical virology: fact or fiction?

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Abstract

Microarrays of DNA probes have at least three roles in clinical virology. These are: firstly, in diagnosis, to recognise the causative agent of an illness; secondly, for molecular typing for (i) patient management, (ii) epidemiological reasons (e.g. investigating routes of transmission), (iii) purposes related to vaccine use; and thirdly, in research, to investigate the interactions between the virus and the host cell. Microarrays intended for syndromic diagnostic purposes require genome specific probes to capture the unknown target viral sequences and thereby reveal the presence of that virus in a test sample. Microarrays intended for typing and patient management, e.g. monitoring antiviral drug resistant mutations require a set of probes representing the important sequence variants of one or more viral genes. Microarrays intended for research into virus-host interactions require probes representative of each individual gene or mRNA of either the virus or the host genome. Diagnostic microarrays are dependent for their utility and versatility on generic, multiplex or random polymerase chain reactions that will amplify any of several (unknown) viral target sequences from a patient sample. In this review, the existing and potential applications of microarrays in virology, and the problems that need to be overcome for future success, are discussed.

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1. Introduction and background

DNA arrays consist of oligonucleotide or PCR amplicon probes immobilised on a solid surface, usually glass or a nitrocellulose or nylon membrane. Membrane based arrays may be in the format of line probe blots. The results of hybridisation between the bound probe and labelled sequences in the sample applied and tested are revealed by scanning or imaging the array surface. High density arrays, which may have thousands of individual probes per cm², are referred to as microarrays. Arrays can be prepared in-house using one of the several arraying instruments available commercially. These will spot synthetic oligonucleotides or PCR amplicons onto glass slides at low or high density. Microarrays of oligonucleotides can also be synthesised on a silicon surface by photolithographic combinatorial chemistry methods similar to those used to make electronic chips. These microarrays on silicon surfaces are therefore known as ‘DNA chips’. An example is the Affymetrix human immunodeficiency virus type 1 (HIV-1) antiretroviral drug mutation detection GeneChip which comprises a set of matched and mismatched probes designed to hybridise with protease and reverse transcriptase (RT) gene sequences.

Detailed technical information about arrays can be found in books (Schena, 1999) and Nature Genetics Reviews (1999, 2002), and the uses of arrays have been reviewed (Cummings and Relman, 2000; Kato-Maeda et al., 2001; Manger and Relman, 2000) see also Table 1. The usual nomenclature for arrays is that the element bound to the solid phase is the ‘probe’, while the ‘target’ is in solution. This is in contrast to Southern or dot blotting where the ‘target’ is bound and the ‘probe’ is in solution. In both cases, though, the target is DNA or RNA sequences derived from the test specimen. The process of making and using an array is illustrated in Fig. 1, and a check list of requirements for an arraying project is given in Table 2.

Reviewing the potential benefits that microarrays or DNA chips may be able to offer to the clinical virologist requires a certain amount of crystal ball gazing, and runs the risk of being overtaken by unforeseen developments. Nevertheless, it is possible to paint a broad picture of how microarrays might fit into the routine of the diagnostic laboratory in the near future:

- First, nucleic acid would be extracted from pathological specimens by means of an automated instrument.

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Table 1
Potential uses of DNA arrays

The genetic polymorphism of the virus
(1) Detection of the presence or absence of specific viruses (e.g. influenza virus (Li et al., 2001) rotavirus (Chizhikov et al., 2002))
(2) Confirmation of the identity of PCR products for:
   Diagnosis (e.g. Rota et al., 2003; Wang et al., 2002)
   Epidemiological surveillance and transmission
   Host range determination
   Monitoring molecular evolution and new strains
   Vaccine coverage surveillance
(3) Antiviral drug resistance mutation screening (e.g. HIV-1 (Kozal et al., 1996; Wilson et al., 2000))
(4) Vaccine quality control (e.g. poliovirus, mumps virus (Amexis et al., 2002; Cherkasova et al., 2003; Proudnikov et al., 2000))
(5) Detection of the presence or absence of specific genes (e.g. those associated with virulence and/or pathogenicity (Chizhikov et al., 2001))
(6) Study of the RNA expression profile of virus following infection, including drug effects in vitro (e.g. HCMV (Chambers et al., 1999), KSHV (Jenner et al., 2001))
(7) Study RNA expression profile of host following infection, including drug effects in vitro (e.g. HCMV (Zhu et al., 1998), PVM (Domachowske et al., 2002), HIV-1 (Corbeil et al., 2001; Gross et al., 2000))

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**Fig. 1.** An illustration of the processes involved in making and using an array. At the top is depicted the route from the virus of interest to the spotted arrays (herpesvirus and enterovirus particles are shown). At the bottom, DNA or RNA is extracted from samples, amplified and labelled with either Cy3-dCTP (green) or Cy5-dCTP (red). When applied to the array bearing the immobilised probes, the target binds to complementary sequences. An example of an array result is shown on the right: the green spots represent hybridisation of the probe only with target sequences labelled with Cy3-dCTP; the red spots represent hybridisation of the probe only with target sequences labelled with Cy5-dCTP; the yellow spots represent hybridisation with both target sequences.

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*a More useful for bacterial pathogens.*
Table 2
Requirements for a viral DNA arraying project

| Requirement                                              |
|----------------------------------------------------------|
| Genomic sequence data from several strains of the target viruses |
| Sets of PCR primers for making amplicons to be bound as probes to the solid phase or sets of synthetic oligonucleotides to be bound as probes to the solid phase |
| An arraying instrument                                   |
| Generic and multiplex PCR assays for amplifying and fluorescently labelling target DNA/RNA |
| Hybridisation and washing chambers                       |
| A scanner to capture readout data from the array         |
| Software to interpret array readout data                 |

- Second, the RNA and/or DNA would be subject to an enzymatic process that selectively amplifies and fluorescently labels sequences from the genome of any virus that is present.
- Third, the amplified nucleic acid would be applied to a DNA chip and hybridize to complementary sequence probe(s) representing known pathogenic viruses.
- Fourth, detection instrumentation and software would interpret the fluorescent hybridisation signals on the chip and provide a readout that identifies the virus present in the original specimen.

How close is this to reality? In that it assumes the existence of a universal ‘panviral’ DNA chip, it is arguable that it makes little diagnostic or experimental sense. Pathological specimens, and viruses, come in all shapes and sizes and clinical judgement should be exercised to assess the probable causes of whatever condition the patient has, leading to an intelligent guess of which viruses to test for. It makes little sense, for example, to search for respiratory viruses in a gastrointestinal sample, or blood borne viruses in a throat swab. From this it follows that informed decisions need also to be made about which extraction protocol and amplification primers should be used. It seems likely, therefore, that rather than universal extraction and amplification methods and a panviral DNA, specialist chips will be developed for the diagnosis of similar clinical conditions.

2. Molecular diagnostic arrays

The development of arrays that will be useful for diagnostic purposes in the clinical virology laboratory is dependent in part on the stage of development of PCRs for the detection of individual viral genomes. Generally, any DNA array system will need to be used in conjunction with a nucleic acid amplification method and, specifically, PCR methods capable of amplifying a wide range of viral genomes will be required. For example, if an investigator wishes to develop a microarray that would be useful in the diagnosis of viral infections of the CNS, it would be necessary to be able to amplify DNA sequences from a range of herpesviruses, enteroviruses, adenoviruses and retroviruses as well as JC, influenza, measles, mumps, rubella and West Nile viruses. The ability to do this is dependent on generic (Table 3), multiplex (Table 4) and random PCR amplification procedures (Bohlander et al., 1992; Elnifro et al., 2000; Wang et al., 2002).

2.1. Generic and multiple sequence amplification

Generic PCR tests are designed so that only one or two pairs of primers are necessary to amplify a target sequence types of such chips are already in use in many laboratories, but there is still a long way to go before they become routine in the way of, for instance, diagnostic ELISA kits. It is also probable that just as automated serological methods are the province of commercial suppliers, diagnostic viral DNA chips and accompanying instrumentation will have to come from that source, because of their cost and the need for consistency and quality control. Research and reference laboratories will, however, be involved in much of the bio-technical development necessary before such commercially supplied chips become available. These laboratories will also need to develop and maintain their own in-house methods for confirmatory purposes, for detecting more obscure viruses, and to monitor the performance of commercial equipment.

Table 3
Examples of generic primer PCR tests

| Virus Type                     | Target            | Reference                                                                 |
|-------------------------------|-------------------|---------------------------------------------------------------------------|
| Papillomaviruses              | L1 region         | (Coutlee et al., 2002; Kletz et al., 1998; Nelson et al., 2000)            |
| Enteroviruses                 | 5′-NCR            | (Clewley, 1995; Shih et al., 2003; Zell et al., 1992)                      |
| Herpesviruses                 | DNA polymerase    | (Calvario et al., 2002; Elders et al., 1999; Minjolle et al., 1999; Odeben et al., 1996; Yamamoto and Nakamura, 2000) |
| Retroviruses                  | Pol region        | (Bumstein et al., 2001)                                                   |
| Lentiviruses (HIV/SIV)        | Pol region        | (Clewley et al., 1998; Courgnaud et al., 2001; Gelman et al., 1992; Masciotra et al., 2002; Miura et al., 1990; Yang et al., 2000) |
| Positive stranded viruses     | RNA polymerase    | (Chen and Plagemann, 1995)                                               |
| Influenza viruses             | NP gene           | (Corazza et al., 2003)                                                    |
| RSV                           | Fusion protein    | (Corazza et al., 2003)                                                    |
| Adenoviruses                  | Hexon protein gene| (Corazza et al., 2003)                                                    |
| Dengue viruses/flaviviruses   | 3′-NCR, NS3 protein gene | (Chow et al., 1993; Hoang et al., 2001; Saah et al., 1995)               |
| Arenaviruses                  | 3′-NCR            | (Lorano et al., 1997)                                                    |
| Rotaviruses                   | VP7 segment       | (Chichkov et al., 2002)                                                  |
from a range of related viruses, for example those from the same genus or family. To develop this type of test, regions of conserved amino acid and nucleotide sequence are identified in the target genome. This is accomplished through multiple alignment of genome sequences from as many strains of the virus as possible. Suitable conserved sequences are found in protein encoding regions (e.g. at the active site of the enzyme) or untranslated regions that fold up to form self-annealed tertiary structures (e.g. ribosome binding sites and the 5′ and 3′ non-coding regions of viral genomes). Generic PCRs have been designed in this way for a conserved region of the DNA polymerase gene of the herpesviruses and for the 5′-NCR of the enteroviruses (Table 3). The primers for generic PCRs are often degenerate, having mixed bases at positions of redundancy in the target sequence, or they may utilise the base inosine at positions of maximum degeneracy (e.g. see, Clewley et al., 1998). The amplification reagents and thermal cycling conditions usually have to be empirically established for successful generic PCR.

In contrast to generic PCR, multiplex PCR involves the combination of several primer sets targeting different sequence regions in one amplification reaction (Table 4). In a PCR for neurological viruses, this might include generic primers for amplifying herpesvirus and enteroviruses together with more specific primers for measles, mumps, rubella and West Nile viruses. In a similar manner to generic PCRs, multiplex PCRs require careful optimisation, and even then it is difficult to make them as sensitive as specific single primer pair amplification reactions. To attain sufficient sensitivity, it may be necessary to split any sample into several aliquots for amplification with selected subsets of the full range of primers. Thus either one complex or several simpler multiplex PCRs containing a mixture of generic and specific probes would be used to amplify and label DNA extracted from CNS or brain tissue.

The microarray that would then be used to detect the product of a multiplex PCR for neurological viruses would contain probes for each individual virus of interest that is, for example, probes for specific EBV, VZV, HHV-6, HSV-1, HSV-2, CMV and HHV-7 sequences in the gene region between the generic primers. It would also have, if possible, a generic ‘catch-all’ probe so that uncharacterised herpesvirus sequences not recognised by the specific probes would not be missed. Similarly, specific and generic enterovirus probes would be included on the array, as well as specific probes for measles, mumps, rubella and West Nile viruses. Control probes on the array would consist of unrelated viral and human cellular DNA sequences.

### Table 4

Examples of multiplex PCR tests

| Viruses | References |
|---------|------------|
| Respiratory | Enterovirus, influenza viruses, RSV, parainfluenzavirus, adenoviruses, *Mycoplasma pneumoniae, Chlamydia pneumoniae* (Coiras et al., 2003; Daum et al., 2002; Echevarria et al., 2000; Grondahl et al., 1999; Li et al., 2001; Na et al., 2002; Stockton et al., 1998; Xu et al., 2000) |
| Neurological | EBV, VZV, HHV-6, HSV-1, CMV, HHV-7, adenoviruses, enteroviruses, BK, JC, SV40 (Calvario et al., 2002; Casas et al., 1999; Fedele et al., 1999; Markionatos et al., 2001; Quereda et al., 2000; Rahaus et al., 2003; Read et al., 2001; Robert et al., 2002; Valassina et al., 2002) |
| Blood borne | HIV-1, HCV, HBV, HTLV-I and II |
| Exanthemas | Measles, B19, rubella (Mosquera Mdel et al., 2002) |
| Gastroenteritis | Adenoviruses, rotaviruses, Norwalk, enteroviruses, HAV |
| STDs | Papillomaviruses (Gravitt et al., 2000; Zheng et al., 1995) |

*See, Elnifro et al., 2000.*
as well as with RNA extracted from nasal lavage specimens from patients with common colds. The RNA was amplified to DNA and labelled using a random PCR procedure (Bohlander et al., 1992), a step that is essential if arrays are to be used successfully for the detection of pathogens of unknown or unrecognised sequence.

A more comprehensive version of this microarray was used in the identification of the SARS virus as a coronavirus (Rota et al., 2003). This microarray contained about 11,000 70-mer oligonucleotides, representing all complete viral genome sequences. The SARS material RNA was amplified and labelled by the random PCR approach, and then hybridised against the microarray. The DNA that hybridised to the array was recovered with a micromanipulator and further PCR-amplified, cloned and sequenced.

2.3. Detection of agents of bioterror

A microarray for the detection of five viruses, 11 prokaryotes and two eukaryotes considered to be pathogens that bioterrorists might employ has been designed by researchers at the Lawrence Livermore National Laboratory, USA (Wilson et al., 2002). Their strategy employed multiplex PCR amplification with primers targeting multiple regions of the genome of each pathogen, and the use of overlapping 20-mer oligonucleotide probes complementary to the entire diagnostic region. The microarray they made in this way had a total of 53,660 probes.

2.4. Other diagnostic arrays

A low density spot array has recently been described for the specific detection of enterovirus 71 (Shih et al., 2003). This array is designed to capture PCR amplicons produced from the 5′-NCR and the VP2 gene of the enterovirus 71 genome.

The development of diagnostic microarrays is not confined to human virology: their use is being investigated for the detection of plant viruses (Boonham et al., 2003; Bystricka et al., 2003). Arrays have also been described for two viruses of economic importance for shrimp farmers, white spot syndrome virus and infectious hypodermal and haematopoietic necrosis virus (Quéré et al., 2002). These latter workers used a membrane based miniarray system with bound PCR amplicons for the capture of complementary strands which were labelled with digoxigenin. Hybridisation of the digoxigenin labelled target DNA to the bound PCR amplicon probes was demonstrated colorimetrically.

Arrays that are used for diagnostic purposes may be considered to be the least discriminatory in the sense that they need to be designed so that they are able to pick up all members of a virus group, and thereby identify novel pathogens as, for instance, was shown by the identification of the SARS agent as a coronavirus (Rota et al., 2003). It is complicated, however, to develop and use this type of array and, at present, there are more examples of arrays that are used for strain typing viral genomes. For example, for typing influenza virus A and B strains, human group A rotaviruses, human papillomaviruses (HPV), hepatitis B and C viruses (HBV and HCV), and human immunodeficiency virus type 1 (HIV-1). Typing arrays exist in the format of glass slides, line probes or blots, and high density chips.

3. Molecular typing arrays

3.1. Influenza virus

An influenza virus microarray based on four haemagglutinin (HA), three neuraminidase (NA) and two matrix protein (MP) gene targets from five different influenza virus strains has been described (La et al., 2001). Twenty-four PCR products (each of about 500 bp) were made from these gene targets and were spotted in duplicate onto glass slides, together with control E. coli amplicons and buffer spots to give an 8 × 8 array. Thus each gene was represented by two or three amplicons. Three multiplex PCRs based on combinations of two to four primers for the four HA, three NA and two MP gene targets were used to generate amplicons labelled with a fluorescent dye (Cy3-dCTP). When tested with fluorescently labelled DNA from different influenza virus strains, the expected correct homologous hybridisation patterns were observed on the array.

3.2. Rotaviruses

A rotavirus microarray based on 47 genotype-specific oligonucleotides designed in variable regions within the conserved VP7 segment, which is used to classify the G genotype of rotaviruses, has been described (Chizhakov et al., 2002). Each rotavirus was identified by an average of nine different specific probes (for the clinically relevant G1 to G4 and G9 genotypes), which were spotted on glass (via an aminolink) in an ordered way. PCR primers in regions of VP7 common to all group A rotaviruses were used to make amplicons fluorescently labelled with Cy5-dCTP. One of the primers was biotinylated, which allowed separation of the two amplicon DNA strands by streptavidin binding. In this way the test sequence could be applied to the array as fluorescently labelled single stranded DNA. When tested with 20 rotavirus isolates, the expected correlation with genotype results determined by sequencing was seen. As with the influenza virus array, details of the routine use of the rotavirus array in a diagnostic laboratory environment have yet to be published.

3.3. Line probe arrays and human papillomaviruses

The immediate precursor of a DNA array suitable for use for typing viral genomes was the reverse hybridisation line probe or blot, developed for HLA genotyping by scientists at Cetus Corporation, where PCR was invented (Bugawan et al., 1994; Saiki et al., 1989). Subsequently,
the first viral application was for the typing of HCV, de-
veloped at Innogenetics (Stuyver et al., 1993, 1996). Be-
sides HCV, line probe/blot assays have been described for
the detection of mutations in the HIV-1 reverse transcriptase
gene (Stuyver et al., 1997), for genotyping and detecting
lamivudine-resistance HBV (Aberle et al., 2001; Swenson
et al., 2001), and for typing HPV (Gravitt et al., 1998; Kleter
et al., 1999, Levi et al., 2002; van Doorn et al., 2002).

Although the details may vary, in general line probe blasts
or strips are made by immobilizing capture (i.e. probe)
oligonucleotides onto nitrocellulose or nylon membranes.
The oligonucleotides are identified from multiple sequence
alignments of the target viral genes. After synthesis of each
oligonucleotide probe, it is tagged with DT residues enzym-
atically or conjugated with BSA, and bound to the mem-
brane. The DT tail or BSA acts as a spacer between the solid
phase and the bases of the oligonucleotide probe that are
complementary to the target viral sequence. This target is
prepared by a PCR in which a specific product is amplified
with a biotin residue incorporated at the end of it through
the use of a biotinylated primer. After denaturation into sin-
gle stranded DNA by treatment with alkali, hybridisation
between the probe and the biotinylated DNA strand is re-
vealed by a streptavidin-alkaline phosphatase colorimetric
detection step.

There are two descriptions of line probe/blot assays for
HPV, which provide a good example of how this technol-
ogy may be used. One of these is known as a line blot assay
(LBA) and makes use of a 450 bp amplicon in the L1 re-
gion of HPV using a primer set called PGMY (Gravitt et al.,
1998, 2000). This primer set is a mixture (pool) of 5 up-
stream and 13 downstream oligonucleotides. The other assay
is known as a line probe assay (LiPA) and it uses primers
called SPF that amplify a small 65 bp amplicon within the
L1 region (Kleter et al., 1998, 1999). The SPF primers are
not a mixture like the PGMY primers, but are consensus
primers based on highly conserved sequences. Both primer
sets can detect similar numbers (25–27) of HPV anogenital
genotypes. A comparison of these two methods has been car-
rried out with a panel of 400 cervical scrapes and, although
minor differences in the detection of some HPV types was
seen, overall they were found to match and be equally suit-
able for diagnostic use (van Doorn et al., 2002). Either of
these two assays could be readily adapted to a microarray
format: the same oligonucleotides would be used but applied
to glass slides rather than to membranes: the PCR products
would be labelled with a fluorescent dye (e.g. Cy3 or Cy5);
hybridisation would be using slide hybridisation chambers;
and an array scanner and software would be used to obtain
the results, instead of colorimetric visualisation.

The two available HPV line probe assays have thus been
shown to be equally useful, though there have been few
published evaluations of line probe assays against other
methods of PCR product characterisation. For this, the gold
standard method against which they must be compared is
sequencing of the amplicon. One evaluation of a line probe
assay compared to sequencing was for detection of drug
resistance mutations in the reverse transcriptase gene of
HIV-1. It concluded that for 40% of the specimens the line
probe assay was insufficiently reliable for use on its own
(Puchhammer-Stockl et al., 1999). One in five samples gave
results that could not be interpreted so that the antiviral drug
genotype had to be determined by sequencing. However,
another study found that although there were some discrep-
ancies in the results obtained by line probe assay, it was
suitable for use in a clinical virology laboratory for HIV-1
antiretroviral drug mutation detection as an alternative to
sequencing (Wilson et al., 2000). This latter evaluation also
found the Affymetrix GeneChip, which detects mutations
in the protease and reverse transcriptase genes of HIV-1, to
be a satisfactory method.

3.4. DNA chips and HIV-1

The Affymetrix HIV-1 GeneChip was the first commer-
cial microarray offered for use in clinical virology. It was ini-
tially developed for genotyping the protease gene, and later
also for the reverse transcriptase gene (Koszal et al., 1996).
Evaluation of its performance compared to DNA sequencing
has been done by several laboratories and, in general, it has
been found that genotyping by the chip is highly concordant
with sequencing (Gunthard et al., 1998; Hanna et al., 2000;
Wilson et al., 2000; Young et al., 1998). The Affymetrix
HIV-1 protease GeneChip consisted of a high density array
of over 12,000 oligonucleotides made by light-directed com-
binatorial chemical synthesis on a glass surface (Lipshutz
et al., 1999). This array of 15-base overlapping oligonu-
cleotides was designed so as to be able to interrogate each
position of the HIV-1 subtype B protease gene through the
means of a set of four oligonucleotides differing only at one
base position for each nucleotide of the protease gene. The
target was prepared from HIV-1 RNA by reverse transcrip-
tase PCR with primers bearing T3 and T7 RNA polymerase
promoters so that the amplicon produced by PCR in this
way had these promoter sequences at each end. It could then
be transcribed into RNA with T3 or T7 RNA polymerase
in the presence of fluorescein-labelled rUTP. After fragmenta-
tion of this RNA, it was hybridised against the DNA chip
so that the pattern of hybridisation could be read out as the
sequence of the protease gene. This is a powerful method
which illustrates two of the challenges that arise in using
oligonucleotide microarrays to fully characterise viral genes.
Firstly, the design and synthesis of the full overlapping set
of redundant oligonucleotides is technically difficult and be-
yond the resources of many clinical virology laboratories.
Secondly, the sequences of the oligonucleotides used on the
chip must be based on a single known sequence, in this case
that of subtype B of HIV-1. This means that separate chips
would need to be made for each of the other subtypes of
HIV-1, for example for subtype C which comprises about
half of all worldwide infections today. The more rapidly
and extensively the target genome mutates, the more diffi-
cult it will be to design sequence specific oligonucleotide chips that are able to catch all of the genome variants that might be present in individuals as HIV-1 quasispecies or already prevalent in the community. Obviously, this is more of a problem for a fast changing genomic target like HIV-1 than for a slowly evolving one like HPV.

3.5. Vaccine quality control

Microarrays, perhaps in the form of generic oligonucleotide microchips, have the potential to be used to examine batches of vaccine to ensure that they are free of viral genomic sequences (Amexis et al., 2002; Cherkasova et al., 2003; Petricoin et al., 2002; Proudnikov et al., 2000). One of the initial hopes for oligonucleotide microarrays was that they could be used for DNA sequencing by hybridisation (Southern, 1996). Although this has not been realised on large scale, small PCR amplicons (about 100bp) can be characterised with arrays of all possible 8-mer oligonucleotides (a total of 4096 probes). The use of an array of this type has been described for detection and quantification of neurovirulent mutants in oral poliovirus vaccine (Proudnikov et al., 2000). A short sequence region of less than 100bp was amplified from the 5′ end of the poliovirus genome from vaccine preparations, labelled with Texas Red fluorescent dye and applied to a microarray containing 4096 chemically immobilised oligonucleotides. The pattern of spots that was observed following hybridisation and washing was characteristic for the sequence applied. In practice this meant that if there were as few as 10% revertants in the vaccine preparation they could be recognised. Further studies using microarrays bearing 9 or 10 base long oligonucleotides are needed to determine whether this approach is applicable for longer PCR products. The alternative to using microarrays of generic oligonucleotides for characterising virus stocks is to use arrays of strain specific oligonucleotides, and this approach is also being investigated for the analysis of vaccine-derived polioviruses, as well as for analysis of the Jeryl Lynn strain of mumps virus (Amexis et al., 2002; Cherkasova et al., 2003).

4. RNA expression arrays

Microarrays have been used in two other ways in virology, firstly, to study the RNA expression profile of the virus following infection, including drug effects in vitro (Chambers et al., 1999; Jenner et al., 2001) and secondly, to study the RNA expression profile of the host following infection, including drug effects in vitro (Corbeil et al., 2001; Domachowska et al., 2002; Geiss et al., 2000; Iizuka et al., 2002; Zhu et al., 1998). The applications of these two complementary approaches in microbiology have been reviewed by (Cummings and Relman, 2000; Kato-Maeda et al., 2001; Manger and Relman, 2000). The RNA expression of HCMV in cell culture in the presence or absence of cycloheximide or ganciclovir was analysed with an array of oligonucleotides representing HCMV ORFs made on glass slides (Chambers et al., 1999). Similarly, an array of 288 amplicons representing 88 HIV-8/KSHV ORFs was made on nylon membranes, and used to compare the expression of viral miRNAs during latency and TPA-induced lytic replication (Jenner et al., 2001). Other groups are studying poxvirus expression in this way (Laasrl et al., 2002) (Evans, personal communication).

Gene expression can be investigated with the help of microarrays of probes (oligonucleotides or cDNA) representing the miRNAs—i.e. the expressed genes—of the host cell (Schena et al., 1995). This type of host cell gene array is most conveniently obtained commercially, e.g. from Affymetrix. To study HCMV infection, the role of cellular genes, before the onset of viral DNA replication, was analysed by hybridising labelled mRNA from infected cells to Affymetrix gene chips containing over 6600 human gene probes (Zhu et al., 1998). The level of 258 mRNAs was found to change by a factor of 4 or more. Similarly, the expression of mouse cellular miRNAs after infection with the paramyxovirus, pneumonitis virus of mice, was analysed using an Affymetrix mouse gene microarray chip (Dornachowske et al., 2002). Gene expression was compared in hepatocellular carcinomas induced by either HBV or HCV (Iizuka et al., 2002). Messenger RNA from HIV-1 infected T-cells was hybridised to 1500 cellular cDNAs to identify genes involved in T-cell signalling, subcellular trafficking and transcriptional regulation (Geiss et al., 2000). In the same manner, the effects of HIV-1 infection on T-cell transcription was examined using Affymetrix gene chips, and up and down regulation, that is differential expression of cellular genes, was observed (Corbeil et al., 2001).

It has been suggested that the hybridisation of labelled target DNA derived from patients infected with pathogens could be used diagnostically (Cummings and Relman, 2000). These authors wrote: “the unique constellation of virulence factors expressed by a specific pathogen will elicit a unique transcription response in the host”. If this unique transcription pattern could be captured as a readout from an array it could be used as a diagnostic signature. Simplicistically, this might be done by hybridising labelled cDNA/mRNA from PBMCs from a patient to an array of sequences representing human genes, giving an array profile diagnostic of a specific pathogen. The particular advantages of this approach, if successful, would be that only one sample would be needed for several pathogens, and that it would allow the detection of early and late infections and of uncultivable pathogens. In addition, it might be possible to infer time since exposure to the pathogen, if the transcription pattern were temporally characteristic (Cummings and Relman, 2000).

5. Practical problems

Aside from their costs and the difficulties associated with designing and making a suitable array, the most obvious
problems with their use are ones of quality control, because of the difficulties of standardisation and reproducibility associated with the large number of probes on an array, and the large number of slides that need to be made. Although it is possible to rehybridize some arrays, this is unlikely to be acceptable for diagnostic use because of the increase in noise and loss of signal that comes with reuse. It is also difficult to relate the signal from an array back to the amount of virus genome that was present in the original specimen, making quantification a problem.

Arrays of oligonucleotides or PCR amplicons spotted on glass slides can be made in-house, but there may be contamination if PCR amplicons are used, as large amounts of these products will be made. There may also be problems with the hybridisation reaction, for example, sequences not hybridising together for any obvious reason, and the probes or the target self-annealing because of internal complementary regions in their sequences. Commercially bought arrays may be inflexible because they are based on just one target sequence, e.g. the Affymetrix GeneChip for HIV-1 subtype B protease mutations (Kozal et al., 1996).

6. The future: protein arrays and biosensors

From the foregoing discussion it can be seen that microarrays have gained a foothold in virology and have the prospect of serving as a useful diagnostic tool. Other similar technologies that are less ready to be able to fulfil this diagnostic role include protein arrays and biosensors. Protein arrays are being prepared with antigens or antibodies bound to a solid phase (analogous to oligonucleotide arrays) and used to capture specific antibodies bound to a solid phase (e.g. an oligonucleotide) and, instead of there being a specific reaction because of the presence of the target analyte binding to a ligand immobilised on a solid phase. A signal is generated when the enzyme catalyses the large number of slides that need to be made. Although it is possible to rehybridize some arrays, this is unlikely to be acceptable for diagnostic use because of the increase in noise and loss of signal that comes with reuse. It is also difficult to relate the signal from an array back to the amount of virus genome that was present in the original specimen, making quantification a problem.

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7. Conclusions

Microarrays that are useful in the clinical virology laboratory will be developed both in the public and commercial sectors. Those in public laboratories will probably fit in with the research interests of the laboratory that develops them, and may not be easily transferable to other laboratories. More likely, it is commercial suppliers who will deliver quality controlled viral diagnostic arrays, just as line probe assays are made available by them at present. Whether their performance will match their probable high cost remains to be seen. They will be fast and easy to automate, but if they are to supplant the present range of genomic and serological diagnostic tests they must also be able to match them in terms of sensitivity and specificity. That may prove a stiffer challenge.

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