

and reactogenicity differences between the available and candidate pertussis vaccines. I leave it to others to decide whether the expense of another trial is justifiable. Certainly one penny-wise but pound foolish alternative is to never know the answers.

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- 1 Poltera AA. Acellular pertussis vaccines. *N Engl J Med* 1996; **334**: 1547–48.
- 2 Ad Hoc Group for the Study of Pertussis Vaccines. Placebo-controlled trial of two acellular pertussis vaccines in Sweden—protective efficacy and adverse effects. *Lancet* 1988; *i*: 955–60.
- 3 Plotkin SA, Cadoz M. The acellular pertussis vaccine trials: an interpretation. *Pediatr Infect Dis J* 1997; **16**: 508–17.
- 4 Schneerson R, Robbins JB, Taranger J, Lagergård T, Trollfors B. A toxoid vaccine for pertussis as well as diphtheria? Lessons to be relearned. *Lancet* 1996; **348**: 1289–92.
- 5 Gangarosa EJ, Galazka AM, Wolfe CR, et al. Impact of anti-vaccine movements on pertussis control: the untold story. *Lancet* 1998; **351**: 356–61.

Early computed-tomography abnormalities in acute stroke

Sir—Martin Grond and colleagues ('Nov 29, p 1595)¹ show that in patients with acute ischaemic stroke, early computed-tomographic (CT) abnormalities are frequently associated with the presence of a large volume of critically ischaemic brain, potentially salvageable by thrombolytic reperfusion.² However, they also imply that tissue hypoattenuation on a CT scan done within 3 h of onset indicates established local infarction and that extensive early CT changes could be a contraindication to intravenous thrombolytic treatment.³ In the PROACT trial,⁴ all five patients with these radiological findings, who were randomised to intra-arterial recombinant pro-urokinase within 6 h of stroke onset, had haemorrhagic transformation. We present a case in which there was dramatic improvement after intra-arterial thrombolysis.

A 44-year-old woman developed a sudden right hemiparesis with dysphasia, and was rapidly transferred from her local hospital to the regional neurosciences centre for imaging. By this time, she had become completely aphasic, had a fixed gaze deviation to the left, her conscious level was deteriorating, and she was beginning to extend on the right side. CT done within 2 h of onset showed hypodensity with swelling of the left hemisphere. Angiography revealed an occlusion of

the left internal carotid artery with thrombus extending into the left middle cerebral artery. Intra-arterial alteplase 50 mg was administered immediately (3 h and 45 min after stroke onset) by a tracker microcatheter and complete thrombolysis was achieved within 1 h. The patient regained consciousness and has since made a complete recovery. A repeat CT scan shows only minor hypodensity.

The patient was excluded from the ECASS II trial of intravenous alteplase versus placebo because of the extensive early CT signs and because of a rapidly deteriorating conscious level. The latter would probably have meant exclusion from the PROACT phase 2 study of intra-arterial pro-urokinase versus placebo, in which extensive radiological signs have also been associated with a high risk of haemorrhagic transformation. The response to alteplase shows that such signs do not always indicate irreversible tissue ischaemia and that topical thrombolytic treatment, at least in the small doses required by the intra-arterial route, may still be safe and effective. The clinico-radiological predictors of reversible brain ischaemia require further investigation.

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- 1 Grond M, von Kummer R, Sobesky J, Schmuelling S, Heiss W-D. Early computed-tomography abnormalities in acute stroke. *Lancet* 1997; **350**: 1595–96.
- 2 Heiss W-D, Grond M, von Stockhausen H-M, Rudolf J. Ischaemic brain tissue salvaged from infarction with alteplase. *Lancet* 1997; **249**: 1599–600.
- 3 von Kummer R, Allen KL, Holle R, et al. Acute stroke: usefulness of early CT findings before thrombolytic therapy. *Radiology* 1997; **205**: 327–33.
- 4 del Zoppo GJ, Higashida RT, Furlan AJ, and the PROACT Investigators. PROACT: a phase II trial of recombinant pro-urokinase by direct arterial delivery in acute middle cerebral artery stroke. *Stroke* 1998; **29**: 4–11.

Human herpesvirus 8 variants

Sir—Published research on a new human herpesvirus, Kaposi's sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8) grows increasingly confusing. Many studies of this virus are based on PCR, which can give false-positive contamination readings, even under the most stringent control conditions, as has happened in

our own laboratory. PCR contamination, especially for nested PCR, is insidious because it can be intermittent or persistent. Sequencing of PCR products for polymorphisms, cannot reliably detect contamination because of the absence of *Taq* polymerase proofreading capacity.

It was delightful to see this technique used to show that greater than 90% of sarcoidosis lesions (Luca Di Alberti and colleagues, Dec 6, p 1655)¹ are infected with HHV8/KSHV. This confirms a theory I have that there is not one virus detectable by PCR, but actually two. KSHV is rare in low-risk populations and is associated with Kaposi's sarcoma, body-cavity-based lymphomas, and a subset of Castleman's disease lesions by PCR, Southern-blot hybridisation, and serological testing. The epidemiology of this virus makes sense, and methods to find it, although far from perfect, are reliable and reproducible.

HHV8, however, is totally different, and *The Lancet* has been in the forefront of publishing on the unusual epidemiology and biology of HHV8. It is a ubiquitous virus but is also responsible for a remarkable range of human diseases. For example, PCR detects HHV8 DNA in various post-transplant skin tumours of different origins,² whereas KSHV is not present in these tumours.³ Published studies show that nested PCR can find HHV8 in over 90% of US HIV-positive semen donors,⁴ whereas none of the 115 semen samples from low-risk healthy UK donors were positive for KSHV.⁵ Beyond its contribution to virology, the finding of HHV8 in sarcoid lesions¹ also could be of historical importance for providing the first carefully analysed phylogenetic dendrogram of *Taq* incorporation errors. In view of the frequency at which PCR water control samples are positive for HHV8, drinking water should be investigated as a source for transmission.

On the basis of published results, HHV8/KSHV could cause skin tumours, sarcoidosis, multiple myeloma, encephalitis, prostate cancer, neurolymphomas, and other degenerative, autoimmune, neoplastic, and hyperplastic disorders. From haemorrhoids to hangovers, the search for HHV8 should proceed unimpeded by cautious trepidation or scientific consistency. In my experience, HHV8 can be detected reliably only by nested PCR, and one should avoid aerosol barrier-pipet tips or PCR machine bleaching to ensure the highest sensitivity. There is a danger here: intellectual fatigue will set in when readers of *The Lancet* find a new

HHV8-related disease in each week's issue. As my colleague from medical school (Charles T Ellis, Homer, Alaska, USA) once prophetically told me: "If you were to take all the water buffalo in the world, and line them up. And then make them walk across a single point, all day long—it would be one helluva boring parade". Alternatively, KSHV and HHV8 could be different names for the same virus. With cautious interpretation and use of appropriately reliable methods, we may yet find additional diseases caused by both viruses.

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- 1 Di Alberti L, Piattelli A, Artese L, et al. Human herpesvirus 8 variants in sarcoid tissues. *Lancet* 1997; **350**: 1655–61.
- 2 Rady PL, Yen A, Rollefson JL, et al. Herpesvirus-like DNA sequences in non-Kaposi's sarcoma skin lesions of transplant patients. *Lancet* 1995; **345**: 1339–40.
- 3 Cathomas G, Tamm M, McGandy CE, et al. Transplantation-associated malignancies: restriction of human herpes virus 8 to Kaposi's sarcoma. *Transplantation* 1997; **64** (1): 175–78.
- 4 Lin J-C, Lin S-C, Mar E-C, et al. Is Kaposi's sarcoma-associated herpesvirus detectable in semen of HIV-infected homosexual men? *Lancet* 1995; **346**: 1601–02.
- 5 Howard MR, Whitby D, Bahadur G, et al. Detection of human herpesvirus 8 DNA in semen from HIV-infected individuals but not healthy semen donors. *AIDS* 1997; **11**: F15–19.

SIR—Luca Di Alberti and co-workers¹ finding of variant HHV8 DNA sequences in a wide range of sarcoid tissue but not in non-sarcoid tissue does not establish a direct aetiological role of HHV8 in sarcoidosis.

HHV8 is associated with Kaposi's sarcoma, HIV-I-associated Castleman's disease, and other disorders.² The characteristic of latency and reactivation of HHV8 may be shared with other members of the herpesvirus family, but unlike these herpesviruses, HHV8 infection is rare in the general population. Serological assays seem to be necessary to distinguish primary infection or reactivation. HHV8 DNA detection on biopsy specimens for the diagnosis of systemic HHV8 disease is controversial. The relation between the detection of HHV8 open reading frame DNA and clinical signs is not clear.

Pulmonary lesions and uveitis are the most common clinical signs of sarcoidosis. The eruption of erythema nodosum can occur concomitant with sarcoidosis. Serological studies suggest an association of antibody to *Chlamydia pneumoniae* and various syndromes including erythema nodosum. We assessed serum antibodies to

C pneumoniae from patients with endogenous uveitis associated with sarcoidosis and other systemic disorders.³ The serum titres of IgA and IgM antibodies to *C pneumoniae* were significantly higher in patients with endogenous uveitis associated with sarcoidosis than in patients with other endogenous uveitis.

Mycobacterium tuberculosis DNA has been detected in sarcoid tissues containing granulomas from patients with sarcoidosis.⁴ We also tried to detect *C pneumoniae* and *M tuberculosis* DNA from bronchoalveolar lavage (BAL) specimens from 15 patients with endogenous uveitis associated with sarcoidosis by PCR.^{4,5} Neither *C pneumoniae* nor *M tuberculosis* DNA was present in BAL specimens.

Raised serum antibodies to *C pneumoniae* and the presence of circulating chlamydia-specific immune complexes have been found in several chronic *C pneumoniae* infections. Chlamydial infections induce inflammatory changes that might induce modulation of secretion of cytokines. To investigate the relation between cell-mediated immune response to *C pneumoniae* and the pathogenesis of sarcoidosis, we measured the peripheral-blood lymphocyte proliferative response to whole chlamydial elementary bodies. The mean stimulation index of the 15 patients with sarcoidosis was higher than that of controls. The 60-kDa heat-shock protein may also have some role in the induction of non-specific hypergammaglobulinaemia, delayed-type hypersensitivity reaction, and autoimmune reaction associated with chlamydial infections. Whether *C pneumoniae* GroEL gene product might have a similar role in pathogenesis is unknown. Immune abnormalities including raised serum cytokines in patients with *C pneumoniae* infection has been shown and could be implicated in the pathogenesis of endogenous uveitis associated with sarcoidosis.

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- 1 Di Alberti L, Piattelli A, Artese L, et al. Human herpesvirus 8 variants in sarcoid tissues. *Lancet* 1997; **350**: 1655–61.
- 2 Rettig MB, Ha HJ, Vescio RA, et al. Kaposi's sarcoma-associated herpesvirus infection of bone marrow dendritic cells from multiple myeloma patients. *Science* 1997; **276**: 1851–54.

- 3 Numazaki K, Chiba S, Aoki K, et al. Detection of serum antibodies to *Chlamydia pneumoniae* in patients with endogenous uveitis and acute conjunctivitis. *Clin Infect Dis* 1997; **25**: 928–29.
- 4 Fidler HM, Rook GA, Johnson NM, et al. *Mycobacterium tuberculosis* DNA in tissue affected by sarcoidosis. *BMJ* 1993; **306**: 546–49.
- 5 Numazaki K, Suzuki K, Chiba S. Replication of *Chlamydia trachomatis* and *C pneumoniae* in human monocytic cell line U-937. *J Med Microbiol* 1995; **42**: 191–95.

Sir—Luca Di Alberti and colleagues¹ report the presence of HHV8 open reading frame 26 DNA in all of eight lung tissues samples and in 26 of 27 (96%) lymph-node samples from patients with sarcoidosis, but they detected these sequences from Kaposi's sarcoma tissue in only ten of 16 (63%) samples.¹

We have analysed 11 tissue samples from seven patients with sarcoidosis with a highly sensitive nested PCR assay with oligonucleotide primers derived from the open reading frame 26.² HHV8 DNA was detected in none of the biopsy samples, although the presence of β -globin DNA was confirmed in all samples. In addition, we analysed serum from 15 patients with sarcoidosis for the presence of antibodies to the recombinant capsid-related HHV8 antigen open reading frame 65.2 in an ELISA and to latent HHV8 antigens in an immunofluorescence assay with the BC-3 cell line.^{3,4} Anti-HHV8 antibodies were found in all of 25 patients with Kaposi's sarcoma but in only three of 15 (20%) patients with sarcoidosis. We therefore cannot confirm a close association between HHV8 and sarcoidosis.

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- 1 Di Alberti L, Piattelli A, Artese L, et al. Human herpesvirus 8 variants in sarcoid tissue. *Lancet* 1997; **350**: 1655–60.
- 2 Cathomas G, McGandy CE, Terracciano LM, Itin PH, De Rosa G, Gudat F. Detection of herpesvirus-like DNA by nested PCR on archival skin biopsy specimens of various forms of Kaposi sarcoma. *J Clin Pathol* 1996; **49**: 631–33.
- 3 Simpson GR, Schulz TF, Whitby D, et al. Prevalence of Kaposi's sarcoma associated herpesvirus infection measured by antibodies to recombinant capsid protein and latent immunofluorescence antigen. *Lancet* 1996; **348**: 1133–38.
- 4 Arvanitakis L, Mesri E, Nador R, et al. Establishment and characterization of a primary effusion (body cavity-based) lymphoma cell line (BC-3) harboring Kaposi's sarcoma-associated herpesvirus (KSHV/HHV-8) in the absence of Epstein-Barr virus. *Blood* 1996; **88**: 2648–54.