A search for Pneumocystis carinii DNA by polymerase chain reaction on bronchoalveolar lavage fluids from patients with Wegener’s granulomatosis

Sir, Recently, involvement of Pneumocystis carinii in the aetiology of Wegener’s granulomatosis (WG) was suggested. Indeed, co-trimoxazole efficacy in the prophylaxis of relapses of this granulomatosis was reported.
[1]. Varela et al. [2] investigated the presence of the parasite in the lungs of four patients with WG using immunoperoxidase assay. Microscopic examination of three open lung biopsies and one post-mortem lung fragment remained negative despite the high sensitivity of the immunodetection. These data were not in favour of a link between the parasite and the aetiology of the granulomatosis; these findings have to be confirmed, however.

Since 1990, the polymerase chain reaction (PCR) has proved the most sensitive technique for detecting P. carinii in pulmonary specimens [3]. Low numbers of P. carinii organisms, which were undetectable by microscopy, have been detected using PCR on bronchoalveolar lavage fluids (BALFs) from immunosuppressed patients without P. carinii pneumonia (PCP). These low levels of parasites were considered to reflect pulmonary colonization [4, 5]. In so far as PCR is not usually used for routine detection of P. carinii, the diagnosis of cases of colonization is frequently missed. To test the hypothesis that WG could be related to pulmonary P. carinii colonization, we investigated the presence of the parasite in four BALFs from three patients with WG using microscopy and PCR.

Examination of these four BALFs was performed in the same manner. Microscopic examination was carried out after Cytospin® centrifugation using methanol-Giemsa stain and an indirect immunofluorescence assay (Diagnostics Pasteur, Marnes-La-Coquette, France). The remaining BALF sediment was stored at −80°C for further analysis by PCR. After a rapid DNA extraction on sediments (Genereleaser®, BioVentures Inc., Murfreesboro, TN, USA), a hemi-nested PCR assay was performed with specific primers pAZ102-H, pAZ102-E (first round) and pAZ102-L2 and pAZ102-E (second round) amplifying the gene encoding the mitochondrial large subunit rRNA [3, 5, 6]. Positive and negative controls were included in each experiment.

The first patient was a 24-yr-old female. She suffered from the first symptoms of WG 5 yr ago and was treated with high doses of corticosteroid, cyclophosphamide and dialysis. She had no past history of PCP and did not receive prophylaxis against PCP. BAL was performed in April 1997 to investigate a relapse of haemoptysis. Pneumocystis carinii was detected by PCR, whereas the microscopic examination was negative. The corticosteroid treatment was carried out in association with long-term therapy with co-trimoxazole within 2 yr. PCP did not occur during this period.

The sensitivity of the hemi-nested PCR procedure has been evaluated previously [5]; 210 BALFs were examined and P. carinii was detected in 36 BALFs by PCR whereas microscopy was negative. These cases reflected pulmonary colonization. Moreover, no false-negative result was observed since the 14 PCP cases were diagnosed by microscopy and confirmed by PCR. Thus, it was highly probable that a negative PCR result was consistent with absence of the parasite in BALFs from the first and second patient included in the present study. Varela et al. [2] failed to provide biological evidence of a relationship between P. carinii and WG aetiology; however, they did not use the most sensitive technique to detect P. carinii in pulmonary specimens. Our attempt to detect P. carinii DNA by PCR on BALFs from two patients with WG was also unsuccessful. However, P. carinii DNA was detected in the lungs of the third patient, who was considered to be colonized. This observation did not confirm those of Varela et al., but the link between P. carinii and WG remains unclear. Nevertheless, at present, PCR for P. carinii detection appears to be an efficient tool for further studies on the aetiology of WG.

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