DNA restriction fragment length polymorphism differentiates recurrence from relapse in treatment failures of *Streptococcus pyogenes* pharyngitis

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Summary. In the evaluation of treatment failure in *Streptococcus pyogenes* pharyngitis it is necessary to distinguish between persistence of the original streptococcus and acquisition of a new strain. We used the analysis of restriction fragment length polymorphism (RFLP) of total DNA and of ribosomal DNA (rDNA) regions (ribotypes) as epidemiological tools to compare 43 pre- and post-treatment *S. pyogenes* strains obtained from 20 patients. In 16 cases pre- and post-treatment strains gave indistinguishable RFLP patterns of total DNA, strongly suggesting relapse with the same strain. However, in four cases different patterns were obtained for the pre- and post-treatment isolates, indicating recurrence due to the acquisition of a new strain. Ribotyping did not improve discrimination among strains. Thus, analysis of DNA RFLP is a promising method for distinguishing recurrence from relapse in failures of pharyngitis treatment.

Introduction

Studies of the efficacy of antibiotic treatment of group A streptococcal pharyngitis have used streptococcal eradication rates as a measure of success. When treatment fails, it is necessary to distinguish between relapse with the persistence of the original strain of streptococcus and recurrence due to the acquisition of a new strain. In this study, the analysis of restriction fragment length polymorphism (RFLP) of total DNA and of ribosomal DNA (rDNA) regions (ribotyping) was used as an epidemiological tool to compare pre- and post-treatment *S. pyogenes* strains obtained from patients with pharyngitis who were thought to be treatment failures.

Materials and methods

Patients and clinical specimens

During the winter and spring of 1990–1991, 240 patients with clinical findings suggestive of acute pharyngitis were seen by private paediatricians in Paris, Brittany and Lorraine (France). After giving informed consent, they were enrolled in a double blind randomised multicentre study comparing treatment with penicillin V, and cefotiam hexetil. Complete results of this study will be published elsewhere. Throat cultures were obtained by rubbing a sterile, rayon-tipped swab over the posterior portion of the pharynx and over both tonsils (or tonsillar fossae). The swab was immediately streaked on a blood agar plate (Trypticase Soy Agar with sheep blood 5%; Diagnostic Pasteur, Marnes-la-Coquette, France). The plate was examined for the presence of β-haemolytic streptococci after overnight anaerobic incubation at 37°C. All patients were asked to return for two follow-up visits, 10 and 30 days after the start of antibiotic therapy. At each follow-up visit, a further throat culture was obtained. Patients presenting with persistent symptoms and with both pre- and post-treatment cultures positive for *S. pyogenes*, were selected for this study.

Bacterial strains

All β-haemolytic streptococcal strains were grouped with the Streptex test (Wellcome Diagnostics, Dartford, Kent). *S. pyogenes* isolates were stored in Todd-Hewitt broth at −70°C.

RFLP analysis

Total *S. pyogenes* DNA was prepared as previously described; 4 μg of DNA was digested with HindIII and PvuII restriction enzymes (Boehringer, Mannheim, Germany) according to the manufacturer’s specifications and analysed by electrophoresis on sub-marinie, ethidium bromide-containing, agarose 0.8% gels in 1 mM Tris-acetate, 1 mM EDTA buffer. DNA-fragment-size marker Raoul I (Appigene, Strasbourg, France) was used. The separated restriction fragments were transferred to a nylon membrane (Gene Screen...
RFLP OF S. PYOGENES

Fig. 1. Identical pre- and post-treatment S. pyogenes HindIII RFLP patterns: A, agarose gel after ethidium bromide staining; B, Southern blot probed with the rDNA probe. Lanes 1 and 2, patient 1; 3 and 4, patient 2; 5 and 6, patient 3; 7, size marker. Identical rDNA RFLP patterns (lanes 3-6) are obtained for strains exhibiting distinct total DNA RFLP patterns (lanes 3 and 5).

Results

A total of 20 patients with persistent pharyngitis and pre- and post-treatment throat cultures positive for S. pyogenes were enrolled in this study. Of the 20 patients, 10 had positive throat cultures at the first follow-up visit (10 days after beginning the antibiotic therapy), seven at the second follow-up visit (at day 30) and three at both the first and the second follow-up visits. A total of 43 strains was obtained from the 20 patients.

RFLP of total DNA

Figs. 1A and 2 show examples of HindIII digestion patterns of DNA obtained for three and two pairs of pre- and post-treatment isolates, respectively. In fig. 1A (patients 1-3), identical total DNA RFLP patterns were observed for the pre- and post-treatment strains. In contrast, fig. 2 shows that the patterns obtained with the pre- and post-treatment strains from patients 4 and 5 were clearly different.

In 16 patients, indistinguishable RFLP patterns of total DNA were obtained for the pre- and post-treatment strains after both HindIII and PvuII digestion. In four cases, the two enzymes generated different RFLP patterns of total DNA for each pre- and post-treatment pair.

RFLP of rDNA regions (ribotyping)

Among the 43 strains studied, ribotyping after digestion with HindIII and PvuII endonucleases distinguished six and five patterns, respectively. Combinations of the HindIII and PvuII patterns improved discrimination among strains by defining eight distinct ribotypes. However, identical ribotypes were observed among several pairs of S. pyogenes strains which
appeared to be distinct by RFLP patterns of total DNA (fig. 1B).

Discussion

Streptococcal pharyngitis remains a common problem in children and teenagers. Its proper management has contributed significantly to the decline of acute rheumatic fever. A While penicillin has been the treatment of choice for four decades, an increasing incidence of clinical relapse or recurrent infection has appeared to be distinct by RFLP patterns of total DNA RFLP analysis after HindIII digestion. Lanes 1 and 2, patient 4; 3 and 4, patient 5.

References