The colonization over time of cystic fibrosis patients by *Aspergillus fumigatus* was investigated using a DNA fingerprinting method. *Aspergillus fumigatus* isolates collected sequentially for more than one year from six patients with cystic fibrosis were typed by Southern blot hybridization with a repetitive DNA sequence. Each cystic fibrosis patient harbored several strains of *Aspergillus fumigatus* that were isolated recurrently over time. Isolates collected from a cystic fibrosis patient with aspergilloma displayed the same genotype, suggesting that the infection was due to a single strain. Continuous isolation of the same genotype in another cystic fibrosis patient, however, was not correlated clinically with an *Aspergillus* infection.

*Aspergillus fumigatus* is the most common fungal species isolated from sputa of patients with cystic fibrosis (1–3). With the exception of the very few individuals developing a true allergic bronchopulmonary aspergillosis (ABPA), the pathogenic significance of the isolation of *Aspergillus fumigatus* among these patients has not been established. It is still unknown whether *Aspergillus fumigatus* isolates obtained from non-ABPA cystic fibrosis patients result from airborne spores trapped in their mucoid secretions without any further development or if they indeed reflect a true mycelial colonization of their respiratory tract by this opportunistic fungal pathogen. To address this question, the DNA fingerprinting system previously used successfully during epidemiological studies of environmental and clinical isolates of *Aspergillus fumigatus* (4–8) was employed to type isolates of *Aspergillus fumigatus* collected sequentially from the same cystic fibrosis patients.

**Materials and Methods.** Six cystic fibrosis patients (4 boys and 2 girls; 5–17 years old) with diagnostes confirmed by sweat chloride values > 60 mEq/l were monitored from March 1993 to December 1995 at the cystic fibrosis clinic of Hospital A. Trousseau. These patients, who attended the hospital regularly (in most cases monthly), were selected on the basis of a positive isolation of *Aspergillus fumigatus* from their sputum at every visit. Patients 1, 2, 3, 4 and 6 had no clinical or radiological features indicative of ABPA. In addition, total IgE levels were in the mean range of the expected value for their age, and *Aspergillus*-Radio-Allergo-Sorbent-Test (RAST) was negative. Patient 5 developed an *Aspergillus* infection, evidenced by chest radiograph and positive serological data. Total IgE level was high (177 kIU/l on 21 February 1994 and 188 kIU/l on 17 May 1994), and a class 2 RAST as well as two to three anti-*Aspergillus fumigatus* precipitin lines were noted on the same dates. A mucoid plug colonized by *Aspergillus fumigatus* was surgically removed by bronchoscopy in February 1994.

*Aspergillus fumigatus* was isolated in petri dishes using Albicans ID medium (bioMérieux, France) inoculated by successive dilutions of the sputum sample dissolved in an equal quantity of Digest-EUR (Eurobio, France). Individual colonies (1 to 4 per petri dish) were transferred to a 2% malt agar tube and kept at room temperature until processed for typing. No random selection was made at this stage, since all colonies on a plate were processed for typing. DNA extraction, EcoRI digestion, electrophoresis and Southern blot hybridization with the repeated λ 3.9 probe were performed as described previously (4–6). This probe contains the repeated DNA sequence *Afut1*, which is a retrotransposon-like element. Previous studies (5, 6) have shown that this element is inactive and does not transpose in the fungal genome. As a result, the same hybridization patterns were found for strains regularly transferred in the laboratory for 20 years or passed several times through mice infected experimentally.

**Results and Discussion.** Twenty-nine to 174 isolates were obtained per patient. The numbers of typed isolates and genotypes identified per patient are shown in Table 1.

Every cystic fibrosis patient harbored several *Aspergillus fumigatus* strains, as indicated by the
Figure 1: Time distribution of *Aspergillus fumigatus* strains isolated from cystic fibrosis patients. Unique genotypes are designated by a one- or two-letter symbol. Only one identity of genotypes from different patients was observed: strain G from patient 2 corresponds to strain G from patient 1 (black bar). The asterisk (*) in the schematic representation of patient 5 indicates the date of diagnosis of the *Aspergillus fumigatus* infection and surgical removal of the mucoid plug.

The number of genotypes per patient varied from four to 15. The same genotypes were found repeatedly (up to 48 times) in the same patient (Figure 1). In most cases (105 of the 132 samples), all

presence of different hybridization patterns among isolates from the same patient (Figure 1).