GC SUBTYPING IN SERUM AND SEMEN AFTER NEURAMINIDASE TREATMENT

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INTRODUCTION

Genetic polymorphism in the group-specific component (GC) of human serum was first described by Hirschfeld (1959) with two alleles, GC*1 and GC*2. Using isoelectric focusing, Constans and Viau (1977) demonstrated that GC 1 is resolved into GC 1F and GC 1S with a double-band pattern. Thus, six phenotypes are coded by three alleles, GC*1F, GC*1S and GC*2. Cleve and Patutshnick (1979) reported that the anodal bands of GC 1F and of GC 1S disappear after removal of sialic acid by neuraminidase treatment.

We have recently observed that in human semen the anodal bands of GC 1F and of GC 1S do not disappear by neuraminidase treatment. This paper describes GC subtyping in serum, semen and seminal stains using isoelectric focusing after treatment of the samples with neuraminidase.

MATERIALS AND METHODS

Blood and semen samples were obtained from 30 male volunteers with known phenotypes. Seminal stains were made on filter paper (Whatman No. 3) and stored at 4°C, room temperature and 37°C. Native serum samples were diluted 1:150 with distilled water. Serum samples were desialylated in 1/10 volume of 1 M potassium phosphate buffer (pH 7.0), containing 50 U/ml neuraminidase from *Clostridum perfringens* (Type V, Sigma), overnight at 4°C, followed by dilution of the mixture, 1:150, with distilled water. Native semen samples were diluted 1:10 with 6 M urea, containing 0.5% BSA. Semen samples were diluted 1:10 with 6 M urea, containing 0.5% BSA, and incubated in 1/10 volume of 50 U/ml neuraminidase. Seminal stains (5 x 5 mm) were extracted with 36 μl 6 M urea, containing 0.5% BSA, and incubated in 4 μl 50 U/ml neuraminidase overnight at 4°C.

Isoelectric focusing was performed using a Bio-Phoresis Electrophoresis Cell (Bio-Rad) and a Model 3000 Xi Power Supply (Bio-Rad). Polyacrylamide gels (230 x 110 x 0.5 mm) were prepared from 20 ml stock solution (5.25% acrylamide/0.25% N,N'-methylenebisacrylamid), containing 1 ml Pharmalyte pH 4.5-5.4 (Pharmasia), 0.3 ml 0.1% riboflavin and 2.5 g sucrose. The electrode paper strips were soaked with 1 M phosphoric acid for the anode and with 1 M sodium hydroxide for the cathode. Ten μl of the samples were applied to the gel surface 2 cm from the cathode using 5 x 6 mm filter

paper (Whatman No. 3). Electrofocusing was conducted at a constant voltage of 2000 V for 240 min. The filter papers were removed after 60 min of focusing. During focusing the gel was cooled by circulating water at 4°C.

A sheet of nitrocellulose membrane (Bio-Rad) was placed on the gel surface and left for 60 min, applying a 1 kg weight. Following blotting the membrane was rinsed in 20 mM Tris/500 mM sodium chloride buffer, pH 7.5 (TBS), for 10 min and immersed in TBS containing 3% gelatin for 30 min. After washing in TBS for 15 min, the membrane was incubated for 60 min in goat anti-human GC serum (Atlantic Antibodies), diluted 500-fold with TBS containing 0.05% Tween 20 (TTBS). Next, the membrane was washed 3 times in TTBS for 30 min and incubated for 60 min in rabbit anti-goat IgG serum conjugated with alkaline phosphatase (Sigma) that was diluted 750-fold with TTBS. After 3 washes in TTBS for 30 min, the membrane was incubated in 50 ml staining solution (1.8 g sodium hydroxide and 3.7 g boric acid/1000 ml) containing 25 mg β-naphthyl phosphate, 25 mg Fast Blue BB salt and 60 mg magnesium sulfate for a few minutes.

RESULTS AND DISCUSSION

Figures 1 and 2 show the isoelectric focusing patterns of GC in samples of native serum, neuraminidase-treated serum, native semen and neuraminidase-treated semen from the same individuals. By neuraminidase treatment of the serum samples, the anodic bands of GC 1F and of GC 1S disappeared whereas the cathodic bands of GC 1F and of GC 1S and the GC 2 band remained unchanged. In semen samples, the GC 2 type exhibited 2 bands: the main GC 2 band and another fast band which focused at the position of

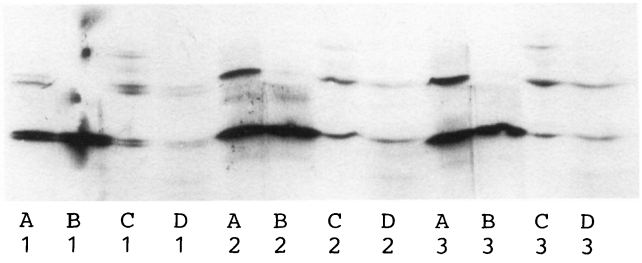

Fig. 1. A: native serum, B: neuraminidase-treated serum, C: native semen, D: neuraminidase-treated semen. 1: 1F1S, 2: 1F, 3: 1S. The anode is at the top.

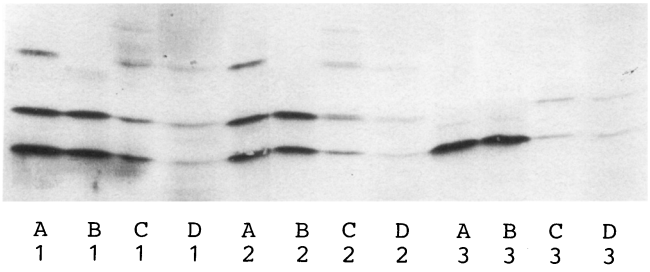

Fig. 2. A: native serum, B: neuraminidase-treated serum, C: native semen, D: neuraminidase-treated semen. 1: 2-1F, 2: 2-1S, 3: 2. The anode is at the top.

the cathodic band of GC 1F. The GC 2-1F type thus showed a three-band pattern, and the GC 2-1S type a four-band pattern. Moreover, some minor bands appeared towards the anode. When the semen samples were incubated with neuraminidase, these minor bands disappeared, but the double bands of GC 1F and of GC 1S as well as the above two GC 2 bands were not altered. It seems therefore that the seminal GC is devoid of sialic acid.

All the seminal stains examined were subtyped for GC at 4°C for periods of up to 10 weeks, at room temperature for periods of up to 8 weeks, and at 37°C for periods of up to 5 weeks. Figure 3 shows the isoelectric focusing pattern of GC in seminal stains stored for 1 week at room temperature. Our results for the determination limits are superior to those of Pötsch-Schneider and Klein (1988) who, using isoelectric focusing in an ultrathin immobilized pH gradient gel, demonstrated the GC patterns in seminal stains stored for 2 weeks at room temperature.

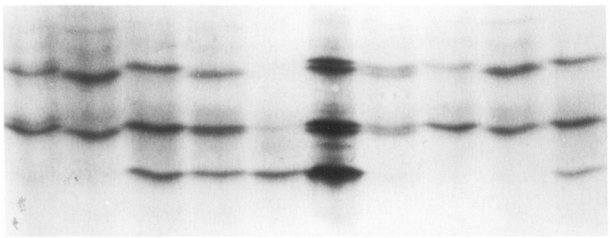

Fig. 3. 1: 1F, 2: 1S, 3: 2-1F, 4: 2-1S, 5: 2, 6: standard (mix of GC 1F1S and 2), 7: 1F1S, 8: 1F, 9: 1S, 10: 2-1F. The anode is at the top.

Isoelectric focusing and immunoblotting combined with neuraminidase digestion permit reliable GC subtyping from seminal stains stored for at least 5 weeks. The technique is simple and economical, and requires no specific equipment as compared with the immobilized isoelectric focusing method. The GC system provides a useful genetic marker for the forensic individualization of seminal stains.

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