progressive systemic sclerosis has prompted us to use this antibody as a probe for identifying centromeric chromatin of chromosomes. We describe here a simple and rapid technique for the identification of centromeric regions of chromosomes by immunofluorescence microscopy. The clinical use of this technique is demonstrated by a case study.

**Method**

The anticentromere autoantibody used in this study was obtained from the frozen serum bank at E W Sparrow Hospital. The titre of the antibody was 1:5120 as determined by routine ANA screening on a Hep-2 cell substrate (Kallestad Laboratories, Austin, Texas). Chromosome spreads were prepared from peripheral blood cultures by the method of Yunis. Briefly, methotrexate synchronised cultures were harvested, fixed, and dropped onto clean glass slides. The chromosome spreads were flooded with a 1:200 dilution of anticentromere antibody in phosphate buffered saline (PBS, pH 7.2) and incubated in a humidified chamber for 30 minutes at room temperature. The slides were then rinsed in two changes of PBS for five minutes each, then flooded with a 1:80 dilution of fluorescein conjugate of antihuman IgG, Fab'2 (Kallestad), and incubated for 30 minutes at room temperature. The slides were washed twice in five minute changes of PBS and examined by fluorescence microscopy on a Zeiss epifluorescence microscope equipped with a 50 watt mercury-arc lamp.

**Case report**

A 38 year old couple was referred to this laboratory with the diagnosis of a rheumatological disorder for which they were being treated at another hospital.

**Detection of centromeric regions of chromosomes by immunofluorescence: procedure and application**

Conventional techniques for the identification of centromeric regions can be time consuming and often produce inconsistent and variable results. The recent description of autoantibodies to centromere antigen in patients with the CREST variant of
for cytogenetic evaluation because of a history of spontaneous abortion. Routine G banding showed the man to have a normal 46,XY karyotype. The woman showed a small supernumerary chromosome in 23 of 30 cells analysed. Silver staining as described by Howell and Black3 showed an active NOR region on one end of this microchromosome. Anti-centromere immunostaining showed a positive centromere region proximal to the NOR+ region (figure). The presence of this centromeric material was confirmed by conventional C banding techniques.

Discussion

The identification of centromeric material by immunofluorescence microscopy using anticentromere autoantibody (anti-C technique) is rapid, simple, and inexpensive. In contrast to conventional C banding techniques, which require long incubation times (frequently up to 24 hours), this procedure can be performed in less than two hours. As shown by this case report, the anti-C technique can provide clinically useful information. We have since used this technique in several other cases and have found it to be reliable and sensitive. Our experience has shown that the anti-C technique gives superior resolution when compared to Giemsa or Wright stained C banding techniques. We are at present evaluating the anti-C technique for its use in identifying the activity of centromeric material.

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References


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