Localization of nRNP Antigen in Mammalian Cells Stained with Peroxidase-Labelled IgG Fraction of Anti-nRNP Antibody Obtained from a Patient with Mixed Connective Tissue Disease (MCTD)

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SAITO, K., SAGAWA, K., NISHIMAKI, T., MORITO, T., YOSHIDA, H. and KASUKAWA, R. *Localization of nRNP Antigen in Mammalian Cells Stained with Peroxidase-Labelled IgG Fraction of Anti-nRNP Antibody Obtained from a Patient with Mixed Connective Tissue Disease (MCTD)*. Tohoku J. exp. Med., 1987, **153** (1), 21-26 — The peroxidase-labelled IgG fraction of anti-nRNP antibody obtained from a MCTD patient stained the nuclei of almost any kind of cells of man and rat. In addition, the cytoplasm of anterior horn cells of the rat spinal cord was exclusively reacted with this antibody. Both cytoplasmic and nuclear staining of the anterior horn cells were reduced after treatment of tissue sections with RNase. ——— nuclear ribonucleo protein antigen; U₁-RNA; anterior horn cells; mixed connective tissue disease; immunohistochemistry

Antigenic structure and biological function of nuclear ribonucleoprotein (nRNP) have been extensively studied with regard to its splicing function of RNA transcript to messenger RNA (Lerner et al. 1980; Rogers and Wall 1980) and to its diagnostic significance for mixed connective tissue diseases (MCTD) (Sharp et al. 1972; Kasukawa et al. 1987).

Localization of nRNP antigen is also interesting to clarify how the anti-nRNP antibody is produced in the patients and how the cells with this antigen could be damaged by this antibody.

The peroxidase-labelled IgG fraction obtained from a MCTD patient with anti-nRNP antibody was used to study the intracellular localization of nRNP antigen.

MATERIALS AND METHODS

Anti-nRNP antibody. Antibody to nuclear ribonucleoprotein (nRNP) was obtained

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from a patient MK with MCTD whose serum contained antibodies to nRNP but not to Sm, SS-B, Og, Jo-1 or Ku.

Fractionation of anti-nRNP serum. The serum from the MCTD patient was precipitated by 30% saturated ammonium sulfate and the precipitate was gel-filtrated through a Sephadex G-200 column. The second peak of this gel-filtration was used as IgG fraction of anti-nRNP serum.

Labelling of IgG fraction of anti-nRNP serum with horseradish peroxidase (HRP). 320 μ g of IgG fraction of anti-nRNP serum was labelled with HRP by the method of Nakane (Nakane and Kawaoi 1974).

Tissue sections. The liver, kidney, stomach and spinal cord were obtained from exsanguinated Wistar rats. Human liver, kidney and stomach were obtained at autopsy. Piece of each tissue were fixed with a PLP (periodate-lysine-paraformaldehyde) solution and then treated sequentially with sucrose solutions with concentration of 10% for overnight, of 15% for 4 hr and of 20% supplemented with 10% glycerol for 2 hr. The tissues were embedded in an OCT compound and frozen in dry ice chilled acetone. The cryostat sections of 4–5 μ m thickness were mounted on the slide glass coated with bovine serum albumin and air dried under an electric fan.

Staining of tissue sections with HRP labelled IgG fraction of anti-nRNP serum. Cryostat sections of various rat tissues were rinsed with chilled phosphate buffered saline (PBS) (pH 7.2) for 15 min, treated with a 5 mM NaN_3 solution for 10 min, washed with PBS and treated with the suitably diluted peroxidase-labelled anti-nRNP IgG fraction for overnight at 4°C. After washing in the chilled PBS, the tissue sections were treated with 0.02% DAB solution (containing 0.02% H_2O_2 and 0.02 M NaN_3 in Tris-HCl buffer pH 7.6) in order to develop brown color of peroxidase and then counterstained with methyl green for nuclei, and the tissue sections were examined under a light microscope.

RNase treatment of the tissue sections. Cryostat sections of PLP fixed tissues were treated with a 0.001% RNase solution for 30, 60 and 90 min at 4°C. After washing with three changes of chilled PBS, the sections were processed for immunohistochemical demonstration of nRNP antigen as described above.

Fig. 1. Double immunodiffusion pattern of the patient serum MK.

Central well: rabbit thymus extract. Peripheral wells (clockwise from the top): 1) Anti-nRNP fraction of the patient serum, 2) a standard serum positive for Sm and nRNP, 3) a standard anti-SS-B serum, 4) anti-nRNP fraction of the patient serum, 5) a standard anti-Scl-70 serum and 6) anti-nRNP fraction of the patient serum.

RESULTS

In the double immunodiffusion plates (Fig. 1), the patient serum formed a single precipitation line with rabbit thymus extract, which was identical to one formed by a standard anti-nRNP antibody, and apparently different from those formed by standard anti-Sm, anti-SS-B and anti-Scl-70 antibodies obtained from

Fig. 2. Immunostaining of the rat kidney with the HRP conjugated anti-nRNP antibody. Note the granular nuclear stain in the proximal tubules. $\times 400$

Fig. 3. Immunostaining of the rat spinal cord with anti-nRNP antibody. In addition to nuclear stain of the cells in both grey and white matter, cytoplasmic stain is seen in the anterior horn cells. $\times 100$

Fig. 4. Pre-treatment of tissue sections with RNase.

Nuclear and cytoplasmic stain of rat anterior horn cells (a) is abolished by pre-treatment of the tissue section with RNase (b). $\times 400$

the American Rheumatism Association.

The HRP labelled IgG fraction of anti-nRNP serum from the patient MK reacted with the nuclei of rat kidney cells. The distribution patterns of reaction products were mostly speckled but occasionally shaggy (Fig. 2). No cytoplasmic stain was observed in the rat kidney and liver tissues. The rat spinal anterior horn cells, however, showed both cytoplasmic and nuclear stain whereas only the nuclear stain was seen in the lateral and posterior horns (Fig. 3). When the rat spinal cord sections were pre-treated with RNase for 90 min, both cytoplasmic and nuclear stains were markedly reduced (Fig. 4). Pre-treatment with RNase also abolished the immunoreactivity to the HRP labelled anti-nRNP antibody in other tissues. It required 60 min for the liver and 90 min for the kidney.

DISCUSSION

By the immunoblotting technique, small nuclear RNP has recently been identified as U₁-RNA and a peptide complex with molecular weight of 61 KD (Lerner and Steitz 1979). The U₁-RNA has been suggested to be involved in the splicing of early transcript RNA polymerase (Sharp et al. 1976).

Cellular localization of nRNP has been determined by the immunofluorescence method. It was localized to the nucleoplasm excluding nucleolei but not to the cytoplasm (Deng et al. 1981; Nyman et al. 1986). In the present study with the PAP method, the kidney, liver, stomach, spleen and spinal cord of the rat and human liver, kidney and stomach were examined for the presence of nRNP. The nRNP was solely localized to the nucleus in these tissues except the rat spinal cord in which the anterior horn cells were positive for cytoplasmic stain.

Two possibilities can be considered to explain the cytoplasmic staining of the anterior horn cells. It may be simply reflecting the presence of cytoplasmic nRNP in these cells. Alternatively, the HRP labelled anti-nRNP IgG fraction used in the present study might have contained a minute amount of antibodies reactive to the unidentified cellular components of the anterior horn cells. Although the double immunodiffusion study on the patient MK serum suggested the absence of antibodies against the Sm, SS-B, Og, Jo-1 and Ku antigens, the presence of a small quantity of these antibodies could not be ruled out when the sensitivity of this method was considered. Since the antigen La (SS-B) has been localized to the cytoplasm and shown to be sensitive to RNase (Mattioli and Reichlin 1974), the cytoplasmic staining in the present experiment may be suggestive of the presence of anti-La antibody in the patient MK serum.

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