IMMUNOHISTOCHEMICAL INVESTIGATION OF KERATAN SULFATE IN THE ARTICULAR CARTILAGE OF C57 BLACK MICE - A STUDY USING ANTI-KERATAN POLYSULFATE ANTIBODY -

Junya Mibe, Kengo Yamamoto, and Yukio Miura
Department of Orthopedic Surgery, Tokyo Medical College

In order to clarify the cause of osteoarthritis (OA), we have investigated the articular cartilage of C57 black mouse (BM) spontaneous OA model by means of histochemical1) and ultrastructural-histochemical2) methods. It has been confirmed that keratan sulfate (KS) played an important role in the initial phase of OA. This time, for the purpose of immunohistochemical staining of KS, we prepared polyclonal anti-keratan polysulfate (KPS) antibody by immunizing rabbits with KPS obtained from the cartilage of shark.

MATERIALS AND METHODS

Into 1 ml of physiological salt solution, 1 mg of KPS and 5 mg of methylated albumin as a carrier were dissolved. The solution was emulsified with the same volume (1ml) of Freund's adjuvant containing 1 mg/ml of dead tuberculous bacilli. The emulsion was injected into the popliteal lymph node of a JW strain rabbit. Four weeks later, whole blood was withdrawn from the animal and antiserum was obtained. Specificity of the antiserum was determined by the competitive ELISA method according to the method of Thonar et al3), and immunohistochemical staining by the ABC method.

RESULTS AND DISCUSSION

By the ELISA method, the reactivity of the antiserum to KPS was determined. The reaction curve is shown in Fig. 1 where KPS was recognized by the antiserum within a range of 10-1,000 µg/ml. When sera of various species were examined, reactivity was detected in the serum of human, monkey, cattle, and horse, but not in the serum of guinea pig, rat, or mouse (Fig. 2).
Mibe J et al: KS in the cartilage of BM

Fig. 2 ELISA method (Animal's sera)
The reactivity was detected in serum of monkey, human, cattle, and horse, but not in serum of mouse, guinea pig, or rat.

By immunohistochemically staining human articular cartilage with the antiserum, the intercellular region was densely stained suggesting the recognition of KS by the antiserum (Fig. 3). After the treatment with chondroitinase ABC, the stainability was almost identical to that before the treatment. On the other hand, keratanase treatment reduced the stainability.

Based on the above results the antibody obtained was considered to be specific to KS. It, however, showed no stainability in articular cartilage of rat or BM. The KS of BM that was confirmed histochemically and ultrastructural-histochemically was not able to be stained with this antibody. Therefore, this KS was considered to have less sulfate groups than the KPS used for the preparation of antiserum.

REFERENCES