A complementary histochemical method for a detection of a carbohydrate antigen

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Introduction

Since Köhler and Milstein¹⁾ established a method to obtain monoclonal antibodies, various kinds of monoclonals against tumor-associated antigens have been obtained. The antigenic determinants recognized by most monoclonals have not been clarified in general. In this paper, we describe a complementary immunohistochemical method to determine whether carbohydrates are related to an antigenic determinant or not using a combination of an avidin-biotin-peroxidase complex method²⁾ and acetylation techniques³⁾.

Materials and Methods

Formalin fixed and paraffin embedded materials of human minor salivary (palatal and labial) glands were used. Two antibodies with known antigenic determinants, NS 19-9 monoclonal antibody (Centocor)* and anti-human keratin polyclonal antibody (DAKO PAP Kit™ K 518), were utilized as primary antibodies in this study. Three kinds of procedures were applied to serial sections in the following order :

I. avidin-biotin-peroxidase complex (ABC) method

1) deparaffinized sections were washed in phosphate buffered saline (PBS) and were incubated with 0.1 % pronase (SIGMA, type VII, diluted with PBS) at 37 °C for 15 min.

2) after a brief wash, endogenous peroxidase activity was blocked with methanol containing 0.3 % H₂O₂ for 20 min.

3) following a wash in PBS, 10 % normal horse serum was incubated for 20 min.

4) without a wash, sections were incubated for 60 min with NS 19-9 (1/500) or anti-human keratin antibody respectively.

5) after washing in PBS, biotinylated horse anti-mouse IgG (1/200, Vector) or biotinylated goat anti-rabbit IgG (1/200, Vector) was applied for 30 min.

6) after another wash, sections were incubated with ABC (ABC Kit, Vectastain™) for 30 min.

7) then, sections were immersed in freshly made 0.005 % 3,3'-diaminobenzidine in 0.05 M HCl buffer (pH 7.6) with 0.001 % H₂O₂ for 10 min.

8) sections were counterstained with Mayer's hematoxylin, dehydrated in graded ethanol, cleared in xylene and mounted.

II. acetylation of tissue sections

1) after pretreatment with 0.1 % pronase, each section was placed in water and immersed in a mixture of acetic anhydride (13 ml) and pyridine (20 ml) for 45 min.

2) sections were washed in tap and distilled water, then in PBS.

3) ABC method was applied to sections as described above.

III. deacetylation of acetylation sections

1) after pretreatment with 0.1 % pronase, each section was placed in water and acetylation of tissue sections were performed as described above.

2) after washing, sections were incubated with 0.1 N NaOH for 45 min.

3) following wash in tap and distilled water, sections were placed in PBS.

* Monoclonal antibody, NS 19-9 was kindly provided by Toray-Fuji Bionics Inc.

Results and Discussion

NS 19-9 reacted immunohistochemically with minor salivary glands in various ways. Positive reaction products were found in the cytoplasm of some mucous and ductal cells and in luminal contents of ducts (Fig. 1 a). CA 19-9 is sialylated lacto-N-fucopentaose II⁵⁾ detected monoclonal antibody, NS 19-9, produced by fusion of mouse myeloma cells with spleen cells from BALB/c mice immunized with colo-rectal carcinoma cell line (SW 1116)⁴⁾. CA 19-9 has been demonstrated in various cancer tissues as well as in some normal tissues by immunohistochemical method^{6,7)}. Atkinson *et al.*⁶⁾ and Arends *et al.*⁷⁾ have described that CA 19-9 was present in normal salivary glands. We⁸⁾ also reported that CA 19-9 was demonstrated in normal salivary glands, especially in minor salivary glands, and in salivary gland tumors. Although no positive reaction product for CA 19-9 was found in the acetylated sections of minor salivary glands (Fig. 1 b), the positive reaction reappeared in deacetylated tissues after acetylation similar to the first sections (Fig. 1 c).

It has been well-known that keratin protein is detected in the cytoplasm of ductal cells (Fig. 2 a). However, no positive reaction products were found in the acetylated and the deacetylated sections (Fig. 2 b,c).

Acetylation techniques are usually combined with periodic acid Schiff (PAS) stain and are useful for the detection of carbohydrates⁹⁾. In the present study we used the ABC method instead of PAS stain and examined the difference of the reactivities between NS 19-9 and anti-human keratin antibody.

Fig. 1

Fig. 2

Positive reaction for CA 19-9 was removed in deacetylated sections. On the other hand no positive reaction for keratin protein was found in deacetylated sections. Although acetylated amino groups can not be returned to amino groups, the 1,2-glycol linkage is returned by deacetylation⁹⁾.

A periodic acid oxidation is commonly used as a method for the detection of carbohydrate antigens, but the presence of periodate resistant carbohydrates have been described⁹⁾.

This acetylation technique combined with ABC method may be a very useful histochemical method in order to discover whether particular antibodies are specific for carbohydrate or non-carbohydrate antigenic epitope.

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