APPLICATION OF MONOCLONAL ANTIBODIES TO IMMUNOHISTOCHEMISTRY

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Since Köhler and Milstein (9) developed a method to prepare the monoclonal antibody (McAb), the antibody has widely used in every field of biological science. As is well known, the McAb recognizes only one determinant of the antigen with high specificity and contains antibody class or subclass in the uniform pattern. Moreover, the hybridoma, once established, produces the homogeneous antibody molecule in the culture media continuously. On the other hand, McAb does not precipitate the antigen in the form of the immune complex of the large molecule, and shows cross-reactivity (11).

In the application of McAb to immunohistochemical studies, it is necessary to pay particular attention to the condition in its utilization on tissue sections, in addition to taking the characteristics of the McAb mentioned above in consideration.

I. Interpretation of the reaction sites

McAb reacts with only one of the determinants on the antigen molecule. The positive reaction site represents the site of a determinant, but does not always show the localization and the configuration of whole antigen molecule. On the assume that an antigen is furnished with three determinants on the molecule and each of them is designated as A, B and C, respectively (Fig. 1). Supposing these determinants are distributed within a cell in such a pattern as shown in Fig. 2, the localization of determinant A is shown as Fig. 3-A, determinant B as Fig. 3-B and determinant C as Fig. 3-C. The immunoreaction site with the polyclonal antibody (PcAb) prepared to the same antigen is shown as Fig. 3-A + B + C, when the PcAb reacts with three determinants equally and does not contain any other rective factors. Under hypothetical conditions, the reactive sites with McAb, shown in Fig. 3 -A, B or C, do not coincide with that of PcAb. But in practice, it might be very rare that each determinant of an antigen distributes itself as scatteringly within a cell as to show wide variation of the localization as is shown in Fig. 3. In other words, the distribution pattern of a determinant shown immunohistochemically by use of McAb almost represents the sites and configuration of the antigen molecule, with some exceptions.

II. Specificity and intensity of the reaction.

The intensity of the immunoreaction on the tissue is usually weaker with McAb than with PcAb, probably because the number of the reacted determinants are less with the former than with the latter. This is the main reason why the PAP method (16), ABC technique (7) or other methods (8, 17) are effective to intensification of the immunoreaction and to decrease background staining. The figures of the positively reactive sites demonstrated by means of McAb are usually far clearer than those by

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FIG. 1. Determinants on an antigen molecule.

FIG. 2. Localization of determinants in a cell.

FIG. 3. Localization of reaction products showing the sites of determinants A, B and C, respectively with monoclonal antibodies, and A+B+C in all with polyclonal antibody.

use of PcAb. This is probably because the specificity of McAb is so high and each antibody molecule has such even binding activity to the target determinant that McAb may more selectively and concentratedly lodge on the sites of the determinants of the antigen molecule than in the immunoreaction with PcAb.

In some cases in the immunohistochemical study, the reaction appears stronger with McAb than with PcAb. As shown in Fig. 4, under the condition that A is the dominant determinant among those of the antigen, the reaction with anti-A McAb is stronger than that with PcAb which reacts with small number of the determinants A, B and C. This kind of reaction pattern may occur when a definite determinant is concentrated in a definite area within a cell.

III. Cross-reactivity

The presence of cross-reactivity in the use of McAb has been pointed out in several reports (3, 4, 12) and summarized by Lane and Kaprowki (11). As previously described, McAb recognizes single determinant of the antigen but not the antigen molecule. Thus the immunoreactive sites imply distribution of the definite determi-

FIG. 4. Explanation for the stronger reactivity with monoclonal antibody than with polyclonal antibody in immunohistochemistry.

nant in the tissue. If the determinant, which is the same as, common with or genetically similar to the determinant to which McAb was prepared, are present in the different kind of tissues, organs and species, the immunoreaction is also positive. This fact is often misunderstood to be that the immunoreaction with McAb abounds in non-specificity. Taking the results of an immunohistochemical study on the distribution of gamma-glutamyl transpeptidase (Gamma-GTP) in rats by use of McAb prepared against rat kidney Gamma-GTP, positive reactions were obtained not only in the kidney but also in liver pancreas, epididymis and so on (19). The reaction sites in rat kidney represent the site of the determinant of 51 Kd polypeptide, but those in the other tissue represent the site of the determinants which are the same as or similar to 51 Kd polypeptide or genetically similar to it (Table 1). Since it is unknown whether the determinant in those tissues is 51 Kd polypeptide or genetically similar to 51 Kd polypeptide, the immunohistochemical results in tissue other than the kidney are explained to be obtained utilizing the cross-reactive nature of McAb. It must be emphasized that the cross-reactivity is strictly distinguished from the non-specific reaction in the immunohistochemistry.

Supposing a McAb was obtained, it is very difficult to know the sphere of the specimens in which the cross-reactivity could be found. The cross-reaction between Thy-1 and intermediate filaments (3), between Vk-TEPG 15 and Thy-1 (12) and bet-

TABLE 1. *Polypeptides recognized by monoclonal antibodies*

Anti-rat kidney γ -GTP (Yasuda et al.)	Anti-rabbit kidney γ -GTP (Komatsu et al.)	Anti-human kidney γ -GTP (Shiozawa et al.)
51 Kd	54.6 Kd	69 Kd

ween each isoenzyme of carbonic anhydrase (4) are reported, but these are found rather unexpectedly or by chance than by investigating wide range of specimens containing various kind of antigens systematically. The immunohistochemistry is in the same situation. There is apparently a limitation in searching for the tissues or organs which are expected to contain the antigens possibly cross-reactive to the McAb. At least it is desirable to observe the immunoreaction with a McAb on the same organ or tissue, from which the antigen was prepared, in different animals or on different organs of the same species of animal, as shown in Table 2.

IV. Miscellaneous remarks in the utilization of McAb in immunohistochemistry

When the immunoreaction cannot be recognized even after making a careful survey on the immunological specificity of the McAb, the selection of the fixative, concentration of the antibody solution, duration of the reaction with the antibody and the selection of the procedure in the preparation of tissue sections, one of the possibilities to be considered is that the target determinant could be hidden by some unknown materials or entangled in the three-dimensional conformation of the antigen molecule. To make the target determinant in the tissue easily reactive to the antibody, several treatments are introduced, and some of them are useful, depending on the nature of the antigen to be examined; treatment with trypsin (2, 6), with pronase (5) with pepsin (13), with urea (1) and with warm PBS (19).

The immunological characteristics must be strictly examined before applying McAb to the immunohistochemical study. Taking Gamma-GTP as an example, the kind of polypeptide which McAb recognizes (Table 1), class or subclass of McAb (Table 3) and ratio of the inhibition of the activity of the antigen by McAb when McAb is prepared against the enzyme (Table 4) must be confirmed. In this respect, the use of McAb prepared in own laboratory is recommended. These characteristics cannot be examined in detail when the McAb from a commercial source is used,

TABLE 2. *Cross-reactivity of monoclonal antibodies*

	Rat			Rabbit			Human		
	Kidney	Pancreas	Epididymis	Kidney	Pancreas	Epididymis	Kidney	Pancreas	Epididymis
anti-rat γ -GTP	+	+	+	—	—	—	+	—	—
anti-rabbit γ -GTP	—	—	—	+	+	+	—	—	—
anti-human γ -GTP	—	+	—	—	—	—	+	+	+

TABLE 3. *Antibody typing*

Anti-rat kidney γ -GTP (19)	Anti-rabbit kidney γ -GTP (10)	Anti-human kidney γ -GTP (15)
IgG ₁ κ	IgG _{2a} λ	IgG ₁ κ

TABLE 4. *Inhibition of enzyme activity by monoclonal antibodies*

Anti-rat kidney γ -GTP (19)	Anti-rabbit kidney γ -GTP (10)	Anti-human kidney γ -GTP (15)
15%	46.7%	86%

because the antigen from which McAb is prepared cannot be obtained.

McAb does not make precipitated immune complex with the antigen. Therefore, the antibody is not absorbed by the antigen by only mixing two in test tube. This fact makes the control experiment more difficult to be performed than in the case of using PcAb. Thus, the culture medium, supernatant of the myeloma cells, omitting the second or third layer in the staining procedure and the irrelevant McAb are used in the control experiment.

The immunohistochemical study with McAb is designed to demonstrate the distribution of a determinant of the antigen but not the antigen molecule itself. Still the positive sites demonstrated show the site and configuration of the antigen in the tissue section. In practice, it is required to take the nature of McAb and the characteristic condition in the application to the tissue section into consideration.

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