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New Aspects of Carcinoembryonic Antigen Defined by Monoclonal Antibodies

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SUMMARY

Several monoclonal antibodies to carcinoembryonic antigen (CEA) were established in our laboratory. Using these monoclonal antibodies, heterogeneity of the antigenic determinants on CEA molecule was defined by serological methods and by immunoperoxidase technique. In addition, immunodiagnostic approaches to gastro-intestinal tumors with the monoclonal antibodies were described. Other papers concerning new aspects of CEA were also briefly reviewed.

Key words: Carcinoembryonic antigen, Monoclonal antibody, Antigenic determinant

INTRODUCTION

Serum carcinoembryonic antigen (CEA) (1) is widely determined in clinical laboratories as a useful marker for monitoring cancer patients. This molecule, however, is known to have a complex antigen system: a number of related antigens, such as non-specific cross-reacting antigen (NCA) (2), nonspecific cross-reacting antigen 2 (NCA2) (3), normal fecal antigen (4), and biliary glycoprotein-I (BGP-I) (5) have been described with the use of polyclonal antisera. Because polyclonal antisera have limitations of specificity and amount for supply, it has been difficult to further analyze the antigenic determinants on CEA or related molecules and to evaluate the results of their immunohistochemical distribution from the view point of the corresponding molecules and/or antigenic determinants. In this brief review, we will first describe the different antigenic determinants on the CEA molecule(s) recognized by a variety of monoclonal antibodies which have been developed in our laboratory and second, we will compare immunohistochemical distribution of the antigenic determinants recognized by these monoclonal antibodies to CEA. And then we will present some of our recent work aiming at developing immunodiagnostic approaches to gastro-intestinal tumors with these monoclonal

Table 1 *Summary of the Biochemical Profile of the Antigens Detected by Monoclonal Antibodies.*

	AS 802 (IgG 1)	AS 210 (IgG 1)	YK 024 (IgG 3)	MT 008 (IgG 3)	AS 001 (IgG 1)
Antigenic structure[1]	200 K + 180 K	200 K + 180 K	200 K + 180 K	200 K + 180 K	200 K
Crossreaction[2] with					
NCA	no	no	no	no	no
NCA 2	no	no	no	no	no
Enzyme treatment[3]					
Trypsin	sensitive	sensitive	resistant	resistant	sensitive
Periodate	resistant	resistant	sensitive	sensitive	resistant
Neuraminidase	resistant	resistant	resistant	resistant	resistant

1) Antigenic structure was determined by SDS-polyacrylamide gel electrophoresis using iodinated CEA preparation (12)

2) Binding assay employing radioiodinated NCA and NCA 2 as described elsewhere (12)

3) ^{125}I-CEA preparation was digested with trypsin (512 unit/mg, Worthington USA, final concentration of 10%) for 60 min at 37℃ or neuraminidase (Sigma, USA, final concentration of 2.5 unit/ug CEA) for 12 hours at 37℃ and then each preparation was used for radioimmunoprecipitation with five monoclonal antibodies and for SDS-PAGE. Tissue sections were incubated with 1% periodic acid solution for 60 min at room temperature and they were processed for indirect immunoperoxidase method.

antibodies.

Heterogeneity of the antigenic determinants detected by monoclonal antibodies to CEA

The molecular profile of the antigens recognized by five monoclonal antibodies has been tested by use of radioimmunoprecipitation and SDS-PAGE. All of these monoclonal antibodies, with the exception of antibody AS 001, immunoprecipitated components with approximate m. w. of 200 K and 180 K daltons from radioiodinated CEA preparations (Table 1). Monoclonal antibody AS 001 immunoprecipitated a component with an approximate m. w. of 200 K dalton. In addition, molecular forms detected by each antibody did not change under either reducing or nonreducing conditions, indicating that 200 K and 180 K dalton forms are not bridged by disulfide bonds. Sepuential immunodepletion experiments were then performed to determine whether these five monoclonal antibodies recognize the same of different molecules. The data strongly suggest that these monoclonal antibodies, except for antibody AS 001, react to the same antigenic structures. Monoclonal antibody AS 001 could not react with a 200 K dalton molecule which had been removed by monoclonal antibody YK 024. In a reverse experiment, antibody YK 024 reacted only with a 180 K dalton molecle after the 200 K dalton molecule was

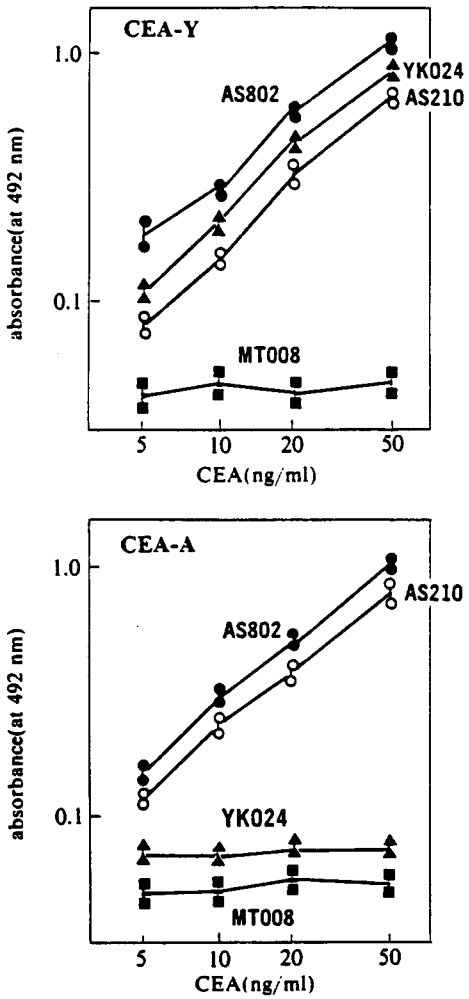

Fig. 1 Antigenic determinants of CEA-Y and CEA-A detected by monoclonal antibodies. In the upper panel, CEA-Y was detected by the antibodies AS 802, YK 024 and AS 210, but not by the antibody MT 008. In the lower panel, CEA-A was detected by the antibodies AS 802 and AS 210, but not by the antibody YK 024 or MT 008.

removed by monoclonal antibody AS 001. Together, these results suggest that monoclonal antibody AS 001 recognizes the 200 K dalton structure, which is a part of the molecules recognized by the other four monoclonal antibodies, and that it also

recognizes a different antigenic determinant on a 200 K dalton molecule from ones recognized by the other four monclonal antibodies, because monoclonal antibody AS 001 immunoprecipitated only 200 K dalton molecules, whereas the other four monoclonal antibodies immunoprecipitated both 200 K and 180 K dalton molecules.

Next, a rosette inhibition assay with CEA-secreting cultured KATO-III cells was performed to compare the antigenic determinants recognized by the three monoclonal antibodies. KATO-III cells, which were coated with monoclonal antibody AS 802 and were capped with goat anti-mouse Ig, were unable to bind monoclonal antibody AS 802 but maintained their reactivity with monoclonal antibody AS 210. In a reverse experiment, cells coated with AS 210 were unable to bind AS 210, but maintained their reactivity with AS 802. Together, these results strongly suggest that monoclonal antibodies AS 802 and AS 210 recognize different antigenic determinants on the cell surface. The same type of rosette inhibition assay suggests that each monoclonal antibody recognizes a different antigenic determinant. Although the rosette inhibition assay reveals different antigenic determinants only on the cell surface, the peroxidate-staining distribution detected by these antibodies on various tissue sections (described in the next session) clearly

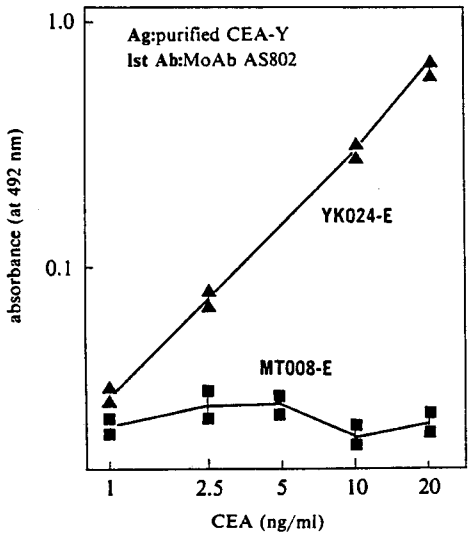

Fig. 2 Double determinants enzyme immunoassay was performed using purified CEA-Y as an antigen. First antibody (AS 802) was incubated with CEA-Y and the second antibodies, which were labeled with peroxidase, were incubated with the immune complex to proceed with the enzyme immunoassay as described (12).

demonstrated that all the monoclonal antibodies recognized different antigenic determinants.

In order to determine whether or not these different antigenic determinants are on the same CEA molecule, enzyme immunoassay was performed using two different CEA preparations (designated CEA-Y and CEA-A). As shown in Figure 1, monoclonal antibodies AS 802, AS 210 and YK 024 strongly reacted with CEA-Y in conjunction with peroxidase-labeled anti-CEA polyclonal antibodies, whereas monoclonal antibody MT 008 failed to react with 50 ng of CEA-Y. On the other hand, monoclonal antibodies AS 802 and AS 210 reacted with CEA-A, but antibodies YK 024 and MT 008 were unreactive to it. Therefore these data suggest that CEA preparation itself may lack the certain antigenic determinant(s) recognized by the monoclonal antibodies which can react with other CEA preapartion(s). To further confirm the lack of the antigenic determinant on CEA-Y, which was recognized by the antibody MT 008, double determinant enzyme immunoassay was carried out. In this assay the first monoclonal antibody AS 802 was attached to the plate before the purified CEA-Y was incubated, and then the second monoclonal antibody which was labeled with peroxidase was added to the plate (Fig. 2). The monoclonal antibody YK 024 bound strongly to the CEA-Y whic was recognized by the antibody AS 802, whereas the antibody MT 008 failed to bind with the CEA-Y. This result indicates that the CEA-Y molecule carries both antigenic determinants detected by the monoclonal antibodies AS 802 and YK 024, but lacks the determinant recognized by the monoclonal antibody MT 008. Therefore it is now clear that CEA molecules may be different from one preparation to another due to the heterogeneous expression of the antigenic determinants.

Recently, von Kleist *et al.* (5) stated that two CEA molecular species of 180 K and 160 K dalton were defined by immunoprecipitation and SDS-PAGE with their monoclonal antibodies. These results supported our date (Personal communication, 5 th Sapporo Cancer Seminar, Abstract, 1985). Primus *et al.* (6) reported that one of their monoclonal antibodies (i. e. NP-4) reacts with a unique determinant expressed on a "subpopulation" of CEA molecules, since it stained only 30% of the primary colorectal adenocarcinoma and it failed to stain most of the nodal and/or liver metastasis from six patients with the monoclonal antibody NP-4 -positive primary tumors. Blaszczyk *et al.* (7) reported that their three monoclonal antibodies 3 d, 4 and C_4 20-32 recognized three different antigenic determinants: antibody 3 d recognized a determinant expressed on 180 K molecule, antibody 4 recognized a different determinant on both 180 K and 160 K molecules, and the antibody $C_4$20-32 reacted with epitope which was expressed on 180 Kd, 160 K, 50 K and 40 K molecules. Hedin (8) *et al.* generated interesting monoclonal antibodies: monoclonal antibodies 9 and 27 recognized the common epitopes

Table 2 *Staining Pattern of Colonic Tissues with Monoclonal Antibodies in Immunoperoxidase Method.*

Colonic tissues	Monoclonal antibodies				
	AS 802	AS 210	YK 024	MT 008	AS 001
Surface epithelium	5/5*	4/5	0/20	0/10	11/13
Well-differentiated adenocarcinoma	10/12	11/12	2/25	4/7	12/12
Moderately or poorly differ. adenoca.	5/10	7/10	9/10	3/10	2/3

* No. positive/No. tested

which both CEA and NCA 2 carry, whereas the antibodies 38 Sl and 49 reacted with an unique epitope which is expressed on CEA molecule. More recently, Kuroki *et al.* (9) described the monoclonal antibodies which bound CEA but did not recognize the epitopes of CEA related molecules such as NCA, NCA-2, NFA-1 and NFA-2. They found that these antibodies did not react with any of five purified CEA prepartions other than that used for immunization. These reports further support our contention that CEA molecules are heterogeneous in their antigenic determinants.

To further analyze the chemical nature of the antigenic determinants detected by these distinct monoclonal antibodies, the CEA preparations were treated with trypsin and were then subjected to radioimmunoprecipiration and SDS-PAGE experiments. It was found that monoclonal antibodies AS 802, AS 210, and AS 001 failed to immunoprecipitate the 200 K and 180 K dalton molecules of the same size. These data suggest that the antigenic determinants recognized by monoclonal antibodies AS 802, AS 210, and AS 001 are of a peptide nature.

Immunohistological distribution of the antigenic determinants

By using the above mentioned monoclonal antibodies, we determined the immunohistological distribution of the antigenic determinants on both cancerous and non-cancerous tissues and on fetal tissues. We found that all of the monoclonal antibodies recognized different antigenic determinants on the tissue section, because the staining distribution on non-cancerous, fetal, and cancerous tissues detected by the five monoclonal antibodies was different (Table 2). These results agree with data obtained from the rosette inhibition assay employing these monoclonal antibodies. It is of interest that three of our monoclonal antibodies stained columnar epithelial cells in morphologically normal colonic mucosa, because this finding agrees with the data obtained by Primus *et al.* (10), indicating that their monoclonal antibody NP-4, reacting with a unique determinant expressed on a " subpopulation " of CEA molecules, stained them. It is most interesting, however,

that one of our monclonal antibodies (YK 024) did not stain any part of the morphologically normal colonic mucosa. This antibody was also found to be unreactive with intestinal metaplasia lesions of the stomach. It is also of interest that it reacted with antigen(s) on fetal stomach but not with those on the fetal colon. Moreover, it was found that this monoclonal antibody mainly reacted with moderately or poorly differentiated carcinoma lesions of both the colon and the stomach. Periodic acid treatment on the antigen (11), may suggest that this antibody recognizes a carbohydrate antigenic determinant in nature, although further study will be needed to reach a firm conclusion regarding the nature of the antigenic determinant, because periodate can also destroy certain amino acids. The details of the chemical composition of this antigenic determinant are now under investigation in our laboratory.

With the exception of monoclonal antibody YK 024, the other monoclonal antibodies stained the majority of well-differentiated adenocarcinoma as well as moderately or poorly differentiated adenocrcinoma of the colon and the stomach. Monoclonal antibody YK 024 stained only 2/25 of welldifferentiated adenocarcinoma of the colon and 2/15 of papillary and tubular well-differentiated adenocarcinoma of the stomach, but stained 9/10 of moderately or poorly differentiated adenocarcinoma of the colon. Preparation of antigens from reactive or unreactive tissues of the five monoclonal antibodies and a structural analysis with SDS-PAGE and western

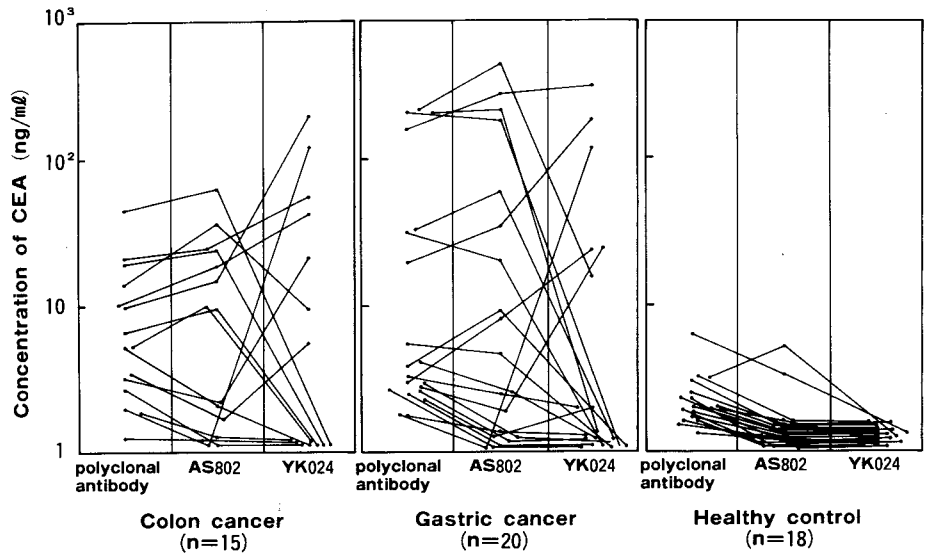

Fig. 3 Radioimmunoassay of immunoreactive CEA with monoclonal antibody AS 802, with monoclonal antibody YK 024 and with anti-CEA polyclonal antibody (Dai-ichi kit). Conditions for radioimmunoassay were essentially the same as described elsewhere (12)

blotting should provide useful information regarding this striking difference.

In any case, these results prompted us to search for a unique antigenic determinant on molecules in the sera from cancer patients.

Immunodiagnostic approaches to gastro-intestinal tumors with the monoclonal antibodies

We have shown that the monoclonal antibody AS 001, which recognizes a peptide antigenic determinant on a 200 K dalton distinct molecule, detected a higher incidence of positivity for sera from gastric, colonic, and lung cancer patients than that obtained by the polyclonal antibody used, and that monoclonal antibody AS 001 could detect the corresponding antigen in sera which the polyclonal antibody failed to detect (12).

We are now using monoclonal antibodies AS 802 and YK 024, and have thus far found that although monoclonal antibody AS 802 showed a similar result as the conventional polyclonal anti-CEA antibody, some cases with the gastro-intestinal tumors showing a low value with the polyclonal antibody demonstrated higher values with the monoclonal antibody YK 024 (Fig. 3). Double determinant enzyme immunoassay using these two monoclonal antibodies is now under investigation in our laboratory.

Another useful application of these monoclonal antibodies to CEA would be radioimmunodetection or "tumor imaging". Buchegger *et al.* (13) showed the potential usefulness of their monoclonal antibodies to CEA for the radio-immunodetection of colon carcinomas: they used Fab and $F(ab')_2$ fragments as well as whole immunoglobulin molecule and found that Fab and $F(ab')_2$ fragments penetrated into the tumor masses whereas the whole molecule mainly stayed on the surace of the tumor tissues. In any case, the technique of radioimmunodetection has certainly developed an area of patient investigation of considerable interest and has a potential for aiding the diagnosis and treatment of cancer. Together with the improving of the imaging techniques, anti-CEA monoclonal antibodies would be more applicable for clinical medicine, since cancer mapping could be of great value to the surgeon or radiotherapist, and precise staging would greatly aid the design and conduct of chemotherapy trials.

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REFERENCES

1. GOLD, P. and FREEDMAN, S. O.: J. Exp. Med. **121,** 439-462 (1965).
2. VON KLEIST, S., CHAVENEL, G. and BURTIN, P.: Proc. Natl. Acad. Sci. USA **69,** 2492-2494 (1972).
3. BURTIN, P., CHAVANEL, G. and HIRSCH-MARIE, H.: J. Immunol. **111,** 1926-1929 (1973).
4. MATSUOKA, Y., HARA, M., TAKATSU, K. and KITAGAWA, M.: Gann **64,** 203-206 (1973).
5. VON KLEIST, S.: Personal Communication, Fifth Sapporo Cancer Seminar Abstract p. 12 (1985).
6. PRIMUS, F. J., NEWELL, K. D., BLUE, A. and GOLDENBERG, D. M.: Cancer Res. **43,** 686-692 (1983).
7. BLASZCZYK, M., PAK, K. Y., HERLYN. M., LINDGREN, J., PESSANO, S., STEPLEWSKI, Z. and KOPROWSKI, H.: Cancer Res. **44,** 245-253 (1984).
8. HEDIN, A., CARLSSON, L., BERGLUND, A. and HAMMARSTROM, S.: Proc. Natl. Acad. Sci. USA **80,** 3470-3474 (1983).
9. KUROKI, MA., KUROKI, MO., MATSUNAGA, A., SHINODA, T., TAKAYASU, T. and MATSUOKA, Y.: J. Immunol. **133,** 2090-2097 (1984).
10. PRIMUS, F. J., KUHNS, W. J. and GOLDENBERG, D. M.: Cancer Res. **43,** 693-701 (1983).
11. YACHI, A., IMAI, K., FUJITA, H., MORIYA, Y., TANDA, M., ENDO, T., TSUJISAKI, M. and KAWAHARADA, M.: J. Immunol. **132,** 2998-3004 (1984).
12. IMAI, K., MORIYA, Y., FUJITA, H., KAWAHARADA, M. and YACHI, A.: J. Immunol. **132,** 2992-2997 (1984).
13. BUCHEGGER. F., HASKELL, C. M., SCHREYER, M., SCHAZZIGA, B. R., RANDIN, S., CARREL, S. and MACH, J. P.: J. Exp. Med. **158,** 413-427 (1983).