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Cross-reactivity in LMI between Gastric and Colorectal Cancer Patients

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SUMMARY

We have reported that the leucocyte migration inhibition (LMI) test was very useful to detect TAA for gastric cancer and colorectal cancer and that a cross-reaction in LMI between gastric and colorectal cancer was observed in some extent. In the present study, the cross-reactivity of LMI between gastric and colorectal cancer was investigated.

The LMI was performed with the modification of Clausen's method, using 3M KCl extracts of gastric cancer, colorectal cancer and the fetuses. The cross-reactivity between gastric and colorectal cancer was found in 15% of tests on gastric cancer patients and in 21% of tests on colorectal cancer patients. Pathologic reaction to fetal extracts was found in 26% of tests on gastric cancer patients and in 34% of tests on colorectal cancer patients. These results suggested that the fetal extracts might induce LIF (Leucocyte inhibition factor) to lymphocytes from gastric and colorectal cancer patients. CEA concentrations of gastric or colorectal cancer extracts were also measured by the Roche kit. Although CEA of 3 kinds of extracts was individually varied from low to high in concentration, the CEA concentration had no relation with LMI reactivity.

In conclusion, it was thought that the fetal extracts might have the common antigen with the gastrointestinal cancer extracts. In addition, CEA concentration of the extracts did not influence LMI reactivity.

Key words: Leucocyte migration inhibition, Gastric cancer, Colorectal cancer, CEA

INTRODUCTION

Leucocyte migration inhibition (LMI) test is one of valuable procedures to detect TAA in human malignant neoplasma. In previous studies on LMI of gastrointestinal cancer patients, it was communicated that a cross-reactivity

between tumors of different histological origins was observed in various incidences (1, 2). But, it has been unclear which fraction of cancer extracts have a great role in the cross-reaction between tumors of different histological origins.

In order to explain the cross-reactivity, we considered that both gastric and colorectal cancer extracts might contain CEA or the other common antigen which sensitize leucocytes from these cancer patients. In the present study, it was also examined whether fetal extracts might possess common antigen to gastric cancer and colorectal cancer extracts or not. In addition, 3 kinds of extracts were investigated biochemically by gel fractionation.

MATERIALS AND METHODS

Blood and tissue samples :

Heparinized blood samples were obtained from 79 patients with gastric cancer and 71 patients with colorectal cancer. As a control, blood samples were also obtained from 10 normal subjects as well as from 20 patients with benign gastrointestinal diseases. Histological confirmation of the diagnosis was obtained in all patients.

Gastric and colorectal cancer tissues as well as fetal tissues were processed immediately after surgery or thrapeutic abortion. Of 18 cancer tissue specimens used in this study, 10 specimens were from gastric cancer patients and 8 from colorectal cancer patients. The 8 fetal tissues (at 10-12 weeks of gestation) were used.

Antigen preparation :

Cancer tissue or fetal tissue extraction was performed according to a modification of 3M KCl extraction procedure described by Reisfeld *et al.* (3). The protein concentration was measured by the procedure of Lowry *et al.* (4).

The optimal concentration of antigens was preliminarily examined with leucocytes of normal subjects. Subsequently, antigens at a final concentration of 0.25 mg/m*l* were selected for the present experiments.

LMI test procedure :

The leucocyte migration inhibition test reported by Clausen (5) was used throughout this study.

Determination of normal range of LMI with gastrointestinal cancer extracts and fetal extracts :

In order to define a pathologic MI, leucocytes from 10 normal subjects were examined with gastric cancer extracts or colorectal cancer extracts or fetal extracts. The average of MI and its standard deviation in normal subjects were obtained using 3 kinds of extracts respectively. The normal range of MIs was determined by calculating the mean$\pm$double standard deviation of normal subjects'

MIs. MIs out of this range were considered to be pathologic.

CEA concentration in gastrointestinal cancer and fetal extracts :

The CEA concentration of these extracts was assessed by the radioimmunoassay using the Roche kit.

Fractionation on Sephadex G-200 :

The 3 kinds of extracts (gastric cancer extract, colorectal cancer extract and fetal extract) were chromatographed on Sephadex G-200 column equilibrated with PBS. Elution was performed by PBS at a flow rate of 10 m*l*/hr. Fractions of 2.4 m*l* were collected and protein peaks were defined by absorption at 280 nm.

RESULTS

Normal range of MI with cancer extracts and fetal extracts :

The normal range of MI was calculated to be between 0.77 and 1.18 with gastric cancer extracts, between 0.83 and 1.19 with colorectal cancer extracts and between 0.71 and 1.31 with fetal extracts.

LMI in gastric and colorectal cancer patients with gastric, colorectal cancer extracts or fetal extracts :

1) LMI with gastric cancer extracts (Table 1).

A pathologic MI was found in 48% of 395 tests on gastric cancer patients and

Table 1 *LMI : Incidence of pathologic MI with 3 M KCl extracts of gastric cancer.*

Leucocyte Donor	No. of Patients	No. of Tests	Total No. of Tests Showing Pathologic MI (%)
Gastric Cancer	79	395	*189 (448)
Colorectal Cancer	20	100	* 21 (21)
Benign Diseases	20	18	* 18 (18)
Normal Subjects	10	52	* 2 (4)

* $p<0.01$(chi square test)

Table 2 *LMI : Incidence of pathologic MI with 3 M KCl extracts of colorectal cancer.*

Leucocyte Donor	No. of Patients	No. of Tests	Total No. of Tests Showing Pathologic MI (%)
Colorectal Cancer	71	355	*175 (49)
Gastric Cancer	20	100	* 15 (15)
Benign Diseases	20	100	* 12 (12)
Normal Subjects	10	50	* 5 (4)

in 21% of tests on colorectal cancer patients.

2) LMI with colorectal cancer extracts (Table 2).

A pathologic MI was found in 49% of 375 tests on colorectal cancer patients and in 15% of 100 tests on gastric cancer patients. Incidence of cross-reactivity between gastric cancer and colorectal cancer was 15% or 21%.

3) LMI with fetal extracts (Table 3).

A pathologic MI was found in 26% or 97 tests on gastric cancer patients and

Table 3 *LMI : Incidence of pathologic MI with 3 M KCl extracts of fetus.*

Leucocyte Donor	No. of Patients	No. of Tests	Total No. of Tests Showing Pathologic MI (%)
Gastric Cancer	26	97	25/97 (26)
Colorectal Cancer	18	74	25/74 (34)
Normal Subjects	6	30	1/30 (3)

Table 4 *Relation between CEA concentration in gastric cancer extract with incidence of pathologic MI of gastric cancer patients.*

CEA Concentration in Gastric Cancer Extracts (ng/mg protein)	≦50	50<
No. of Tests	16	19
Total No. of Tests Showing Pathologic MI	4	7
Incidence of Pathologic MI (%)	25	37

Table 5 *Relation between CEA concentration in colorectal cancer extracts with incidence of pathologic MI of colorectal cancer patients.*

CEA Concentration in Colorectal Cancer Extracts ng/mg protein	≦50	50<
No. of Tests	160	110
Total No. of Tests Showing Pathologic MI	73	53
Incidence of Pathologic MI (%)	46	48

in 34% of 74 tests on colorectal cancer patients. A pathologic MI was observed significantly more in gastric cancer patients or colorectal cancer patients than in normal subjects ($P<0.001$).

Relation between CEA concentration in gastric, colorectal cancer extracts or fetal extracts with the incidence of pathologic MI.

The incidence of pathologic MI was 25% of tests on gasric cancer patients, and 46% of tests on colorectal cancer patients with extracts whose CEA concentration was lower than 50 ng/mg protein, and 37% of tests on gastric cancer patients and 48% of tests on colorectal cancer patients showed pathologic MI with the extracts whose CEA concentration was higher than 50 ng/mg protein (Table 4, 5). CEA concentration of every fetal extract was lower than 20 ng/mg protein, and CEA concentration of fetal extracts had no relation to incidence of pathologic MI in gastric or colorectal cancer patients.

Gel fractionation of cancer extracts (gastric cancer or colorectal cancer) or fetal extracts on Sephadex G-200 column :

The gastric cancer extracts and colorectal cancer extracts showed similar pattern on chromatography, having four protein peaks. On the other hand, the fetal extracts showed to have only two peaks which were seen in the cancer extracts. (Fr. 1 : M. W. about 400,000~500,000, Fr. 4 : M. W. about 10,000) (Fig. 1).

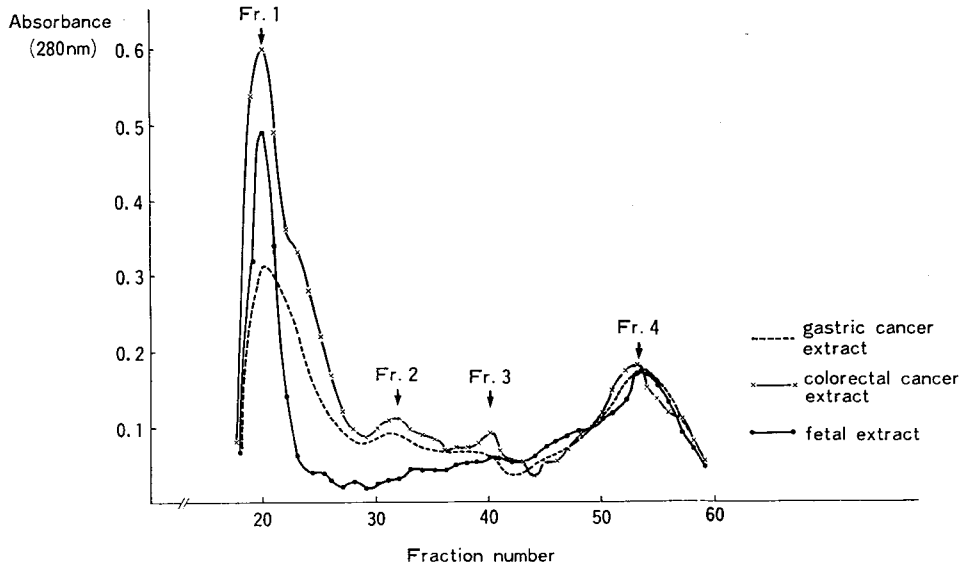

Fig. 1 Chromatography on Sephadex G-200 of 3 M KCl extracts of gastric cancer, colorectal cancer and fetus.

DISCUSSION

LMI has been reported to be depend on tumor associated antigen (TAA) in numerous human neoplasmas. In our previous studies (6, 7), the LMI was useful to detect the TAA in gastric and colorectal cancer patients. A pathologic MI was seen in 15% of tests on gastric cancer patients with colorectal cancer extracts, and in 21% of tests on colorectal cancer patients with gastric cancer extracts. It is thought that the cross-reactivity between gastric and colorectal cancer patients is induced by various factors and that the 3 M KCl cancer extracts may possess several different antigens such as organ related antigen or wide-spread antigen in addition to TAA.

In view of the organ related antigen, Zöller *et al.* (2) reported that patients with non-malignant colorectal diseases were more reactive with colorectal cancer extracts than patients with noncolorectal diseases. In our previous study (7) as to LMI with gastric cancer extracts, chronic gastric ulcer patients with various grade of atrophic gastritis showed to be more reactive than patients with non-gastric diseases. But, these reactivities due to the organ related antigen could not so frequently be observed. On the other hand, it is supposed that wide-spread antigen might affect the LMI reactivity on cancer patients. Emmrich *et al.* (8) reported that CEA induced LIF in leucocytes from gastric and colorectal cancer patients. But our results indicated that CEA concentration of the extracts didn't affect the incidence of pathologic MI in cancer patients. These results were similar to Matzku's results (9) that 3M KCl fetal extracts induced LIF in leucocytes from some kinds of cancer patients, such as lung, stomach and colorectal cancer, although the incidences of pathologic MI in the present study were lower in gastric (25%) and colorectal cancer patients (36%), comparing to the incidence seen in Matzku's report (10). The incidences of pathologic MI with fetal extracts were lower than those with corresponding cancer extracts. The results indicate that fetal extracts might possess several common antigens which can stimulate lymphocytes from gastric and colorectal cancer patients.

Finally, the three kinds of extracts had same two protein peaks at 1×10^4 and 4 to 5×10^5 M. W. in gel fractionation. To analyse the antigenicity of the two protein peaks, further investigation on immunological as well as biochemical study is required.

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