Eur J Cancer Clin Oncol, Vol. 18, No. 7, pp. 617–621, 1982.
Printed in Great Britain.

0277-5379/82/070617-05$03.00/0

Lactosylsphingosine-reactive Antibody and CEA in Patients with Colorectal Cancer*

WŁADYSŁAW JÓŹWIAK and JERZY KOŚCIELAK†
Department of Biochemistry, Institute of Haematology, Warsaw, Poland

Abstract—*The sera of* 71 *patients with colorectal cancer were examined for lactosylsphingosine-reactive antibody and CEA. Fifteen of those patients were studied repeatedly over extensive periods of time. The antibody was determined by the semiquantitative radioimmunoabsorption technique using lactosylsphingosine–polyacrylamide conjugate and* [^{125}I]*-labelled anti-human IgG. Excessive antibody levels were invariably found in serum samples of all* 39 *patients who were examined before or within* 2 *months after surgery. Serum samples of certain patients became negative for the presence of high antibody levels usually between* 3 *and* 6 *months after surgery. This occurred in* 13 *out of* 41 *operated patients. The follow-up study revealed that* 11 *such patients have been free of any signs of cancer relapse up until the time of the follow-up examination, i.e. for* 12–28 months, *mean* 19 months. *In contrast, only* 4 *out of* 28 *patients who remained positive for the excessive antibody longer than* 2 months *after surgery are at present free of the disease. The high antibody levels which persist more than* 6 months *after surgery are almost always associated with cancer recurrences or metastases. This was true for* 21 *out of* 22 *such patients. The high levels of the antibody preceded other signs of cancer relapse, including increased concentrations of CEA in about* 40% *of the operated patients.*

INTRODUCTION

IMMUNODIAGNOSIS of cancer is a rapidly expanding field. The detection of circulating antibodies to tumor or tumor-associated antigens is potentially a sensitive and simple technique. The first such antibodies to be reported were against common antigens of melanoma[1, 2] and osteosarcoma[1, 3]. Subsequently, circulating antibodies to other tumor-associated antigens in a number of diseases were described [4–8]. In addition, elevated titers of antibodies against supposedly oncogenic viruses and virus-associated antigens were reported in patients with various kinds of malignancies [9–12]. Further evidence for the humoral response possible to tumor or tumor-associated antigens is provided by the presence of circulating immune complexes [13, 14] or autoantibodies [15, 16] in patients' sera. Antigens expressed at the surface of tumor cells may in some instances reduce titers of pre-existing antibodies, as is the case with breast cancer and anti-T antibody, i.e. against Thomsen–Friedenreich antigen [17].

Lactosylceramide is a simple glycosphingolipid which is a normal membrane component of most mammalian cells but accumulated in certain tumors [18–21]. Previously we reported the presence of increased amounts of IgG reactive with the artificial lactosylsphingosine–polyacrylamide conjugate in sera of patients with gastrointestinal and uterine cancers [21–23]. These patients were examined prior to or within the three weeks following surgery. Here we report the antibody levels in a larger group of patients with colorectal cancer who were examined also at various times after surgery, some of them serially.

MATERIALS AND METHODS

The sera were randomly selected from the collection of the Institute of Oncology in Warsaw. They were stored at −20°C. A total of 154

Accepted 2 February 1982.
*This study was supported by the Polish National Cancer Programme, Grant No. 1317/13.
†Requests for reprints should be addressed to Prof. J. Kościelak, Institute of Haematology, Department of Biochemistry, Chocimska 5, 00-957 Warsaw, Poland.

serum samples from patients with histologically proven colorectal cancer were investigated. All samples were coded. The patients ranged in age from 25 to 80 years with a median of 56 years. For the detailed description of cases see Legends to Table 1 and Fig. 1. Antibodies reactive with lactosylsphingosine–polyacrylamide conjugate were determined by the

Table 1. *Lactosylsphingosine-reactive antibody and CEA in sera of patients with colorectal cancer*

Sera	No. of cases	Lactosylsphingosine-reactive antibody		CEA	
		Positive	Negative	Positive	Negative
Unoperated cases	16*	16	0	10	6
Examined within 2 months after surgery	14†	14	0	2	12
3–6 months after surgery	9‡	6	3	2	7
7–18 months after surgery	17§	15	2	8	9

*Carcinoma coli, 8; carcinoma recti, 5; carcinoma sigmae, 3.
†Carcinoma recti, 9; carcinoma coli, 3; carcinoma sigmae, 2.
‡Carcinoma recti, 5; carcinoma coli, 4.
§Carcinoma recti, 10; carcinoma sigmae, 4; carcinoma coli, 2; carcinoma caeci, 1.

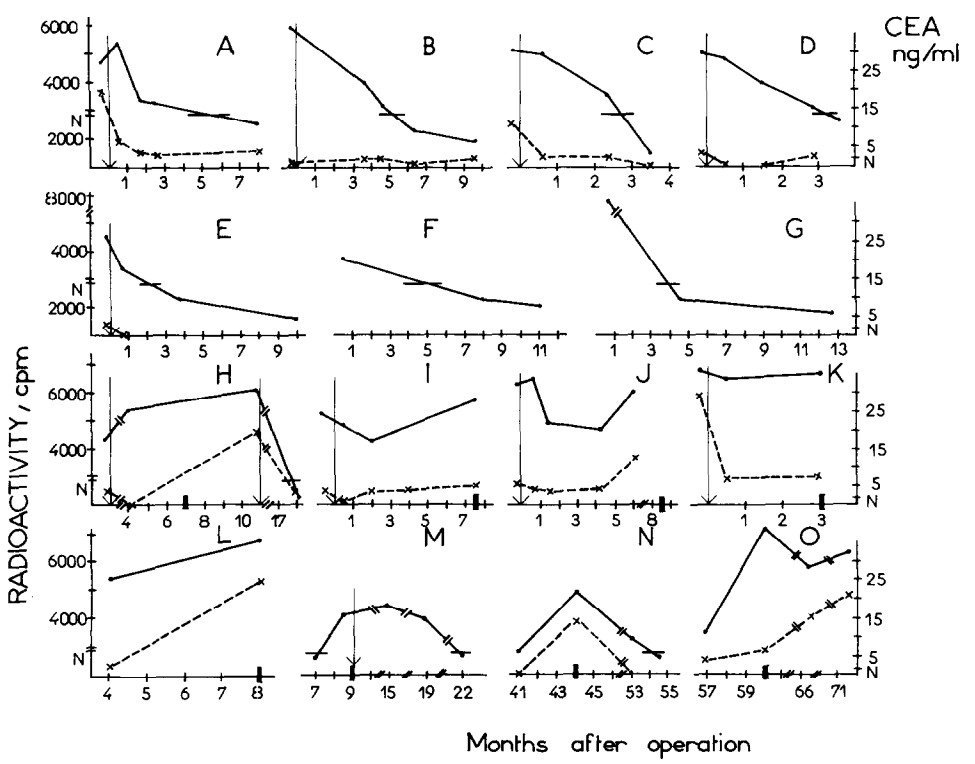

Fig. 1. *Lactosylsphingosine-reactive antibody and CEA in sera of patients with colorectal cancer examined at various times after surgery.* —— *Lactosylsphingosine-reactive antibody;* --- *CEA;* ↓ *-surgery;* ▮*-cancer recurrence.* (*A*) *Carcinoma recti, 50-year-old male;* (*B*) *carcinoma coli, 49-year-old male;* (*C*) *carcinoma recti, 64-year-old male;* (*D*) *carcinoma coli, 49-year-old male;* (*E*) *adenocarcinoma sigmae, 72-year-old male;* (*F*) *carcinoma recti, 71-year-old male;* (*G*) *carcinoma rectosigmoidei, 39-year-old male;* (*H*) *carcinoma recti, 45-hear-old female, twice operated on;* (*I*) *carcinoma recti, 42-year-old female;* 7.5 months *after surgery tumor spread to the liver;* (*J*) *carcinoma coli, 35-year-old male; inoperable cancer recurrence diagnosed* 8.5 months *after surgery;* (*K*) *carcinoma recti, 65-year-old female;* 3 months *after surgery tumor spread to the liver;* (*L*) *adenocarcinoma sigmae, 69-year-old female; inoperable cancer recurrence diagnosed* 8 months *after surgery, and tumor spread to the liver;* (*M*) *carcinoma caecum, 65-year-old female; cancer recurrence diagnosed* 9 months *after surgery and operated on for the second time;* (*N*) *carcinoma sigmae, 55-year-old female; inoperable cancer recurrence diagnosed* 44 months *after surgery;* (*O*) *carcinoma recti, 68-year-old male; inoperable cancer recurrence diagnosed* 60 months *after surgery.*

semiquantitative sandwich radioimmunological technique as previously described [23], using the same batches of the conjugate and anti-human IgG. Briefly, 0.1 ml of serum and 0.5 mg of lactosylsphingosine–polyacrylamide conjugate were incubated with slow stirring for 1 hr at 37°C and subsequently for 24 hr at 0–4°C. The residue was then washed four times with 5 ml portions of 0.01 M phosphate buffer, pH 7.1, containing 0.5 M NaCl, and a fifth time with NaCl-free buffer. Next, the residue was treated with 50 μl of the buffer and 20 μl of anti-human IgG [^{125}I] of goat containing 0.02 μCi of radioactivity. Samples were incubated overnight at 0–4°C with gentle stirring and subsequently washed as above. The radioactivity in the residue was measured on a Packard counter (model 5360). Normal values of the antibody in blood donors amounted to 1253 ± 318 counts/min (540–1988 counts/min) [23]. Samples were considered positive when they exceeded the mean normal value by more than 1590 counts/min (318×5). Carcinoembryonic antigen was determined with the aid of Abbott kits as described by the manufacturer.

RESULTS

The results of single analyses performed on sera from 56 patients are presented in Table 1. Sera of the patients before surgery and of those examined within two months after surgery invariably exhibit elevated antibody levels. Normal levels of the antibody were observed only in certain patients examined more than 2 months after surgery.

In the follow-up study, three patients whose sera contained normal levels of lactosylsphingosine-reactive antibody between 3 and 6 months after surgery were still well in March 1981 (11.5, 12 and 18.5 months respectively after surgery). Among 6 patients of this group, but with elevated antibody levels, 3 were also found free of any signs of cancer relapse for 17, 20 and 28 months respectively. In two other patients, metastases to the liver were diagnosed at the 11th and 12th months after surgery. Follow-up data on the 6th patient are not available.

One of the patients with normal titers of lactosylsphingosine-reactive antibody as determined between 7 and 18 months is still well after an additional 22 months (35 months after surgery). All track of the other patient has been lost. Thirteen patients with elevated antibody titers had local cancer recurrence or metastases within 11–29 months (mean 16 months) of surgery. Four of them died. In 10 of these patients the diagnosis of cancer recurrence was made roughly at the time of testing for lactosylsphingosine-reactive antibody, but in 3 patients the positive antibody test preceded other symptoms of cancer by 5, 10 and 18 months respectively. The serum of one patient was positive for increased titer of lactosylsphingosine-reactive antibody at the 11th month after surgery, yet the patient is still well after an additional 14 months. Follow-up data on one patient with a high level of the antibody between 7 and 8 months are not available.

Figure 1 shows the results of serial determinations of the antibody levels in 15 operated patients. In those who were free of any clinical symptoms of cancer recurrence or of metastases, the antibody level declined between 3 and 6 months (Fig. 1, A, B, C, D, E, F, G and M) and stayed within the normal range (Fig. 1, B, E, F and G). Tumor relapse or an unsuccessful operation were always accompanied by elevated titers of the antibody (Fig. 1, H, I, J, K, L, M, N and O). In several instances the elevated antibody preceded other symptoms of cancer recurrence, including high levels of CEA (Fig. 1, H, I, J, K, L and M). In two cases the fall of the level of lactosylsphingosine-reactive antibody was observed after initiation of chemotherapy at the 11th and 44th months after surgery (Fig. 1, H and N respectively). In one patient the antibody level was normal at the 7th month after surgery and then rose again (Fig. 1, M). The patient was operated on a second time and again the antibody declined, but only after an additional 11 months.

In the follow-up study, 6 patients have been found well for 12–26 months after surgery (mean, 20 months) (Fig. 1, B, C, D, E, F and G). These were the same patients who originally exhibited low levels of lactosylsphingosine-reactive antibody between 3 and 6 months after surgery. One patient with low antibody titers relapsed after 19 months (Fig. 1, A). The patient who was operated on twice is still well 32 months after the first surgery (Fig. 1, M).

DISCUSSION

In our previous study we tested for lactosylsphingosine-reactive antibody in 162 normal people and 115 patients with various neoplastic and non-neoplastic diseases [23]. Only those with gastrointestinal cancer (21, 100%), uterine cancer (8, 100%), extensive skin burns (7, 70%) and autoimmune hemolytic anemia (2, 20%) exhibited elevated antibody titers. The sera of 11 patients with breast cancer and 15 patients with ulcerative colitis or gastric and duodenal

ulcers were all negative. Recently we have also examined the sera of 9 patients with lung cancer which all exhibited normal antibody levels (Jóźwiak and Kościelak, unpublished data). Results on patients with other kinds of neoplastic diseases were indecisive, owing to the fact that some of them were treated with cytostatic drugs and the groups were small. Thus our test for lactosylsphingosine-reactive antibody seem to be positive only with certain types of cancer.

Here we examined 154 more sera of 71 patients with colorectal cancer and found them all positive, providing that the tests were performed either before or within two months of surgery. Therefore, our test should be more sensitive than the titer of CEA, which is elevated only in 62% of the unoperated patients.

More than 2 months after surgery, serum samples of certain patients became negative for the presence of high antibody level. The combined data of Table 1 and Fig. 1 suggest that prognosis should be favorable rather for patients with normal levels of lactosylsphingosine-reactive antibody as found within 3 to 6 months of the surgery and beyond this period. Eleven out of 13 patients of this group survived for 12–28 months after operation (mean 19 months) and were still well at the time of the follow-up study. The recurrence of cancer occurred only in two patients. Elevated antibody levels within 3 to 6 months after surgery may not necessarily be a poor prognosis omen. Indeed, 3 out of 11 patients of this group recovered quite well. These findings can be readily explained on the assumption that the rate of decline of the antibody level after successful surgery may vary among different individuals. The remaining 8 patients had later cancer recurrence or metastases. Our data show that high levels of lactosylsphingosine-reactive antibody which persist more than 6 months after the operation are usually associated with cancer recurrence or metastases. Only one out of 22 such patients is presently free of any symptoms of cancer. It is, however, debatable whether this patient should be treated as 'false positive'. His serum was tested for the antibody at the 11th month after surgery. It may be that the level of the antibody in this patient declined at a slower rate or the recurrence of cancer was imminent.

In summary, it may be stated that high levels of the lactosylsphingosine-reactive antibody in both operated and unoperated patients are most likely associated with active tumor growth. Thus our test may be valuable for the detection and monitoring of patients with colorectal cancer. Obviously the test should not be applied to patients subjected to chemotherapy.

The possible value of the test for monitoring operated patients is highlighted by the fact that in about 40% of them the elevated levels of antibody preceded other symptoms of cancer recurrence, including increased concentration of CEA, by 2–18 months (mean 6.5 months).

It should be pointed out that the cause of the formation of lactosylsphingosine-reactive antibodies is unknown. The antigenic stimulus may be provided by lactosylceramide or other antigens [23]. Antibodies to a number of carbohydrate structures are known to occur in normal sera [24].

It would be interesting to know the level of lactosylsphingosine-reactive antibodies in adenomas and polyps of the large bowel, which may be precancerous [25]. We studied only two such patients. One was initially diagnosed as suffering from benign adenoma, and later from adenocarcinoma. The titer of the antibody was already elevated at the first examination. In another patient who had a polyp of the rectum, the level of the antibody was normal. This patient remained in perfect health for 26 months of observation. This problem clearly requires a separate study.

Acknowledgements—We wish to express our gratitude to Prof. Janusz Szymendera and Dr. Janina Kamińska from the Institute of Oncology, Department of Nuclear Medicine, Warsaw, Poland, for supplying samples of sera and valuable discussion. They also determined CEA levels.

REFERENCES

1. MORTON DL, EILBER FR, JOSEPH WL. Immunological factors in human sarcomas and melanoma: a rational basis for immunotherapy. *Ann Surg* 1970, **172**, 740–749.
2. STEPLEWSKI Z, HERLYN M, HERLYN D, CLARK WH, KOPROWSKI H. Reactivity of monoclonal anti-melanoma antibodies with melanoma cells freshly isolated from primary and metastatic melanoma. *Eur J Immunol* 1979, **9**, 94–96.
3. THORPE WP, ROSENBERG SA. Serological analysis of human sarcoma. *Science* 1973, **181**, 440–442.
4. YOUNG WW, HAKOMORI SI, LEVINE P. Characterization of anti-Forssman (anti-Fs) antibodies in human sera: their specificity and possible changes in patients with Cancer. *J Immunol* 1980, **123**, 92–96.

5. WOOD WC, KORNBLITH PL, QUINDEN EA, POLLACK EA. Detection of humoral immune response to human brain tumors. Specificity and reliability of microcytotoxicity assay. *Cancer* 1979, **43**, 86–90.
6. VON KLEIST S, KING M. Detection of circulating antibodies directed against NCA. In: LEHMAN F-G, ed. *Carcino-embryonic Proteins.* Amsterdam, Elsevier 1979, Vol. II, 885–891.
7. GOLD P. Circulating antibodies against carcinoembryonic antigens of the human digestive system. *Cancer* 1967, **20**, 1663–1667.
8. TACHEUCHI S, HIGUCHI M, TAKEUCH Y, TOKUNAGA A, NABESHIMA Y. Immunochemical characterization of antibody-like substance in the sera from the patients with gynecologic malignancies and from pregnant or pregnancy related patients reacted with oncofetal antigen on UCLASO M14 cell mambrane. In: LEHMAN F-G, ed. *Carcino-embryonic Proteins.* Amsterdam, Elsevier, 1979, Vol. II, 873–878.
9. HENLE W, HENLE G, HO HC. Antibodies to Epstein–Barr virus in nasopharyngeal carcinoma, other head and neck neoplasms, and control groups. *J Natl Cancer Inst USA* 1970, **44**, 225–231.
10. HOLLINSHEAD AC, TARRO G. Soluble membrane antigens of lip and cervical carcinomas—reactivity with antibody for Herpes-virus nonvirion antigens. *Science* 1973, **179**, 698–700.
11. WITKIN SS, SARKAR NH, KINNE DW, GOOD RA, DAY NK. Antibodies reactive with the mouse mammary tumor virus in sera of breast cancer patients. *Int J Cancer* 1980, **25**, 721–725.
12. HERBRINK P, MOEN JET, BROUWER J, WARNAAR SO. Detection of antibodies cross-reactive with type C RNA tumor viral p 30 protein in human sera and exudate fluids. *Cancer Res* 1980, **40**, 166–173.
13. YOSHIDA R, ZAWADZKI ZA. Circulating immune complexes in patients with neoplastic disorders. *Oncology* 1980, **37**, 152–156.
14. CHAPUIS B, LOUIS J, BARRELET L, DIALDAS J, DRAGON V, CERROTTINI JC. Circulating antibody complexes. *Schweiz Med Wochenschr* 1980, **110**, 796–801.
15. NELSON DS. Autoantibodies in cancer patients. *Pathology* 1977, **9**, 155–160.
16. ZAULI D, BIANCHI BB, GARDELLI T, VOLTA U, MANCINI AF, PISI E. Smooth-muscle antibodies in children with neuroblastoma. *Cancer* 1980, **46**, 497–499.
17. SPRINGER GF, MURTHY MS, DESAI PR, SCALON EF. Breast cancer patient's cell-mediated immune response to Thomsen–Friedenreich (T) antigen. *Cancer* 1980, **45**, 2949–2954.
18. PACUSZKA T, JÓŹWIAK W, MILLER-PODRAZA H, KOŚCIELAK J. Neutral glycolipid composition in human cancer. *Cancer Biochem Biophys* 1980, **5**, 1–6.
19. SIDDIQUI B, WHITEHEAD JS, KIM YS. Glycosphingolipids in human colonic adenocarcinoma. *J Biol Chem* 1978, **253**, 2168–2175.
20. NARASIMHAN R, MURRAY RK. Neutral glycosphingolipids and gangliosides of human lung and lung tumor. *Biochem J* 1979, **179**, 199–211.
21. KOŚCIELAK J, JÓŹWIAK W, PACUSZKA T, MILLER-PODRAZA H. Glycolipid composition of human cancer and possible role of lactosylceramide as tumor antigen. In: SCHAUER R, ed. *Glycoconjugates. Proceedings of the Fifth International Symposium (Kiel,* 1979). Stuttgart, George Thieme Publishers, 1979, 619–620.
22. JÓŹWIAK W, KOŚCIELAK J. Possibility of using antibodies to lactosylsphingosine in diagnosis of neoplasm. *Acta Haemat Pol* 1979, **10**, 307–310.
23. JÓŹWIAK W, KOŚCIELAK J. Occurrence of lactosylsphingosine-reactive antibodies in sera of cancer patients. *Acta Haemat Pol* 1980, **11**, 173–179.
24. BIRD GWG, ROY TCF. Human serum antibodies to melibiose and other carbohydrates. *Vox Sang* 1980, **38**, 169–171.
25. MORSON BC. Polyps and cancer of the large bowel. In: YARDLEY JH, MORSON BC, ABELL MR, eds. *International Academy of Pathology Monograph. The Gastrointestinal Tract.* Baltimore, Williams and Wilkins, 1977, 101–108.