ABSTRACT

A new monoclonal antibody, CSLEX1, directed against sialylated Lewis^x^ was tested in parallel with a monoclonal antibody, CSLEA1, directed against sialylated Lewis^a^ antigen. In tests with a solid-phase radioimmune sandwich assay, the sialylated Lewis^a^ monoclonal antibody detected sera from certain cancer patients that were negative with the sialylated Lewis^a^ monoclonal antibody. Some sera from cancer patients showed the reverse reaction. We conclude that the combined use of these two monoclonal antibodies detects a wider range of sera from cancer patients than the use of a single antibody alone. It should be possible in the future to use multiple monoclonal antibodies to increase detection.

INTRODUCTION

Sears et al. (12) showed that a monoclonal antibody against the sialylated Lewis^a^ hapten reacted to sera of cancer patients but was essentially nonreactive to sera from normal persons. The sialylated Lewis^x^ antigen is present in various normal tissues, but its presence in sera is diagnostic of malignant growths. Recently, we described a different monoclonal antibody which reacts with the sialylated Lewis^a^ hapten (1) which is present in the sera of cancer patients but is absent in sera from normal persons. Sera from as many as 45% of patients with lung adenocarcinomas reacted positively with the monoclonal antibody CSLEX1 by the reverse passive hemagglutination test.

We wish to describe here tests using a radioimmune sandwich assay and the CSLEX1 monoclonal antibody combined with CSLEA1 which is similar to the monoclonal antibody described by Sears et al. in that it reacts with sialylated Lewis^x^ (2). By testing with the 2 monoclonal antibodies, we have attempted to see whether a different group of sera from cancer patients reacts and whether a higher percentage of the sera from cancer patients can be detected.

MATERIALS AND METHODS

Antibodies. The monoclonal antibodies were prepared according to the method of Kohler and Milstein (7) as described earlier (1). Ascites prepared from various hybridomas were used as starting materials. One ml of ascites was purified by using a Bio-Gel hydroxyapatite column with a high-pressure liquid chromatography system from Bio-Rad. A linear gradient of 0.1 M PO_4^-0.3 mm CaCl_2 (pH 7.0) to 0.3 M PO_4^-0.01 mm CaCl_2 (pH 7.0) was used to eluate the antibodies. The eluted fractions were evaluated for their antibody activity by ELISA. Fractions with the most antibody activity were pooled, and the protein concentration was determined by using the Bio-Rad protein assay.

One-half ml (1 mg/ml) of purified antibodies was labeled with 1 mCi of ^125^I by using the iodogen method (11). The free label was removed by a desalting column of Bio-Gel P-50G (Bio-Rad) and then extensively dialyzed against phosphate-buffered saline.

Sera. Sera samples from 247 cancer patients and 49 healthy persons were kindly supplied by Dr. Steve Lemkin, Dr. Barry Rosenbloom, Dr. Kenneth Tokita, Dr. Lia Katz, Dr. Melvin Avedon, and Dr. Leonard Goldberg. Most of the sera were from Stages III and IV. The sera were ultracentrifuged at 40,000 rpm for 30 min, placed in serum panels, and then frozen at -60°.

Sandwich Assay. The sandwich method was performed in the following manner. Fifty ^125^I antibodies (50 to 100 mg/ml) in sodium bicarbonate buffer (0.1 M, pH 9.6) were added to ELISA wells (Immulon I; Dynatech) for overnight at 4°, and the wells were blocked by 1% bovine serum albumin in sodium bicarbonate buffer for 1 hr at 37°. The wells were then stored at -20° until used. Then, 50 ^125^I of sera (normals or patients) were added to the wells and incubated at 37° for 2 hr. The sera were then aspirated, and the wells were washed with phosphate-buffered saline (0.01 M PO_4^-0.15 M NaCl, pH 7.4) with 0.05% Tween 20. ^125^I-labeled antibodies (-50,000 cpm) were added and incubated for 2 hr at 37°, and the wells were thoroughly washed again. The radiolabeled antibodies bound to the wells were counted by a gamma counter. The results were expressed as a ratio of the cpm of sample to the average cpm of normals for each individual experiment. A sample was considered positive when the value was greater than the mean ± 2 S.D. of all the normal samples tested for that particular antibody.

Inhibition of 19-9 by Antibodies. The inhibitory activity of our monoclonal antibodies against antibody 19-9 was determined by using the Centocor CA 19-9 radioimmunooassay kit (Centocor, Malvein, PA) with a small modification. Just prior to the addition of ^125^I-labeled 19-9 antibody, our antibodies at various concentrations were incubated with plastic beads. The amount of inhibition was calculated according to a decrease in ^125^I binding on the beads.

RESULTS

Specificity of Antibodies. CSLEA1 and CSLEX1 reactivities against target cells (primary gastric adenocarcinoma for CSLEX1 and cultured colon line 205 for CSLEA1) were neuraminidase sensitive by ELISA.

Since the 19-9 antibody of Magnani et al. (8) was directed against sialylated Lewis^a^ antigen, the CSLEA1 antibody was tested for inhibitory activity. Twenty ^125^I of CSLEA1 inhibited 84% of 19-9, suggesting that the epitope was similar but probably not identical. The CSLEX1 antibody did not inhibit 19-9.

Reproducibility of Sandwich Assay. To test the reproducibility of the sandwich assay, the same sera in 2 separate experiments done in triplicate yielded 5150 ± 422 (S.D.) and 5262 ± 926 cpm, with 312 ± 57 and 358 ± 52 cpm for CSLEX1. Similar
results were obtained for CSLEX1.

**Antigen Detection in Sera.** The results of the sandwich assay for antibody CSLEA1 are shown in Chart 1. The 41 sera from normal persons were negative (less than 2 S.D. from the mean). Some sera from rheumatoid arthritis and miscellaneous nontumor patients were slightly positive. The following percentages were positive in the sera from 184 tumor patients: colon, 39%; stomach, 43%; breast, 18%; and lung, 50%. The results of testing the CSLEX1 monoclonal antibody on the sera of 199 cancer patients’ sera are shown in Chart 2. Positive reactions were noted in the following percentages of sera from cancer patients: lung, 34%; colon, 35%; stomach, 19%; and breast, 52%. Among 37 sera from normal persons, one (3%) was positive. However, 3 of 21 (14%) sera from patients with non-neoplastic diseases gave a weak positive reaction.

These 2 monoclonal antibodies were tested for the presence of these antigens in a randomly selected panel of 136 tumor patients. The results are shown in Chart 3. Twenty-two % of the sera were positive to both tumor markers, and with CSLEA1, 16% were positive, and for CSLEX1, 16% were positive. Using a combination of the 2 antisera, 54% of all tumor patients were positive.

**DISCUSSION**

With the aid of monoclonal antibodies, many sugar groups present on gangliosides have been shown to be associated with tumors. For example, the asialo GM1 antigen is present in common acute lymphoblastic leukemia (9). The glycolipid PK antigen is found on Burkitt’s lymphoma cells (10) and on esophageal cancer cells.6

The Lewis* hapten in its sialylated form is of special interest, because this antigen appears in cancer patients’ sera and is absent in sera from normal persons (12). The present study shows that the sialylated Lewis+ hapten, which also has a similar association with tumors (1), reacts with the sera of a different group of cancer patients. Although many of the sera react with both the sialylated Lewis* and sialylated Lewis+ monoclonal antibodies, there were many other sera that reacted with either one or the other antibody. Of particular interest is the extra reactivity observed in sera from breast cancer patients with CSLEX1 antibody as compared with the CSLEA1 antibody. In contrast, with stomach cancers, the CSLEA1 antibody reacted more often by itself than did the CSLEX1 antibody. It is obvious that the 2 antibodies do not produce the same reactions and that their combined use, or eventually mixed as one reagent, should increase the range of sera detectable in cancer patients.

The increase of sialyl transferase activity in the serum of cancer patients is well documented (3). In addition, the special role of sialic acids in cancer has often been reviewed (14, 15, 17). It would appear that the sialylated form of the tumor-associated glycolipid or glycoprotein is present as an abnormal feature in cancer patients. The nonsialylated Lewis* hapten is increased in many cancers (16), and several different investigators report that monoclonal antibodies produce monoclonal antibodies to Lewis* against teratoma cells (13), myeloid leukemia cells (5), and human cancer cell lines (4). One can then postulate that, during the malignant process, these antigens are sialylated and spilled into the sera. The sialylated form of glycoproteins is known to be

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cleared less rapidly from the circulation (6).

From the experiments described, the sialylated Lewis*-specific antibody should be a useful adjunct together with the sialylated Lewis* antibody in the diagnosis of cancer. Although most of the sera in this study have been from patients with cancers of Stages III or IV, it should be possible to increase the sensitivity of the test to detect sera from patients with earlier stages of cancer with the use of the new monoclonal reagents.

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