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Detection of Ovarian Cancer by the Humoral Leukocyte Adherence Inhibition Test Using a Purified Tumor-associated Antigen*

HANS KR. KOTLAR,†‡§ SUZANNE KNAUF,|| JACKSON BEECHAM,|| KJELL KJØRSTAD¶ and TORE SANNER†

†*Laboratory for Environmental and Occupational Cancer and* ¶*Department of Gynecology, The Norwegian Radium Hospital, Oslo, Norway and* ||*Department of Obstetrics and Gynecology and Medical Center, University of Rochester, Rochester, NY, U.S.A.*

Abstract—*The defined, purified ovarian cancer-associated antigen, NB/70K, was used as tumor antigen in the humoral leukocyte adherence inhibition (H-LAI) assay. Bell-shaped dose-response relationships were obtained with increasing concentrations of NB/70K and constant serum concentration from ovarian cancer patients. The concentrations of NB/70K used in this study were 100–250 times less than what had been used in previous H-LAI studies with crude antigen extracts. The response rate of ovarian cancer patients was greatly influenced by the stage of the disease. Thus 75% (6/8) of the patients with disease restricted to the pelvis (stages I and II) reacted while only 20% (5/24) of the patients with more advanced stages (stages III and IV) gave response. Of patients with other gynecological cancers, 17% (1/6) were positive and only 8% (1/13) of patients with other malignant diseases reacted. One of the 11 controls (9%) was found to give positive response. The importance of the present investigation was the finding that a defined, purified, cancer-associated antigen elicited reactivity in the H-LAI assay and that a high sensitivity for detection of early stages of ovarian cancer was obtained with this antigen. The results suggest that TAAs involved in immune recognition of cancer can be identified by the H-LAI assay.*

INTRODUCTION

AN ANTITUMOR immunity test has recently been developed in our laboratory [1, 2]. The experimental procedure of this test has some similarity to that of the leukocyte adherence inhibition assay [3–5]. However, in contrast to the original leukocyte adherence inhibition assay, which appears to give an expression of cellular immunity [3, 4], the new test is based on the use of serum from the patients under study and gives a measure of humoral antitumor immunity. Promising results have so far been obtained with the humoral leukocyte adherence inhibition (H-LAI) test in patients with breast and lung cancer [1, 2]. Of particular interest is the finding that sera from persons who later have developed lung cancer produced a response against lung cancer antigen several years prior to diagnosis [6]. The results obtained suggest that the test may be useful in early detection of cancer.

In previous studies with the H-LAI assay, crude antigen extracts from lung and breast cancer cell lines were used as antigen. Although the sensitivity with these antigens was high, some cross-reactions were found [1, 7]. It was therefore of interest to investigate the use of a purified tumor-associated antigen (TAA) in the test.

TAAs of human ovarian cancer have been purified by several laboratories. Knauf and Urbach [8] have reported a method for the purification of a 70,000 dalton glycoprotein (NB/70K) from ovarian cancer tissue. Significant levels of serum NB/70K have been detected in patients with advanced disease, as measured by radioimmunoassays using both polyclonal [9] and monoclonal [Knauf *et al.*, in preparation]

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*Supported by the Norwegian Cancer Society.
‡Fellow of the Norwegian Cancer Society.
§To whom requests for reprints should be addressed at: Laboratory for Environmental and Occupational Cancer, NHIK, The Norwegian Radium Hospital, Montebello, 0310 Oslo 3, Norway.

anti-NB/70K antibodies. In these studies NB/70K has been demonstrated to have specificity for malignant ovarian tumors. In the present communication the H-LAI test has been used to study ovarian cancer with the NB/70K antigen.

MATERIALS AND METHODS

Patients

Blood samples were obtained from patients admitted to the Norwegian Radium Hospital. The blood samples were drawn the day after admission. The samples were coded before being analyzed. The medical record was examined and the patient's tumor status, histology and treatment were recorded. Blood from controls was obtained from employees of the hospital and from Røde Kors Blodsenter, Oslo.

Cancer antigen

NB/70K was extracted from ovarian cancer tissue with perchloric acid. The extract was subjected to further purification with affinity and immunosorbent columns followed by gel filtration and acrylamide gel electrophoresis as previously described [8, 10].

H-LAI technique

The H-LAI assay has been described in detail previously [1, 2]. Briefly, serum (0.5 μl) from the person under study was mixed with NB/70K (25 and 50 ng protein) and indicator leukocytes (10^6 cells) were added. All substances were dissolved in Eagle's minimum essential medium. The total volume was 200 μl. Trypsinized leukocytes from control persons were used as indicator cells. After incubation for 30 min at 37°C a small sample was transferred to a hemocytometer and incubated for 60 min at 37°C. The cells were then counted, the cover glass removed, the surface washed and the same areas recounted. The LAI index was calculated from the formula:

$$\frac{A_a - A_p}{A_a} \times 100,$$

where A_a and A_p represent the percentage of adherent cells in the absence and presence of antigen respectively. An index above 10 is considered a positive response.

RESULTS

The effect of the concentration of the ovarian cancer-associated antigen, NB/70K, on the H-LAI index has been determined for two patients with cancer of the ovary, for one control and for one patient with cancer of the lung (Fig. 1). Bell-shaped dose-response curves were obtained with serum from patients with cancer of the ovary.

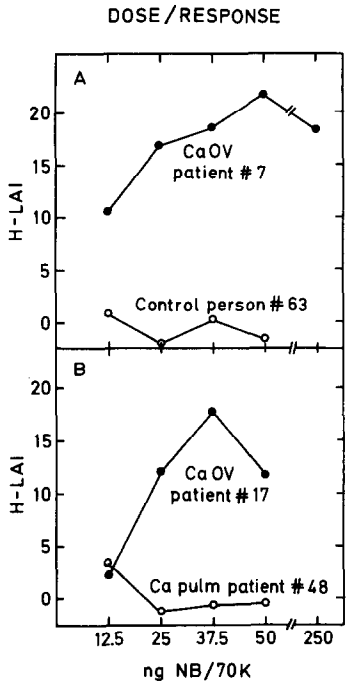

Fig. 1. Effect of antigen concentration on the H-LAI response with serum from patients with cancer of the ovary and a control (A), as well as with serum from a patient with cancer of the lung (B).

With serum from patient 17 a maximum LAI-index was obtained with 37.5 ng NB/70K, while with serum from patient 7 the maximum LAI response occurred at a antigen concentration between 50 and 250 ng. No response was obtained with serum from the control or from the patient with cancer of the lung. On the basis of the results it was decided to use concentrations of 25 and 50 ng NB/70K in the test. These concentrations are 100-250 times less than that previously used in the H-LAI assay with crude antigen extracts.

The results obtained with sera from patients with ovarian cancer as well as with sera from patients with other types of cancer and control persons are shown in Table 1. For most patients with ovarian cancer the response increased with the NB/70K concentration. However, sera from some patients did give a higher response with 25 ng than with 50 ng NB/70K. The fact that in some cases the response was higher at the lower antigen concentration reflects that the concentration for maximum response may differ and that in certain cases the maximum is passed at the higher antigen concentration (e.g. patient 17; Fig. 1).

Using an H-LAI index above 10 as indicative of a response, 11 of the 32 patients (34%) with ovarian cancer responded. The response rate was greatly influenced by the stage of the disease (Fig. 2). Thus 75% (6/8) of the patients with disease restricted to the pelvis (stages I and II) reacted while only 20% (5/24) of the patients with more

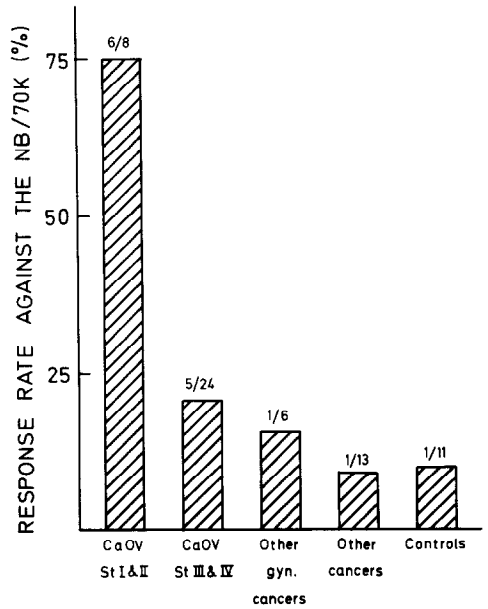

Fig. 2. Response rate in the H-LAI reaction using NB/70K and serum from patients with cancer of the ovary, as well as patients with other cancers and controls.

Table 1. H-LAI response against the ovarian cancer antigen NB/70K

Patients	Stage of disease	NB/70K 25 ng	NB/70K 50 ng	Response
Ovarian cancer				
1	I	3	2	-
2	I	20	1	+
3	II	2	25	+
4	II	24	20	+
5	II	12	21	+
6	II	6	25	+
7	II	16	22	+
8	II	-2	-2	-
9	III	-1	0	-
10	III	11	6	+
11	III	9	8	-
12	III	13	9	+
13	III	2	2	-
14	III	0	-1	-
15	III	7	1	-
16	III	0	0	-
17	III	12	12	+
18	III	-11	6	-
19	III	27	3	+
20	III	8	6	-
21	III	-4	7	-
22	III	31	21	+
23	III	0	-2	-
24	IV	-6	-8	-
25	IV	5	-2	-
26	IV	-16	6	-
27	IV	-1	6	-
28	IV	-8	-7	-
29	IV	10	5	-
30	IV	0	-4	-
31	IV	-12	5	-
32	IV	5	2	-
Other gynecological cancers				
33 fallopian tube		4	10	-
34 endometrial, stage I		30	31	+
35 endometrial, stage I		-1	5	-
36 endometrial, stage IV		2	7	-
37 cervical		-4	2	-
38 pelvis		-13	-19	-
Other cancers				
39 breast		10	7	-
40 ,,		2	2	-
41 ,,		-3	9	-
42 ,,		7	4	-
43 ,,		7	5	-
44 ,,		2	0	-
45 ,,		14	11	+
46 lung		-3	1	-
47 ,,		13	1	+
48 ,,		-8	10	-
49 ,,		2	6	-
50 colon		-3	7	-
51 malignant lymphoma		1	-9	-
Controls				
52		0	-1	-
53		-4	-5	-
54		-3	-3	-
55		1	1	-
56		14	-1	+
57		2	7	-
58		-2	0	-
59		2	1	-
60		4	5	-
61		1	-1	-
62		-5	0	-

advanced stages (stages III and IV) did. Of patients with other gynecological cancers 17% (1/6) were positive, and only 8% (1/13) of patients with other malignant diseases reacted. One of the 11 controls (9%) was found to give a positive response. The present results thus show a high response rate in the early stages of ovarian cancer, while for more advanced stages and for other types of cancer the response rate was low.

DISCUSSION

Symptoms of ovarian cancer are unspecific and early diagnosis is difficult [11]. Therefore the stage distribution of patients with this disease is extremely unfavorable, as 2/3 of them have advanced disease at the time of diagnosis. Serum antigens associated with ovarian cancer have been shown to be present in increased concentrations in more advanced stages of the disease [10]. Significant serum levels of NB/70K were not found in the early stages and the measurement of NB/70K did not seem to be of particular value in detection of early stage ovarian cancer [8-10]. Therefore it was of interest that the response rate

against NB/70K in the H-LAI assay was high among patients with stage I or II disease.

With purified NB/70K as antigen in the H-LAI test, bell-shaped dose-response relations were obtained with serum from ovarian cancer patients. This is in contrast to what we have observed with crude antigen extracts [1, 2]. Crude antigen extracts in high concentrations resulted in an increasing influence of unspecific and cross-reacting antigenic reactions and the response increased continuously with the antigen concentration. Possibly, histocompatibility antigens may also affect the reactions. With the purified antigen, 100–250 times less protein relative to that in the crude extract was needed for a positive reaction. Moreover, the use of the purified antigen resulted in more specific reactions with the same high sensitivity.

In the H-LAI measurements on sera from patients with ovarian cancer, it was found that the response rate decreased in the more advanced cases. This is in agreement with earlier studies using the original LAI assay in breast cancer [12] but is different from studies of lung cancer patients, where it was found that the response rate was about 90% regardless of the stage of disease [13].

The mechanism causing the decreased response rate in more advanced stages of ovarian and breast cancer is not known. One possibility could be the dominance of less immunogenic tumor cell phenotypes in advanced disease. However, at least in the case of ovarian cancer this seems unlikely since the level of NB/70K in serum increases in more advanced cases [10]. A more likely possibility is that the increased concentration of NB/70K could bring about blocking of the H-LAI serum factor.

The results of this investigation show the presence of a serum factor with affinity for the NB/70K antigen in women with ovarian cancer. The importance of this finding is that a defined purified human tumor associated antigen is active in this type of *in vitro* assay of humoral immunity. The results presented here therefore suggest that TAAs, important in immune recognition of cancer, can be identified by the H-LAI assay.

REFERENCES

1. Kotlar HK, Sanner T. Humoral antitumor immune responses in patients with breast cancer measured with the leukocyte adherence inhibition technique. *JNCI* 1981, **66,** 265–271.
2. Sanner T, Kotlar HK, Eker P. Immune responses in lung cancer patients measured by a modified leukocyte adherence inhibition test using serum. *Cancer Lett* 1980, **8,** 283–290.
3. Halliday WJ. Historical background and aspects of the mechanism of leukocyte adherence inhibition. *Cancer Res* 1979, **39,** 558–563.
4. Powell AE, Sloss AM, Smith RN. Leukocyte adherence inhibition: a specific assay of cell-mediated immunity dependent on lymphokine-mediated collaboration between T-lymphocytes. *J Immunol* 1978, **120,** 1957–1966.
5. Thomson DMP, Tataryn DN, Lopez M *et al.* Human tumor-specificity immunity assayed by computerized tube leukocyte adherence inhibition. *Cancer Res* 1979, **39,** 638–643.
6. Kotlar HK, Sanner T, Eker P *et al.* Immune anti-tumor response in the preclinical period of lung cancer. *Eur J Cancer Clin Oncol* 1982, **18,** 317–319.
7. Kotlar HK, Boysen M, Sanner T. A serum immune factor in detection of an occupational group with increased risk for lung and nose cancer. *Eur J Cancer Clin Oncol* 1982, **18,** 957–965.
8. Knauf S, Urbach GI. Identification, purification, and radioimmunoassay of NB/70K, a human ovarian tumor-associated antigen. *Cancer Res* 1981, **41,** 1351–1357.
9. Knauf S, Taillon-Miller P, Helmkamp BF *et al.* Selectivity for ovarian cancer of an improved serum radioimmunoassay for human ovarian tumor-associated antigen NB/70K. *Gynecol* 1984, **17,** 349–355.
10. Knauf S, Urbach GE. Purification of human ovarian tumor-associated antigen and demonstration of circulating tumor antigen in patients with advanced ovarian malignancy. *Am J Obstet Gynecol* 1977, **127,** 705–710.
11. Kottmeier HL. *Annual Report on the Results of Treatment in Gynecological Cancer.* Stockholm, Radiumhemmet, 1982, Vol. 18.
12. Sanner T, Kotlar HK, Eker P *et al.* Early detection of breast cancer by leukocyte adherence inhibition assay. *Cancer Detect Prev* 1983, **6,** 443–450.
13. Kotlar HK, Eker P, Brennhovd I *et al.* Leukocyte adherence inhibition assay in human pulmonary neoplasia. *Eur J Cancer Clin Oncol* 1982, **18,** 141–146.