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Leukocyte Adherence Inhibition Assay in Human Pulmonary Neoplasia*

HANS KR. KOTLAR, PER EKER, IVAR BRENNHOVD, HERMAN HØST, JOHAN HØIE and TORE SANNER†

Norsk Hydro's Institute for Cancer Research, Montebello, Oslo 3 and The Norwegian Radium Hospital, Montebello, Oslo 3, Norway

Abstract—*The hemocytometer leukocyte adherence inhibition technique was used to study cell-mediated immuno-activity of patients with lung cancer. KCl extracts (3.5 M) from the lung cancer cell line Calu-1 and the breast cancer cell line MCF-7 were used as antigens. Of 138 patients with lung cancer, 85% showed a positive response against the Calu-1 antigen. The response was independent of the histological type of the tumor and was the same among untreated patients, patients undergoing different types of treatment and patients who died within 3 months after blood collection. Twenty-five percent of the untreated lung cancer patients also reacted against the breast cancer antigen. Among lung cancer patients undergoing different types of treatment, 36% reacted while 50% of the patients who died within 3 months after blood collection reacted against the breast cancer antigen.*

INTRODUCTION

PROMISING results have been obtained with the leukocyte adherence inhibition (LAI) test in cancer detection. The assay is based on the finding that leukocytes from sensitized individuals show a reduced adherence to glass as compared to leukocytes from non-sensitized individuals when mixed *in vitro* with the relevant antigen [1]. A high degree of specificity has been reported on different types of cancer using various modifications of the LAI assay [2–11]. The hemocytometer LAI test is assumed to give an *in vitro* correlate of cell-mediated immunity. Evidence has been presented that the response is mediated through the release of a lymphokine from T-lymphocytes [12–16].

Carcinoma of the lung is a highly malignant disease and most cases are not resectable when diagnosed. Moreover, many patients develop progressive disease after surgery despite an apparent localized tumor at the time of diagnosis. Except for a very recent study of Thomson and co-workers [17] using the tube LAI test, no systematic study has been reported on the use of the LAI assay in relation to lung cancer.

The purpose of the present work was to study the response of leukocytes from patients with lung cancer in the hemocytometer LAI test. The specificity of the assay was evaluated with antigen extracts from cell lines established from human carcinomas of the lung (Calu-1) and breast (MCF-7).

MATERIALS AND METHODS

Patients

Blood samples from male and female patients with lung and breast cancer were obtained from the Norwegian Radium Hospital. The blood samples were drawn the day after admission to the Hospital. All results presented represent data obtained with leukocytes from different patients. For patients subjected to surgery, radiotherapy, chemotherapy or combined therapy, the time lapse between the last day of treatment and the LAI measurement was at least 3 weeks. Blood from normal donors was obtained from people working in the Hospital and from Røde Kors Blodsenter, Oslo, Norway.

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†Reprint requests to: Dr. Tore Sanner, Laboratory for Environmental and Occupational Cancer, Norwegian Radium Hospital, Montebello, Oslo 3, Norway.

Peripheral blood leukocytes

Twenty milliliters of venous blood were collected from each patient in 500 U of heparin. The mononuclear cells were isolated according to the method of Bøyum [18]. The cells were washed in Eagle's Minimum Essential Medium (EMEM) and, if necessary, hemolyzed with an isotonic Tris-buffered NH_4Cl solution [19]. The leukocytes were subsequently diluted to 20×10^6 cells/ml in EMEM.

Antigen extracts

The immune responses of the leukocytes against lung carcinoma antigen were tested with a 3.5 M KCl extract [20], prepared as previously described [10], of the cell line Calu-1. This cell line was established from a squamous cell carcinoma of the lung by Dr. J. Fogh, Sloan–Kettering Institute, N.Y. A 3.5 M KCl extract of the cell line MCF-7, derived from a pleural effusion of a breast carcinoma patient, was used as breast cancer antigen. MCF-7 cells were provided by Dr. M. Rich, Michigan Cancer Foundation Detroit, MI. The protein concentration of the antigen extracts were determined by the method of Wadell [21].

LAI-technique

The LAI assay was carried out by the method of Halliday and Miller [22] as described by Powell *et al.* [5]. A cell suspension (0.05 ml; 10^6 cells), EMEM (0.10 ml) and the antigen extract (0.05 ml; 7.5 μg protein) were mixed and incubated at 37°C for 30 min in an atmosphere of 5% CO_2 in air. Aliquots were subsequently transferred to a hemocytometer and incubated at 37°C for an additional 60 min in a moist chamber. The cells were counted in two pre-determined squares on each side of the hemocytometer using a Zeiss Microscope (× 10) and an Alpha Omnicon Bausch & Lomb Image Analyzer. About 1600 cells were counted per hemocytometer. The hemocytometers were subsequently immersed in Hank's Balanced Salt Solution (HBSS) to float off the cover slip, and the surface was gently rinsed by immersion and withdrawal. The same squares were counted again. Each analysis was done in duplicate. The number of adherent cells was expressed as a percentage, and the mean of the eight individual percentages (2 duplicates × 4 squares per hemocytometer) was calculated. The number of adherent cells in the absence of tumor extract varied from 80 to 95%. The hemocytometers were cleaned after use, as described by Halliday *et al.* [2].

The results are expressed as:

$$\text{LAI index} = \frac{A_a - A_p}{A_a} \times 100,$$

where A_a is percentage of adherence cells in the absence of antigen and A_p is percentage of adherence of cells in the presence of antigen.

RESULTS

The LAI assays were performed with KCl extracts of Calu-1 cells (lung carcinoma antigen) and MCF-7 cells (breast carcinoma antigen). Figure 1 shows the effect of antigen concentration on the LAI response. It is apparent (part A) that the adherence of leukocytes from the lung cancer patient decreased much more rapidly with increasing concentration of Calu-1 antigen than with MCF-7 antigen. Thus, the LAI index was significantly higher with lung cancer antigen than with breast cancer antigen (part B). From these data, as well as on the basis of other data [10, 23, 24], it was decided to use an antigen concentration in the LAI assay mixture corresponding to 7.5 μg protein.

Figure 2 summarizes the LAI indices obtained with antigen extract from Calu-1 cells and leukocytes from patients with lung cancer and from normal donors. Based on previous studies [5, 10, 23, 24], an LAI response higher than 10 is considered positive. Of the 138 patients with lung cancer, 117 (85%) gave a response higher than 10. The leukocytes from normal donors all gave indices below 10.

A series of LAI measurements was perfor-

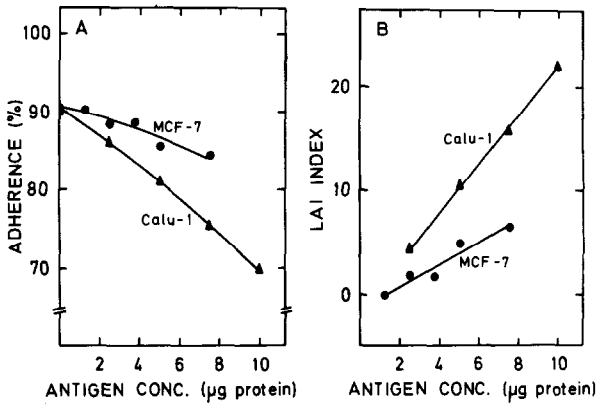

Fig. 1. Effect of antigen concentration on the LAI response of a patient with a squamous cell carcinoma of the lung. A. Leukocyte adherence as a function of antigen concentrations. B. LAI index as a function of antigen concentration. The LAI response was measured with 3.5 M KCl extract from Calu-1 and MCF-7 cells. The protein concentration represents the amount of protein extracts used in each test.

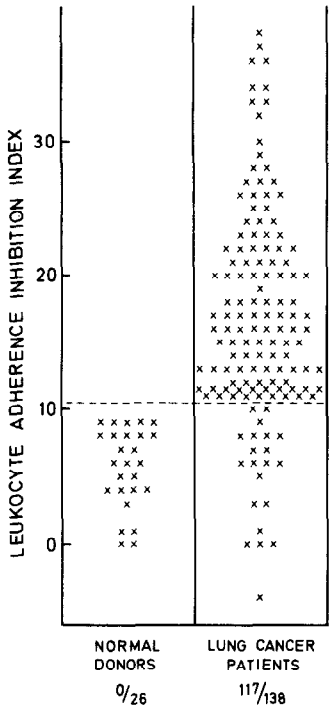

Fig. 2. LAI reactivity of leukocytes from lung cancer patients and from normal blood donors. The response was measured with a 3.5 M KCl *extract of Calu-1 cells.*

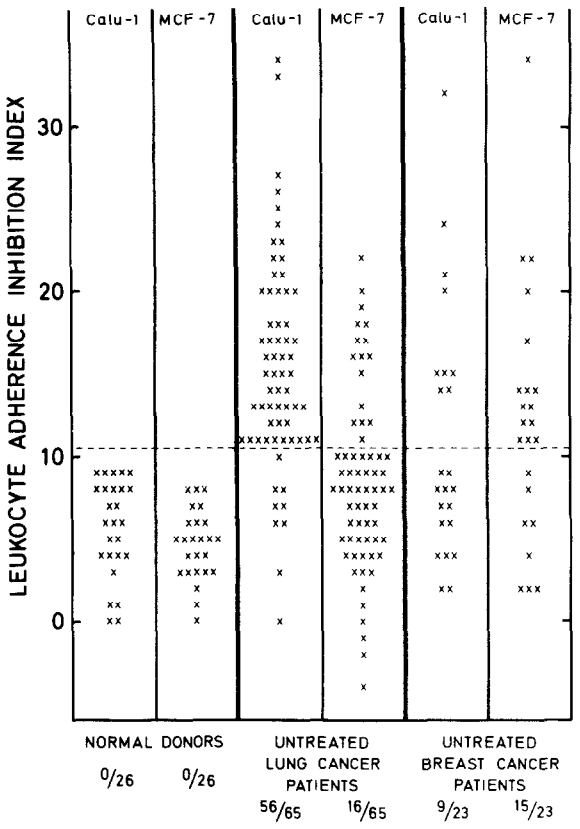

Fig. 3. LAI reactivity of leukocytes from untreated patients with lung and breast cancer and from normal blood donors. The responses were measured with 3.5 M KCl *extracts from Calu-1 cells and MCF-cells.*

med using both lung and breast carcinoma antigens and leukocytes from untreated lung and breast cancer patients, as well as from normal donors (Fig. 3). Among the untreated lung cancer patients, 86% reacted against lung cancer antigen while only 25% reacted against breast carcinoma antigen. None of the lung cancer patients who were negative against Calu-1 antigen reacted against MCF-7 antigen. In the case of untreated breast cancer patients, 65% reacted against MCF-7 antigen and 39% was also positive with Calu-1 antigen. None of the normal donors reacted against the two antigens used.

Since the Calu-1 cell line was established from an epidermoid cell carcinoma of the lung, it was of interest to determine whether the LAI reactivity of the patients would be restricted by the histological subclasses. Table 1 shows the scores of positive response of untreated lung cancer patients with different histological types of the tumor. Based on the experimental data it is concluded that the reactivity against both Calu-1 and MCF-7 antigen was the same for epidermoid cell carcinoma, small cell anaplastic carcinoma, large cell carcinoma (only 3 patients) and adenocarcinoma.

Studies have been carried out to evaluate the role of different treatment of the lung cancer patients on the LAI response. It is apparent from Table 2 that patients treated by surgery, radiotherapy, chemotherapy or combined therapy showed the same LAI response as untreated patients against Calu-1 antigen. However, the response to MCF-7 antigens was increased (36%) after the therapy as compared to the response of untreated patients.

The data in Table 3 show the LAI response obtained with leukocytes from lung cancer patients who died less than three months after collection of the blood samples and for patients who, in addition to lung cancer, also had another type of cancer. It is apparent that the response of these patients against Calu-1 antigen is the same as for untreated patients. On the other hand, the percentage of positive response against MCF-7 antigen was significantly higher (50%, $P = 0.05$) among the patients who died within three months than for untreated patients and for patients undergoing different types of treatment. Moreover, of the 6 patients who had an additional cancer and reacted against the Calu-1 antigen, only one did not respond to the MCF-7 antigen.

*Table 1. LAI responses of leukocytes from patients with untreated lung cancer of different histological types**

Antigen	Histological type			
	Epidermoid cell carcinoma	Small cell anaplastic carcinoma	Large cell carcinoma	Adenocarcinoma
Calu-1	33/37(89)†	11/15(73)	3/3(100)	6/7(86)
MCF-7	10/35(29)	2/15(13)	2/3(67)	1/7(14)

*All blood samples were collected the day after admission of the patients to the Hospital. The histological diagnoses are based on histopathologic examination according to established guide lines.
†Number of patients with LAI index above 10/number of patients tested. The number in parentheses gives the percentage of patients with positive LAI response.

*Table 2. LAI response of leukocytes from lung cancer patients subjected to different types of treatment**

Antigen	Treatment			
	Surgey	Radiation therapy	Chemotherapy	Combined therapy
Calu-1	6/6(100)†	32/38(84)	21/25(84)	9/11(82)
MCF-7	2/6(33)	13/38(34)	10/23(43)	3/11(27)

*All blood samples were collected the day after admission of the patients to the Hospital. The patients were tested at least 3–4 weeks after surgical resection. Patients receiving chemotherapy or radiation therapy were tested no sooner than 3 weeks following their last dose.
†Number of patients with LAI index above 10/number of patients tested. The number in paretheses gives the percentage of patients with positive LAI response.

*Table 3. Effect of different factors on the LAI response of leukocytes from patients with lung cancer**

Antigen	Death within 3 months after blood collection	Additional cancer
Calu-1	20/24(83)†	6/7(86)
MCF-7	12/24(50)	5/7(73)

*Blood samples were collected the day after admission of the patients to the Hospital. Patients with additional cancers include patients with cancer of the prostate, cancer of the bladder or chronical lymphatic leukemia besides cancer of the lung.
†Number of patients with LAI index above 10/number of patients tested. The number in parentheses gives the percentage of patients with positive LAI response.

DISCUSSION

The present results show that about 85% of the patients with lung cancer gave a positive reaction in the LAI assay to a crude extract from the cell line Calu-1 derived from an epidermoid cell carcinoma of the lung. The response was not related to the histological type, the treatment or the stage of the disease. This latter finding is in contrast to recent results on lung cancer by Thomson *et al.* [17] using the tube LAI assay. About 25% of the untreated patients with lung cancer also reacted against an extract from the breast carcinoma derived cell line, MCF-7.

The finding (Table 1) that patients with lung cancer of different histological types all reacted to the same extent in the LAI assay against antigen extracts from the cell line Calu-1 is in

agreement with previous results obtained with the tube LAI [17], as well as with the migration inhibition technique [25]. This finding suggests that various types of lung carcinoma share common tumor antigens. It is of interest that the extent of non-specific reaction against the breast carcinoma antigen seemed to depend on the dissemination of the disease. Thus, while 25% of the untreated lung cancer patients reacted against breast carcinoma antigen (Fig. 3), 36% of the patients undergoing treatment (Table 2) and 50% of the patients that died within 3 months after blood collection (Table 3) showed non-specific reaction.

The mechanism underlying the non-specific reaction is not known. The possibility that weak, common tumor-associated antigens exist in different types of cancer should be considered. Impairment of the immune system due to treatment and also the general condition of the advanced cancer patients may possibly result in more non-specific reactions against a variety of different antigens. Thomson *et al.* [26] argue that the increase of non-adherent cells in the presence of non-specific antigens found with leukocytes from patients with disseminated disease may be due to binding of tumor antigen *in vivo* to the responsive cells.

Several authors, using other immunological methods, have also obtained non-specific immune reactions which seem to some extent to depend on the type of cancer studied [27–31]. Thus, it has been reported that breast cancer patients react against a variety of different antigens, while lung cancer patients seem to show a much higher degree of specificity [24, 26]. This is supported by our observations, showing that only 25% of the untreated lung cancer patients reacted against breast carcinoma antigen while 39% of the untreated breast cancer patients reacted against lung carcinoma antigen (Fig. 2). It is also of interest that non-specific reactions are only observed among patients who also react against the tumor antigen of their own type. Thus, none of the patients with lung cancer who showed a negative response against lung carcinoma antigen reacted against breast carcinoma antigen. Similar results have been obtained with breast cancer patients.

In previous studies of women with breast cancer [10] we found that cellular anti-tumor immune response against breast carcinoma antigen appears to be related to risk factors for development of breast cancer. A similar study will be carried out concerning risk factors for development of lung cancer. The present results indicate that the LAI assay will be a promising tool in detection of lung cancer.

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