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Letter to the Editor

Immune Anti-tumor Response in the Preclinical Period of Lung Cancer

H. KR. KOTLAR,* T. SANNER,†* P. EKER,* A. PIHL,‡ H. ØRJASÆTER,‡ S. HARVEI,‡ L. THEODORSEN‡ and E. JELLUM‡

**Laboratory for Environmental and Occupational Cancer, NHIK, Radiumhospitalet, Oslo 3, Norway, and ‡The JANUS Committee, The Norwegian Cancer Society, Huitfeldtsgt. 49, Oslo 2, Norway*

RECENTLY, we have developed a new test based on the detection of an anti-tumor immune factor present in the serum of cancer patients [1, 2]. In this humoral leukocyte adherence inhibition (H-LAI) test, serum from the person under study is mixed with the relevant antigen, incubated with trypsinized leukocytes from healthy blood donors and the reduction in the adherence of these leukocytes to a glass surface is subsequently measured. With the H-LAI test we have found positive responses in 70–90% of patients with manifest breast and lung cancers, using breast cancer and lung cancer antigen respectively [1, 2].

In 1973 the Norwegian Cancer Society started the JANUS project [3, 4], in which serum samples from healthy blood donors were collected and stored in the frozen state. The purpose of this ongoing project is to provide premorbidity blood samples for examination of early indicators of disease. During the first seven years, four of the blood donors have developed lung cancer. In this preliminary report it is demonstrated that, in three of these patients, the H-LAI test was positive in sera collected even several years before the cancer became manifest.

Serum specimens were obtained from the JANUS serum bank [3, 4], where the samples had been stored for up to seven years at −25°C. Fifteen coded serum samples from the four lung cancer patients and from age- and sex-matched controls were provided for H-LAI testing.

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†Mailing address: Dr Tore Sanner, Lab Environ and Occup Cancer, NHIK, Radiumhospitalet, Montebello, Oslo 3, Norway.

Extracts (3.5 M KCl) of the human lung cancer cell line Calu-1 (from Dr. J. Fogh, Sloan Kettering Institute, NY) and the breast cancer cell line MCF-7 (from Dr. M. Rich, Michigan Cancer Foundation, Detroit, MI) were used as antigens [5, 6]. The H-LAI test was carried out as previously described [1, 2]. Briefly, serum (0.5 μl) and antigen (5 μg protein) are incubated in a total volume of 150 μl Eagle's Minimum Essential Medium at 4°C for 1 hr. Leukocytes from healthy blood donors (1×10^6 in 50 μl), pretreated with trypsin, are added to the above mixture and incubated for 30 min at 37°C. Aliquots of the cell suspension are subsequently transferred to hemacytometers and incubated for another hour at 37°C. At the end of the incubation the cells are counted prior to and after removal of the non-adherent cells by gently rinsing. The response of the test is expressed in the LAI index:

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

where A_a and A_p represent the percentage of adherent cells in the absence and presence of antigen respectively. On the basis of previous experience, an LAI index greater than 10 is taken as a positive test [1, 2, 5, 6].

The results are shown in Table 1. In three of the four patients the LAI index was greater than 10 when tested against the lung cancer antigen. In the six samples from these three patients only one sample gave a positive response with the breast cancer antigen, which was used as a test of the specificity of the assay. The samples from the matched controls were all negative.

The data on these three patients show two

Table 1. H-LAI measurements on sera collected prior to development of lung cancer

		Patients				Control persons*	
				LAI index		LAI index	
No.	Sex	Age at time of diagnosis	Time from serum coll. to diag. (months)	Lung cancer antigen	Breast cancer antigen	Lung cancer antigen	Breast cancer antigen
1	M	58	58	21	8	3	7
			47	12	14	1	2
			30	15	8	5	2
2	M	41	29	7	6	0	−1
			23	26	9	4	5
3	F	54	13	17	9	5	3
4	M	58	47	−1	3	11	15
			23	2	3	NR†	NR

*Sera from age- and sex-matched control persons were tested together with sera of the pateints. The sera had been drawn at approximately the same time as in the corresponding cancer patients. All the analyses were performed in a blind manner.

†Sera samples were not received.

interesting features. In patient 1 the test was clearly positive in a sample drawn 58 months before the diagnosis was established, suggesting a long, subclinical development of the disease. In patient 2, a young man, 41 years of age at the time of the diagnosis, the first sample taken 29 months before diagnosis was negative, whereas the second sample, drawn 6 months later, was positive. Thus, in this case the immunological response appeared within a well-defined time period. All the three LAI-positive patients suffered from large cell carcinoma of the lung.

The fourth patient had a negative LAI index in the two samples collected at different times. By coincidence, the sex- and age-matched control had a positive response both against the lung and the breast cancer antigen. Since we have no clinical information about this control person (except that he had no registered cancer), no explanation can be afforded for this positive response.

Patient 4 had a lung tumor, and the biopsy showed adenocarcinoma. The different histology in this patient cannot explain the negative reaction, since in a separate study [5] it has been found that the response rate against the Calu-1 antigen is the same for the major histological types of lung cancer. The patient was treated with irradiation and the lung tumor disappeared. He died from symtoms of brain metastases, but on autopsy no metastases or lung tumor was found. It is apparent from the patient's record that the pathologists had been unable to ascertain from the biopsy the origin of the tumor. The possibility must therefore be considered that this lung tumor may have been secondary.

A number of tests are now available where the presence of different tumor markers in the blood is assayed. The present test is based on an entirely different principle, viz. the presence of a specific humoral anti-tumor immune factor. To our knowledge this is the first example where a positive humoral immune response has been detected in cancer patients long before the appearance of clinical symptoms. Although the material presented admittedly is small, we feel that it is still worth reporting as the results are rather striking and seem to open new possibilities. If the results can be confirmed in a larger material, the H-LAI test may have obvious practical applications.

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