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A Serum Immune Factor in Detection of an Occupational Group with Increased Risk for Lung and Nose Cancer

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Abstract—*Epidemiological studies have demonstrated that workers in the nickel refinery industry have an increased risk for respiratory tract carcinoma. In the present study, serum from 51 workers at a Norwegian nickel refinery have been tested against lung, nasal and breast carcinoma antigens in the humoral leukocyte adherence inhibition test. The breast cancer antigen was used as a non-specific antigen. The frequency of positive response against the lung carcinoma antigen was significantly higher among the refinery workers (21/51) than in the controls (3/17) (P = 0.07). Moreover, among workers employed for 10 years or more, the response was higher than found for workers with shorter employment. Of the nickel workers with nasal dysplasia, 56% (15/27) gave a positive reaction against the lung carcinoma antigen compared to 25% (6/24) of the workers without dysplasia (P = 0.03). The same trends were also found for the nasal carcinoma antigen. The study gives further support for the usefulness of the humoral leukocyte adherence inhibition test in identification of individuals with an increased risk for developing cancer.*

INTRODUCTION

CONSIDERABLE effort has currently been centered on the development of tests for early diagnosis of cancer and for identification of persons at high risks for cancer. These tests are either based on the detection of specific proteins released from the tumors [1–5] or on assays which may reflect antitumor immunity [6–11].

Recently, a new test, the humoral leukocyte adherence inhibition assay (H-LAI) for detection of a humoral immune factor in sera of cancer patients, has been developed [12, 13]. The serum factor responsible for the observed reaction is antigen-specific, i.e. it is directed against and forms stable complexes with the cancer-related antigen [14, 15].

Promising results have been obtained with the new H-LAI assay in studies on patients with breast and lung cancer. With serum from these two types of cancer, a response rate of 80–90% has been obtained [12, 13]. Of particular interest is the demonstration of positive reactions on serum samples drawn up to five years before the clinical diagnosis of lung cancer [16]. This finding suggests that specific cancer-related serum immune factors can be detected even in preneoplastic stages.

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For further evaluation of the H-LAI assay, it was of interest to study the possible response in sera from a group of persons at a high risk of developing cancer. Workers occupationally exposed to nickel have a high risk for nasal and pulmonary carcinoma [17–21]. The development of these malignancies are probably preceded by epithelial dysplasia [22–24].

In the present study, sera from nickel refinery workers with nasal dysplasia were tested against lung and nasal carcinoma antigens and the results compared to groups of workers without nasal dysplasia, controls and patients with nasal carcinoma. A breast cancer antigen was used as a nonspecific antigen.

MATERIALS AND METHODS

With the exception of 4 persons with nasal carcinoma, all individuals participating in this study were or had been employed in Falconbridge Nickelverk A/S, Kristiansand, Norway. Two groups (I, II) of nickel-exposed workers were selected and compared to a group (III) of age-matched, non-exposed individuals and patients with verified nasal carcinoma (IV). None of the control persons or nickel workers had any known malignancies when the study was started; neither had they developed any

malignant tumors one year following the sampling of blood and nasal mucosa specimens.

Clinical material

I. Short-term employees. This group comprised 18 randomly selected persons employed for 6–10 years and not previously biopsied.

II. Long-term employees. These were 33 randomly selected active workers employed for more than 10 years. In 25 of these, previous nasal biopsies showed epithelial dysplasia. With the exception of 2 persons, the dysplasia had been verified in 2 or more biopsies. This group further included 8 workers without noticeable nasal dysplasia in two or more biopsies.

III. Controls. The controls were made up of 17 randomly selected office workers not previously involved in the refinery process.

IV. Persons with cancer nasi. Group IV was made up of 6 patients with verified nasal carcinoma. The histological type were in all cases squamous cell carcinoma. Two of the patients had an occupational history of nickel exposure.

Collection of samples

Biopsy specimens. Biopsies were taken from the anterior curvature of the middle nasal turbinate in local anesthesia (xylocain 2% and adrenalin 0.1%). In patients with malignant tumors several biopsies were taken from the tumor. All specimens were fixed in buffered 6% formaldehyde (pH 7.4), embedded, sectioned and stained with hematoxylin–eocin. Histological grading and scoring was performed according to the concept of gradual epithelial transformation as outlined by Torjussen *et al.* [22, 23]. Pseudostratified epithelium was scored as 0, metaplasia scored as 1–4, dysplasia as 5 and carcinoma 7.

Serum samples. Blood was drawn on the same day as biopsy sampling. The serum was immediately frozen at −25°C. All sera were coded.

Clinical information

Occupational history, including duration of nickel exposure, smoking habits, alcohol consumption, medication, and previous and present illnesses, in particular, allergic diseases and chronic lung diseases, were evaluated from a questionnaire and interview. Two persons with allergic diseases were omitted from the material due to corticosteroid medication at the time of investigation.

H-LAI analyses

Indicator cells. Blood from normal, healthy blood donors was purchased from Røde Kors Blodcentral, Oslo. The mononuclear cells were separated according to the method of Bøyum [25]. The cells were washed and treated with 0.025% trypsin as described elsewhere [12, 13].

Freezing procedure. With minor modifications, the procedure described by Oldham *et al.* [26] was used. Briefly, to a suspension of freshly prepared trypsin-treated indicator cells [10^7 cells/ml in Eagle's Minimum Essential Medium (MEM)] equal volumes of a freezing solution [20% dimethylsulphoxide (DMSO) and 20% fetal calf serum (FCS) in MEM] were added dropwise and the mixed solution was left on ice for 10 min before freezing at a rate of approximately −1°C/min. The cells were stored at −80°C and used within one month.

Thawing procedure. The tubes were melted at 37°C until the last ice crystals disappeared. They were then placed on ice for 2 min. Doubling volumes of a room temperature thawing solution (20% FCS in MEM) were added at 1-min intervals until a DMSO concentration of 4% was achieved. After 5 min on the bench the cells were washed in Hank's Balanced Salt Solution and subsequently in MEM, and reconstituted in MEM to a final concentration of 2.5×10^7 cells/ml. Viability was tested by trypan blue incorporation and varied between 92 and 98%.

Cancer antigens. Potassium chloride (3.5 M) extracts of the cell lines Calu-1 and MCF-7 were used as lung and breast cancer antigens. The Calu-1 cell line had been isolated from a squamous cell carcinoma of the lung by Dr. J. Fogh, Sloan Kettering Institute, New York. The MCF-7 cell line was derived from pleural effusion of a breast carcinoma and was kindly provided by Dr. M. Rich, Michigan Cancer Foundation, Detroit. Nasal cancer antigen was prepared by KCl extraction from a squamous cell carcinoma of a former nickel worker.

H-LAI technique. The H-LAI test was performed without access to clinical and histological information following the procedure described by Kotlar and Sanner [12]. In brief, serum (0.5 μl) and antigen (5 μg protein) were incubated in a total volume of 150 μl of MEM at 4°C for 1 hr. Cryopreserved trypsin-treated indicator cells (10^6) were added to the above mixture and incubated for 30 min at 37°C. Aliquots of the cell suspension were subsequently transferred to hemocytometers and incubated for another hour at 37°C. At the end of the incubation the cells were counted in nine predetermined squares on each side of the hemocytometer. Between 4000 and 5000 cells were counted by the use of an image analyser. The cover glass was removed, the surface was gently rinsed to remove nonadherent cells and the

same squares were recounted. Each test was performed in duplicate. The response of the test is expressed by 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 was taken as a positive test [12, 13].

The adherence of the cryopreserved indicator cells was found not to be as reproducible as freshly made cells. Due to this fact, a standard normal serum was tested together with each series of analyses. The LAI indices obtained with this serum against the relevant antigens were subtracted from the LAI indices found for the serum under investigation.

Statistics. Contingency tables (two-way classification) with chi-quadrate statistics were used for evaluation of the results.

RESULTS

Table 1 presents the age, years of employment at the nickel refinery, the histological score of the nasal biopsies and the H-LAI indices for all participating persons. The same samples were tested against lung, nasal and breast carcinoma antigens. The breast carcinoma antigen was included in the study as a nonspecific antigen.

The H-LAI responses are summarized in Table 2. Both for the lung and nasal carcinoma antigens, the percentage of positive responses was higher in the nickel workers than in the controls. A still higher response rate was found in persons with nasal carcinoma. Interestingly, the H-LAI response rate was higher among long-term employed workers than in short-time employees. The finding that the sera from 5 of the 6 patients (83%) with nasal cancer reacted with the nasal carcinoma antigen shows that this antigen has a high sensitivity. However, the specificity was less satisfactory since 24% of the controls also responded.

Three of the 17 control persons (18%) showed a positive response against lung carcinoma antigen. This response rate was considerably higher than found in previous studies [12, 13]. Two factors may contribute to this discrepancy. All except two of the control persons were smokers. Moreover, it should also be noted that in the present study cryopreserved lymphocytes were used as indicator cells. A greater variation and a slightly higher response rate have been observed with cryopreserved cells compared with freshly prepared indicator lymphocytes.

The percentage of positive responders against the breast carcinoma antigen found for the long-term nickel workers was higher than among the controls and the short-term employees. As most of the persons who reacted against the breast carcinoma antigen also showed reaction against both the lung and nasal carcinoma antigens (see Table 1), the response could be due to shared common cancer-associated antigenic determinants between the nasal, lung and breast cancer antigens. It should also be stressed that crude antigen preparations were used. These preparations contain most of the surface proteins of the tumor cell, including histocompatibility antigens.

The percentage of positive responses in controls and in nickel workers was similar when comparing persons over and under 50 years of age (Table 3). Thus there is no reason to assume that the difference in response rate between short and long-term employees could be caused by differences in age distribution.

The H-LAI responses in nickel workers with nasal epithelial dysplasia were compared with the results obtained with nickel workers without dysplasia (Table 4). While only 25% of the nickel workers without dysplasia reacted against the lung carcinoma antigen, the percentage of positive responders among the nickel workers with dysplasia was 56%, which is significantly higher ($P = 0.03$). Similarily, the response rate against the nasal carcinoma antigen was higher among nickel workers with nasal dysplasia than without dysplasia.

DISCUSSION

Epidemiological studies have demonstrated that workers in the nickel refinery industry have an increased risk for respiratory tract carcinoma [17–21]. For men employed in roasting/smelting and electrolyses departments in a nickel refinery in Norway for more than 3 years, the ratio of observed to expected was 7 : 1 for lung cancer and 40 : 1 for nasal cancer [18]. The risk of nasal cancer increased with the duration of employment, being 168 : 1 for workers employed for 25 years or more [27]. While lung cancer among Norwegian men is approximately 26 times as frequent as nasal cancer, lung cancer in nickel workers exceeded nasal cancer by a factor of 3.5 only.

As part of a preventive health program, histological examination of nasal biopsies has been made on a large scale since 1976 [22, 23, 28]. From these studies it is concluded that nickel

Table 1. H-LAI responses and histological score of nasal biopsies

Subject No.	Age	Years of employment	Histological score	LAI index Lung carcinoma antigen	LAI index Nasal carcinoma antigen	LAI index Breast carcinoma antigen
			Controls			
701	50	0	1	6	6	5
702	52	0	3	8	6	7
703	62	0	1	18	10	9
704	57	0	2	9	20	10
705	60	0	1	9	10	7
706	29	0	1	6	6	5
710	47	0	2	17	20	10
711	42	0	0	10	10	3
712	52	0	3	5	14	6
713	66	0	3	5	3	2
715	52	0	2	14	12	11
716	52	0	0	2	5	0
717	52	0	1	8	6	8
721	50	0	0	9	9	10
732	51	0	1	1	4	4
733	54	0	1	10	3	8
734	36	0	2	9	4	4
			Short-term employees			
610	64	8	5	21	12	12
611	38	6	1	14	14	8
612	31	8	1	16	14	9
613	28	7	3	6	5	2
621	51	9	3	6	3	9
622	34	8	2	3	5	9
623	33	7	2	6	10	6
625	58	9	3	9	4	6
626	33	8	3	20	11	5
629	29	8	0	6	7	7
630	35	10	0	17	3	4
631	27	8	3	6	−6	9
632	27	7	2	2	0	3
634	36	7	4	9	9	9
640	53	8	5	8	4	6
643	36	8	2	10	6	4
644	36	10	2	3	12	0
645	30	10	0	6	5	3

			Long-term employees			
123	62	28	5	17	28	16
133	52	26	5	8	18	5
415	54	26	3	6	0	−3
419	58	13	5	12	10	2
435	58	30	3	9	8	−8
447	45	14	3	10	7	0
448	53	26	3	8	5	0
455	45	22	4	6	8	0
466	49	14	4	35	1	11
605	46	22	5	15	11	15
624	52	29	0	12	15	5
638	29	22	1	9	13	5
694	64	30	5	11	12	13
696	54	26	5	18	6	9
697	59	20	5	15	12	0
698	51	17	5	12	5	6
700	70	25	5	2	3	0
707	52	29	5	4	10	9
708	67	29	5	−1	0	7
709	49	27	5	5	2	2
714	53	26	5	9	3	2
718	67	11	5	20	18	22
719	62	32	5	18	2	3
720	57	30	5	15	7	8
722	47	27	5	19	12	10
724	46	22	5	17	11	8
725	39	16	5	6	3	5
726	46	24	5	7	5	6
727	42	17	5	1	4	5
728	51	28	5	5	1	13
729	43	16	5	20	11	5
730	64	28	5	6	4	1
735	48	29	5	13	6	10
			Persons with nasal cancer			
320	57	0	7	11	11	−1
384	68	34	7	10	9	0
440	72	0	7	4	13	1
441	60	0	7	18	18	2
442	83	0	7	25	20	10
776	77	28	7	14	13	18

Table 2. H-LAI responses in nickel refinery workers and persons with lung cancer

Group	No. of subjects	Mean age (range)	LAI index Lung carcinoma antigen	Nasal carcinoma antigen	Breast carcinoma antigen
Controls	17	51 (29–66)	3/17 (18%)	4/17 (24%)	1/17 (6%)
Nickel-exposed workers	51	48 (27–78)	21/51 (41%)	16/51 (31%)	7/51 (14%)
Short-term employment	18	38 (27–64)	5/18 (28%)	5/18 (28%)	1/18 (6%)
Long-term employment	33	54 (39–78)	16/33 (49%)	11/33 (34%)	6/33 (20%)
Persons with nasal cancer	6	70 (57–83)	4/6 (67%)	5/6 (83%)	1/6 (17%)

refinery workers have a high frequency of epithelial dysplasia and that these lesions should probably be interpretated as preneoplastic. Cytological studies have shown that preneoplastic epithelial changes due to nickel exposure also occur in the bronchial epithelium [24].

Recent cytogenetic studies showed that nickel workers have an increased incidence of sister chromatid exchange compared to nonexposed persons [29]. In the present study attempts have been made to use immunological methods in identifying a group with increased risk of respiratory tract carcinomas.

The original hemocytometer leukocyte adherence inhibition test has proved useful in the detection of women with increased risks of breast carcinoma [30]. Previous studies with the H-LAI assay have shown that humoral immune reactivity against cancer antigen can be demonstrated several years before the clinical diagnosis. Thus in 3 out of 4 cases of lung cancer, positive response has been obtained in the H-LAI assay up to 5 years prior to cancer diagnosis [16]. In the present study, 56% of the nickel workers with nasal epithelial dysplasia reacted against lung carcinoma antigen and 37% against nasal carcinoma antigen. The higher reaction rate against lung carcinoma antigen may be related to the higher probability of developing lung cancer than nasal cancer. Since none of the persons participating in the study had developed cancer one year following the sampling of blood, the observed positive H-LAI response may be due to the presence of preneoplastic lesions.

The finding that 4 out of 6 (67%) patients with nasal carcinoma reacted against lung cancer antigen might in part be explained by the high risk of these persons of developing lung cancer. Thus nasal epithelial dyplasia in the nickel refinery worker may occur simultaneously with similar changes in the lung. The fact that 3 workers with nasal dysplasia have developed lung cancer gives support to this hypothesis (Torjussen, personal communications). The high reaction rate with the lung cancer antigen may also suggest cross-reactivity between the lung and nasal carcinoma antigens. In previous studies we have found cross-reactivity between lung and breast cancer antigens [12–13]. There are several hypotheses concerning the existence of cancer-related antigens in humans. Common cancer-related antigens have also been suggested in different types of cancer [31]. It is of interest that the reaction against the breast carcinoma antigen, which was included in the study as a non-specific antigen, was significantly higher in the group of workers with the greatest probability of developing cancer and that most of the persons who reacted against the breast carcinoma antigen also reacted against the lung and nasal carcinoma antigens.

The present work shows that there is a higher response rate to lung cancer antigen in the long-term employees when compared to controls ($P = 0.03$) and short-term employees ($P = 0.15$). Interestingly, the response rate was significantly higher among nickel workers with nasal epithelial dysplasia than in those without ($P = 0.03$). Although not statistically significant, the same trend was found for the nasal carcinoma antigen. The results support the hypothesis that changes take place in the bronchial epithelium in the preneoplastic stage, with appearance of antigenic determinants shared with those found in lung carcinoma. Moreover, the present data strengthen the usefulness of the H-LAI test for identification of individuals with an increased risk of developing cancer.

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Table 3. H-LAI response according to age groups

Group	No. of subjects	LAI index: Persons < 50 years: Lung carcinoma antigen	Persons < 50 years: Nasal carcinoma antigen	Persons < 50 years: Breast carcinoma antigen	Persons > 50 years: Lung carcinoma antigen	Persons > 50 years: Nasal carcinoma antigen	Persons > 50 years: Breast carcinoma antigen
Controls	17	1/6 (17%)	1/6 (17%)	0/6 (0%)	2/11 (18%)	2/11 (18%)	1/11 (9%)
Nickel-exposed workers	51	10/26 (38%)	8/26 (31%)	2/26 (8%)	11/25 (44%)	8/25 (32%)	5/25 (20%)

Table 4. H-LAI response on persons with or without dysplasia

Group	No. of subjects	Mean age (range)	LAI index Lung carcinoma antigen	Nasal carcinoma antigen	Breast carcinoma antigen
Nickelworkers without dysplasia	24	41 (27–64)	6/24 (25%)	6/24 (25%)	1/24 (4%)
Nickelworkers with dysplasia	27	54 (43–78)	15/27 (56%)	10/27 (37%)	6/27 (22%)

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