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**DISCUSSION**

**Dr. Rasp:** We found that the nylon adherence of alveolar macrophages from cigarette smokers is uniformly decreased. Did you quantitate adherence of smokers' alveolar macrophages to the glass coverslips? Could you correlate the increased percentage of flat cells from smokers with any alteration of phagocytic or adherence activity?

**Dr. Davis:** In most smokers, 45 minutes' incubation causes 80 percent or more adherence of cells. The data accumulated so far are insufficient to draw conclusions about form vs function.

**Dr. Scoggin:** Shouldn't you incubate your system for a longer period or add a synergistic compound such as testolactone before you conclude flattening is not caused by cyclic-AMP?

**Dr. Davis:** We haven't done this so far.

**Dr. Musson:** Have you checked to see if the lavage fluid itself from smokers and non-smokers will change the adherence or morphology of cells?

**Dr. Davis:** No, we haven't.

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**The Primary Immune Response of Patients with Different Stages of Squamous-cell Bronchial Carcinoma**

*Class Specific Antibody Response and in-vitro Lymphocyte Stimulation after Primary Immunization with Helix pomatia Hemocyanin*

**Henk M. Jansen, M.D.; T.H. The, M.D.; and N.G.M. Orie, M.D.**

In human lung cancer patients, many attempts have been made to investigate the immune responsiveness in relation to the development and course of the disease. An in vitro parameter such as the antigen-induced lymphocyte transformation reaction, can be used in these studies as a test of T-lymphocyte function. The possible importance of the role of the humoral immune response in lung cancer is less frequently studied. The assessment of class-specific antibody titers to the primary test antigen, α-helix-pomatia-hemocyanin (HPH) in healthy adults, was recently studied in our laboratory using the enzyme-linked immunosorbent assay (ELISA). The aim of this study, using this technique, was to investigate the class specific humoral immune response of patients with bronchial carcinoma, in relation with the specific cellular immune reactivity after immunization with HPH.

**METHODS**

Thirty patients with primary squamous-cell bronchial carcinoma, classified in TNM stages, according to the criteria for lung carcinoma proposed by UICC (1974), and a control group of 15 individuals with low grade chronic obstructive lung disease (COLD), were immunized at the time of the diagnosis and before any treatment was given, with 1 mg HPH subcutaneously. Sera for antibody titer determinations were collected before and 2, 8 and 14 weeks after immunization.

Antibody determination was performed using an indirect ELISA technique in round bottom microtiters plates, as described by Weits et al. The results are expressed in terms of the log of the highest dilution of each serum which resulted in a positive reaction. At two weeks after immunization, HPH-induced lymphocyte transformation tests (LTT) were also performed in the same patients and control subjects, using a microculture technique as described by Du Bois et al. Results are expressed as dpm/mm³ of peripheral blood using the formula:
In patients with bronchial carcinoma with disease, IgM (7.88 ± 2.04) antibody titers, compared with the control subjects (respectively 11.03 ± 0.82, and 10.36 ± 1.31), (Student t-test: P < 0.001 and P < 0.001), whereas the IgM anti-HPH titers (9.42 ± 1.49) did not differ from the control subjects (10.09 ± 1.17).

In patients with localized disease (stage 1), there was no difference in anti-HPH-antibody response, compared with the controls. In ten patients with localized disease who underwent thoracotomy between two and eight weeks after immunization, in an additional group of seven individuals who underwent thoracotomy in the same period for non-malignant diseases, and in 14 control subjects with COLD, serial ELISA tests could be performed 8 and 14 weeks after immunization. Figure 2 shows graphically that at 14 weeks after immunization, in the patient group, the percentage of IgA titer decline (24.78 ± 3.31 SEM) and of IgG titer decline (24.85 ± 3.75 SEM) is statistically significant, more pronounced than in the control group (COLD, IgA 18.89 ± 2.31; IgG 12.69 ± 2.9; “benign” thoracotomy control subjects IgA 17.35 ± 2.8; IgG 16.95 ± 2.7) (P < 0.001). There was no difference in decline of the IgM antibody titers found in patients, compared with controls. Serum IgC, IgA and IgM levels were found within the normal range in the patient group as well as in the control groups.

**DISCUSSION**

This study revealed, two weeks after primary immunization with HPH, a decreased class-specific IgG and IgA anti-HPH-antibody response in stage 3, bronchial carcinoma patients, in combination with a relatively high IgM response. Although in stage 1 patients normal IgC and IgA anti-HPH-antibody titers were found at the peak response at two weeks, a more rapid decline in these titers was shown at 8 and 14 weeks after immunization, compared with the controls. This effect was not induced by the thoracotomy.

The humoral immune response to a thymus-dependent antigen such as HPH requires a helper T-cell system. Since IgG and IgA antibodies are in general thymus-dependent and IgM antibodies more thymus-independent, our findings may reflect a decreased T-helper function in bronchial carcinoma patients. The concomitant decreased HPH-induced LTT may be an additional argument of a defective T-cell function. Recently it has been shown that hyperactive suppressor T-cells may inhibit antibody dependent T-cell cytotoxicity directed against tumors. IgG immune complexes suppressed the generation of plasma cells in activating suppressor T-cells. In a previous study we have suggested the existence of circulating IgG-immune complexes in bronchial carcinoma patients. Induced hyperactivity of suppressor T-cells by these complexes may be the cause of the described changes in the humoral and cellular anti-HPH-immune response in squamous-cell bronchial carcinoma.

**REFERENCES**

Lung Lining Material As a Chemotactant for Alveolar Macrophages*

Lester W. Schwartz, D.V.M., Ph.D., and Claudia A. Christman, B.A.

Critical functions performed by alveolar macrophages (AMφ) as defenders of the pulmonary gas exchange surface include migration, phagocytosis, cytotoxicity, and generation of soluble mediators. Accumulation of AMφ within the lung serves as an indicator of pulmonary insult. In the face of acute insult, recruitment and enhanced mobility aid in confinement of particulates and protection of the lung. Persistence of increased numbers of these inflammatory cells for prolonged periods upon the delicate alveolar membrane may proceed to be more detrimental than protective to the host.

Our study was designed to evaluate the influence of lung lining material on directional migration of AMφ collected from rhesus monkeys. Motivation for the present study was derived from previous morphologic observations made on lungs of rodents and nonhuman primates exposed to low levels of oxidant air pollutants. Our observations indicated that the AMφ was the principal inflammatory cell which accumulated within the lung during initial phases of insult and, in

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DISSCUSION

Dr. Lawrence: Did you measure the proliferative response in the patient's own serum?
Dr. Jansen: It was measured in 25 percent inactivated pooled human donor serum.

Question: Are the suppressor cells sensitive to indomethacin?

Dr. Jansen: Induction of lymphocyte response seems to be monocyte-dependent. In these patients, suppression of prostaglandin synthesis with indomethacin leads to increased lymphocyte response.

Dr. Goodman: Is there a defect in surveillance of killer cells here?

Dr. Jansen: We looked for NK cells and found no difference.