Immunocompetence in Lung Cancer

Relationship to Extent of Tumor Burden and Histologic Type

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In vitro **assays of immunocompetence were done in 60 patients with differing extents of tumor load and various histologic types of lung cancer and were compared to values obtained for 60 normal controls. Profound alterations in monoclonal antibody-defined T-lymphocytes and circulating B-cells were seen. All patients showed impaired blastogenic response to the mitogens used with the exception of a normal response to pokeweed mitogen (PWM) in patients with localized disease. Increased levels of serum IgG were seen in patients with localized disease whereas high levels of IgA was seen in patients with more advanced disease. Distant metastases were associated with low IgM levels. All patients studied regardless of stage and histologic type had elevated levels of circulating immunocomplexes. These findings indicate gross immunologic abnormalities in these patients.**

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IMMUNOLOGIC REACTIONS play an important role in the natural defense mechanism against tumor cells.[1] Several researchers have supplied evidence that tumor-bearing individuals mount a specific immunoresponse against their own tumor cells.[2,3] Neoplastic proliferation results from an impairment of the dynamic equilibrium between the inherent growth potential of the neoplasm and its ability to survive in the host. Results are varied regarding the correlation between immunocompetence of the patient and prognosis,[4,5] and we had demonstrated previously that general and tumor-specific immunoreactions are depressed with extension of disease.[6]

Lung cancers form approximately 11.5% of cancers in males and 6.5% of all cancers recorded at our institute.[7] Previous studies on immunologic parameters in patients with lung cancer have been contradictory and varied, and few of them address the problem with respect to stagewise and histologic classification of the disease.[8–11] In the current study, we have evaluated some immune parameters that involve both cell-mediated and humoral aspects. These include analysis of lymphocyte populations, transformation responses of lymphocytes to mitogens, levels of serum immunoglobulins, and circulating immunocomplexes in patients with various stages and histologic types of lung cancer.

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Materials and Methods

Patients

The study population included 60 patients with various grades and histologies of lung cancer. Patients were grouped based on the extent of disease[12] (localized, regional extension, and distant metastases) and type of cell (adenocarcinoma, squamous cell carcinoma, and small cell carcinoma). As a control group, 60 apparently normal persons in the same age group (40 to 70 years) were selected. Details are given in Table 1.

Blood Collection

Twenty ml of blood was collected from each patient and control by venipuncture in sterile conditions. Of this, 15 ml was collected in sterile heparinized tubes and 5 ml in sterile siliconized tubes without any anticoagulant.

Preparation of Peripheral Blood Lymphocytes

Boyum's[13] method was used for this preparation. Briefly, blood was diluted with an equal volume of Hanks Balanced Salt Solution (HBSS, Gibco, Chagrin Falls, OH) and centrifuged over a cushion of Ficoll Hypaque (Lymphoprep, Nyegaard, Norway) at 400 g for 25 minutes.

TABLE 1. Subjects Included in Study: Extent of Disease and Histology

Extent of disease and cell type	No.	Age range (yr)
Localized (disease confined to one lung and/or main stem bronchus)	10	40–57
Adenocarcinoma	4	
Squamous cell carcinoma	6	
Regional extension (disease extending to pleura, trachea, esophagus, with neurologic involvement, hilar nodes)	24	51–65
Adenocarcinoma	8	
Squamous cell carcinoma	9	
Small cell carcinoma	7	
Distant metastasis (disease extending to both lungs, ribs, sternum, contralateral hilar nodes, cervical nodes, other distant, involvement)	26	50–70
Adenocarcinoma	10	
Squamous cell carcinoma	6	
Small cell carcinoma	10	
Normal controls	60	40–70

The mononuclear cell band was harvested, washed twice in HBSS, and resuspended in RPMI 1640 medium buffered with 20 μM/l Hepes and supplemented with 10% heat inactivated human AB serum, 100 μg/ml streptomycin, 100 U/ml penicillin, 5 μg/ml of Fungizone, and 300 μg/ml of fresh glutamine (complete medium).

Depletion of Adherent Cells

To be depleted of monocytes, mononuclear cells were incubated for 1 hour in plastic dishes coated with heat inactivated fetal bovine serum (FBS, Gibco, Chagrin Falls, OH) at 37° C. Nonadherent cells were collected by repeated washing with complete medium. More than 95% of these cells were determined by peroxidase staining to be lymphocytes. The percent viability of the lymphocytes was indicated as more than 95% by Trypan Blue dye exclusion test.

Analysis of Lymphocyte Subpopulations

Purified lymphocytes depleted of adherent cells were analyzed by indirect immunofluorescence technique for quantitation of subsets. The monoclonal antibodies used were OK T3, OK T4, and OK T8 (Ortho Diagnostics, Raritan, NJ) that identify total T-cells (CD 3+), helper/inducer T-cells (CD4 +), and suppressor/cytotoxic T-cells (CD 8 +). Binding of antibody to cells was located using an antimouse IgG conjugated to fluorescein isothiocyanate (FITC) (Kallested Labs, Chaska, MN). Cells incubated with the second antibody alone served as negative controls. B-lymphocytes were detected by surface immunofluorescence using $F(ab)_2$ portion of antihuman immunoglobulin conjugated to FITC. The cells were examined for fluorescence under a Leitz Orthoplan Fluorescence Microscope (Leitz, Wetzlar, Germany).

Blastogenic Response of Lymphocytes to Mitogens

The ability of lymphocytes to respond to optimal concentrations of mitogen was assessed by standard techniques[14] in which 1×10^5 lymphocytes were cultured in plastic tubes with the appropriate mitogen for 48 hours. The mitogens used were phytohaemagglutin (PHA, Bacto, Difco, Detroit, MI), pokeweed mitogen (PWM, Difco, Detroit, MI) and Concanavalin A (Con A, Sigma, St. Louis, MO). The optimal dilution of PHA and PWM that produced the maximum response was determined by the reconstitution of mitogens according to manufacturers' directions, and by a dose response curve that used twofold dilutions beginning with 1:5. The optimal dilution for Con A was 10 μg/0.1 ml. Cultures were incubated at 37° C in a 5% CO_2 atmosphere. For the last 18 hours of the culture, cells were pulsed with 1 μCi of ^3H thymidine (BARC, Bombay, India), harvested, and counted on a liquid scintillation counter.

Serum Immunocomplexes

Sera were assayed for soluble immunocomplexes by a method modified from Digeon *et al.*[15] This procedure combines precipitation of immunocomplexes with 3.75% polyethylene glycol 6000 (PEG 6000) and measurement of the protein content in the washed PEG 6000 precipitate by the Lowry[16] procedure. Serum samples for this assay were stored in separate 2 ml vials at −70° C and were not thawed until the day the assay was done. All the immunocomplex assays were done on large panels with the use of single lots of reagents prepared freshly for the particular run. An internal quality assurance standard was used to ensure assay reproducibility.

Serum Immunoglobulins

Serum IgG, IgA, and IgM were quantitated by the Mancini type radial immunodiffusion (RID) method with commercially available RID plates (Hoechst, Bombay, India).

Data Analysis

Statistical analysis of data was done with the Student's *t* test.

Results

Lymphocyte Subpopulations

The results of monoclonal antibody-defined phenotypic analysis of T-cells, and an enumeration of B-cells in the

TABLE 2. Lymphocyte Populations in Lung Cancer Patients With Localized Disease

Category	CD3+ cells/mm³	CD4+ cells/mm³	CD8+ cells/mm³	CD4/CD8	B cells/mm³
Normal controls (n = 60)	1770 ± 320	1084 ± 232	540 ± 150	2 ± 0.4	539 ± 170
Localized disease total (n = 10)	1540 ± 200 *	960 ± 186 *	700 ± 178 *	1.4 ± 0.3 *	630 ± 170 *
Adeno (n = 4)	1490 ± 145 *	970 ± 126 *	730 ± 200 *	1.4 ± 0.4 *	648 ± 205 *
Squamous (n = 6)	1700 ± 185 NS	900 ± 140 *	700 ± 220 *	1.4 ± 0.3 *	545 ± 220 NS

All values are mean ± SD. NS: not significant. * $P < 0.05$.

various grades and histologic types of lung cancer are stated in Tables 2, 3, and 4. Patients with localized disease showed reduced total T-cell (CD3+) counts with an exception of squamous cell carcinomas. The CD4+ cells showed a decrease along with concomitant increase in CD8+ cells. The CD4/CD8 ratio was altered in all cases. B-cell counts were increased significantly in patients with localized disease with adenocarcinoma and small cell carcinoma (Table 2). Similarly, the total T-cell counts were decreased significantly in patients with regional extension of disease as well as in patients with distant metastases. Significant decrease in CD4+ cells accompanied by increase in CD8+ cells was seen. B-cells showed a significant increase in all patients who had regional extension of disease, whereas they were in normal limits in patients with distant metastases (Tables 3 and 4).

Blastogenic Responses to Mitogens

Of the three mitogens used, response to PHA was impaired significantly in all 60 patients analyzed regardless of the stage or histologic type of the disease. Response to PWM was normal in patients with localized disease whereas a depressed response was evident in other groups. A similar significant depression of mitogenic response to Con A was evident in all patients (Table 5). This impairment showed no correlation with the histology of the cancer.

Serum Immunoglobulins

Levels of these immunoreactive proteins varied in lung cancer patients. Significant increase in IgG was seen in patients with localized disease whereas levels of IgM and IgA remained unaltered. However, patients with regional extension of disease and those with distant metastases had significant increases in IgA levels, but showed no change in IgG levels. Patients with distant metastases had reduced IgM levels also. However, in this group, the reduction in IgM levels was not seen in patients with squamous cell carcinoma (Table 6).

Circulating Immunocomplexes

Levels of circulating immunocomplexes assessed by protein content of PEG 6000 precipitates showed significant increase in all patients irrespective of the stage or histology of the disease (Table 6).

Discussion

This study and related reports emphasize the importance of broadfield analysis of immunologic parameters

TABLE 3. Lymphocyte Populations in Lung Cancer Patients With Regional Extension of Disease

Category	CD3+ cells/mm³	CD4+ cells/mm³	CD8+ cells/mm³	CD4/CD8	B cells/mm³
Normal controls (n = 60)	1770 ± 320	1084 ± 232	540 ± 150	2 ± 0.4	539 ± 170
Regional extension total (n = 24)	1460 ± 297 *	900 ± 270 *	770 ± 178 *	1.3 ± 0.3 *	620 ± 189 *
Adeno (n = 8)	1500 ± 177 *	930 ± 148 *	700 ± 178 *	1.4 ± 0.4 *	640 ± 200 *
Squamous (n = 9)	1580 ± 168 *	970 ± 180 *	740 ± 162 *	1.4 ± 0.3 *	600 ± 146 *
Small cell (n = 7)	1400 ± 186 *	977 ± 148 *	756 ± 177 *	1.3 ± 0.4 *	636 ± 177 *

All values are mean ± SD. NS: not significant. * $P < 0.05$.

TABLE 4. Lymphocyte Populations in Lung Cancer Patients With Distant Metastases

Category	CD3+ cells/mm³	CD4+ cells/mm³	CD8+ cells/mm³	CD4/CD8	B cells/mm³
Normal controls (n = 60)	1770 ± 320	1084 ± 232	540 ± 150	2 ± 0.4	539 ± 170
Distant metastases total (n = 26)	1270 ± 200 *	780 ± 188 *	830 ± 142 *	1.2 ± 0.3 *	545 ± 120 NS
Adeno (n = 10)	1300 ± 170 *	800 ± 130 *	852 ± 110 *	1.3 ± 0.4 *	548 ± 100 NS
Squamous (n = 6)	1370 ± 120 *	760 ± 137 *	800 ± 112 *	1.3 ± 0.3 *	540 ± 97 NS
Small cell (n = 10)	1200 ± 140 *	700 ± 146 *	815 ± 130 *	1.2 ± 0.4 *	535 ± 77 NS

All values are mean ± SD. NS: not significant. * $P < 0.05$.

that show the specific and nonspecific responses to neoplastic disease. This investigation shows substantial differences in the profile of lymphocyte phenotypes in the various stages of the disease and its various histologic types. A decrease in CD3+ cell population is evident in most patients. The CD4+ cell count is found to be reduced significantly with a concomitant increase in CD8+ cells and thus results in a decrease in the CD4 to CD8 ratio. The maintainence of the normal CD4/CD8 ratio is responsible in part for maintaining immunohomeostasis. Such findings have been reported previously.[17,18] All types of lung cancer studied showed depressed CD3+ cell counts except for patients who have squamous cell carcinoma with localized disease. A similar finding was reported by Shanker *et al.*[8] who showed that the proportion of peripheral blood lymphocytes that form rosettes with sheep erythrocytes were significantly higher in squamous cell carcinoma compared with small cell anaplastic lung cancer.

Previous reports have documented that patients with lung cancer display immunodeficiency, as proved by impaired reactions of delayed cutaneous hypersensitivity,[9] depressed reactivity of lymphocytes to lectins,[10] impaired natural killer cell activity,[11] and altered monocyte-macrophage functions.[19] However, most of these studies did not look into the stagewise or histologic type of the disease. Our findings show a depressed response to the T-cell mitogens, PHA, and Con A by all groups of patients. However, the response to PWM was normal in patients with localized disease irrespective of the histologic type. A similar finding along with defective interleukin 2 production was reported in patients with small cell carcinoma by Masuno *et al.*[20] This probably suggests an inherent or acquired T-cell defect in these patients.

The current study indicated changes in humoral response parameters as proved by alterations in levels of serum immunoglobulins and immunocomplexes. An increase is seen in IgG levels in patients with localized disease. Serum level of IgG is dependent on the intensity of the antigenic stimulation and the functional capacity of the antibody-producing mechanism.[21] Consequently, the increase in IgG in this group of patients could be due to intensive antigenic stimulation that is due possibly to the neoplastic process. However, the decrease in IgM seen in patients with distant metastases is probably a result of immunologic deterioration due to age.[21] Most of these patients are in the group 50 to 70 years of age . The elevation of IgA in advanced stages of the disease may be significant. Similar findings have been reported previously.[22,23] At least one group has described an IgA-like blocking factor in the sera of patients with nasopharyngeal carcinoma.[24] Basler, *et al.*[25] and Abraham and Balaram[26] have reported persistent elevation of IgA immunocomplexes in patients with head and neck cancer. Thus, it appears that these data indicate the possible importance of a local immunoresponse of the mucosal immune system to malignancies associated with this defense barrier.

Another significant finding was the demonstration of elevated immunocomplex-like material that uses the PEG 6000 precipitation method of Digeon *et al.*[11] All serum specimens tested were assayed as randomized blind coded specimens, and data reduction awaited completion of all serum-based testing procedures. Elevated immunocomplex levels could be seen in all three groups of patients studied. Lack of correlation between immunocomplex levels and stage of the disease was reported earlier by Abraham and Balaram.[26] Circulating specific blocking factors have long been related to impaired cellular immunity,[27–29] and the role of immunocomplexes feature prominently in these studies. A qualitative analysis of these immunocomplexes would help to clarify its antigenic relation to the tumor.

Our study has shown that host immunocompetence is affected profoundly and, therefore, additional studies should attempt to rectify this depressed state. Preliminary investigations in this field have been encouraging.[30,31] The

TABLE 5. Blastogenic Responses of Lymphocytes to Mitogens in Lung Cancer

Mitogen	Normal controls (n = 60)	Localized disease			Regional extension				Distant metastases			
		Total (n = 10)	Adeno (n = 4)	Squamous (n = 6)	Total (n = 34)	Adeno (n = 12)	Squamous (n = 10)	Small cell (n = 12)	Total (n = 26)	Adeno (n = 10)	Squamous (n = 6)	Small cell (n = 10)
PHA	40000 ± 20450	30120 ± 17640 *	27380 ± 9700 *	34000 ± 10150 *	24700 ± 14300 *	22000 ± 12400 *	26400 ± 11800 *	23700 ± 18200 *	20000 ± 14000 *	22000 ± 10640 *	25000 ± 12000 *	20000 ± 9700 *
PWM	13760 ± 8000	12400 ± 6780 NS	13460 ± 9760 NS	12970 ± 8680 NS	9970 ± 5540 *	10000 ± 7700 *	9880 ± 5440 *	9000 ± 4800 *	9560 ± 5580 *	9770 ± 4880 *	10000 ± 5880 *	8990 ± 4760 *
Con A	27650 ± 12700	20000 ± 10460 *	22000 ± 11650 *	20760 ± 12430 *	17600 ± 10760 *	18000 ± 11200 *	17880 ± 14200 *	17770 ± 10210 *	17100 ± 10000 *	17850 ± 8950 *	17800 ± 9760 *	16430 ± 9000 *

All values are mean ± SD (CPM). NS: not significant. * $P < 0.05$.

TABLE 6. Serum Immunoglobulins and Circulating Immune Complexes in Lung Cancer

Serum protein	Normal controls (n = 60)	Localized disease			Regional extension				Distant metastases			
		Total (n = 10)	Adeno (n = 4)	Squamous (n = 6)	Total (n = 24)	Adeno (n = 8)	Squamous (n = 9)	Small cell (n = 7)	Total (n = 26)	Adeno (n = 10)	Squamous (n = 6)	Small cell (n = 10)
IgG (mg/dl)	1570 ± 221	2000 ± 440 *	2080 ± 320 *	1970 ± 300 *	1560 ± 266 NS	1680 ± 281 NS	1497 ± 300 NS	1650 ± 282 NS	1580 ± 300 NS	1600 ± 270 NS	1576 ± 188 NS	1620 ± 170 NS
IgA (mg/dl)	250 ± 41	246 ± 48 NS	257 ± 40 NS	260 ± 38 NS	307 ± 60 *	298 ± 47 *	301 ± 50 *	296 ± 57 *	340 ± 70 *	298 ± 32 *	308 ± 42 *	300 ± 51 *
IgM (mg/dl)	160 ± 37	170 ± 52 NS	166 ± 38 NS	173 ± 44 NS	176 ± 60 NS	162 ± 44 NS	170 ± 38 NS	158 ± 44 NS	115 ± 37 *	110 ± 42 *	167 ± 60 NS	107 ± 56 *
CIC (μg/ml)	175 ± 70	560 ± 146 *	480 ± 100 *	510 ± 140 *	530 ± 138 *	500 ± 130 *	470 ± 146 *	488 ± 156 *	620 ± 140 *	588 ± 156 *	590 ± 180 *	600 ± 178 *

All values are mean ± SD. NS: not significant. * $P < 0.05$.

treatment of cancer is successful to a certain extent because of precise analysis of the immune status of the patients and the mechanisms by which immunodeficiency is generated. This latter aspect needs to be dealt with in detail, and is being investigated currently by our group.

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