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IMMUNOLOGIC ASSESSMENT OF PATIENTS WITH PULMONARY METAPLASIA AND NEOPLASIA

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SUMMARY

Immune profiles have been obtained on 206 individuals including 57 controls, 50 lung cancer patients, and 99 uranium miners with well-defined sputum cytologies ranging from normal to carcinoma *in situ*. Little effect of smoking, uranium mining or a combination of mining plus smoking on immune function was observed if sputum cytology was normal. In heavy smokers there was a suggestion that total T cells are increased while T cell function is slightly depressed. Immunologic abnormalities were noted in the moderate atypia group where 40% had one or more abnormal immunologic parameters. Immunologic abnormalities were detected in 68 to 70 patients with marked atypia, carcinoma *in situ*, or invasive carcinoma. The most sensitive test was the rosette inhibition test which may be detecting changes associated with very early neoplastic transformation. Immunologically, marked atypia and carcinoma *in situ* are indistinguishable suggesting that marked dysplasia actually represents an irreversible neoplastic change. Further sequential study of the uranium miner population is necessary to define more precisely the predictive value of immunologic testing, and the role of early identification of high risk individuals in the early institution of definitive therapy, such as surgery or immunotherapy. Long-term prospective analysis of this population may also provide the answer to the question of whether alterations in immune function precede, or result from the appearance of cells committed to the development of neoplasia.

INTRODUCTION

A vast and often conflicting literature has appeared in recent years concerned with the interactions between host immune system and developing or established neoplasms. Much of this work in tumor immunology resulted from the theory of immunosurveillance which originally proposed that host immunocompetent cells are capable of recognizing and reacting to neoantigens expressed by developing neoplastic cells, with the resultant elimination of the malignant cells.[1] Although in the light of recent advances in the understanding of basic immunologic processes the theory *per se* appears oversimplistic,[2] it is clear that host immunologic reactivity against tumors can and does occur. Despite this capability, tumor-specific immune responses and general immunocompetence are usually depressed when neoplasms are clinically evident and become progressively more abnormal as the neoplasm increases in size.

Much research today is directed toward determining when and why immunologic capacity is lost in cancer patients and how it might be restored. Underground uranium miners represent one human population of particular interest in this regard. In this population group exposure to radon, the environmental carcinogen associated with uranium mining, and cigarette smoking results in a synergistic or co-carcinogenic effect leading to a markedly increased incidence of lung cancer. This population has also been extensively studied by Saccomanno and colleagues [3,4] who have utilized sputum cytologic examination to describe the progressive changes in the respiratory tract that occur during the development of lung cancer. Following exposure to inhaled carcinogens, the bronchial epithelium undergoes a series of gradual changes from squamous metaplasia to progressively more atypical metaplasias characterized as mild, moderate and marked, leading to noninvasive carcinoma *in situ* and finally invasive, clinical lung cancer. Similar delayed

progressive devlopment of cells in the bronchial tree has been demonstrated by Schreiber in hamsters[5] and by Kato in the dog[6] after carcinogen exposure. Of particular interest and importance in the study of uramium miners was the finding that individuals may exfoliate markedly atypical metaplastic cells or cells representing carcinoma in situ for long periods of time (4-5 years) before progression to invasive carcinoma occurs, making early detection a real possibility. Such a well-defined population of individuals with identifiable premalignant cytologic changes also provides the long-sought after opportunity to study the timing, type and extent of immunologic changes that occur in the early stages or premalignant stages of lung cancer development.

Our specific interest in the immunologic evaluation of lung cancer dates back to an earlier study.[7] In this study it was found that circulating thymus-dependent lymphocytes (T cells) as measured by spontaneous rosette formation with sheep red blood cells (SRBC)[8] was significantly depressed in a group of patients with localized Stage I lung cancer. Of further interest was the observation that a newer test involving the inhibition of rosette formation by anti-lymphocyte globulin[9] was profoundly depressed and represented a much more sensitive measure of immune status than T cell levels alone. These results suggested that with the appropriate test one might detect immunologic changes prior to the development of clinically detectable cancer. Other investigators have studied immune function in patients with different clinical stages of lung cancer with a variety of different immunologic techniques.[10-14] Although most report detection of abnormalities in a high proportion of lung cancer patients, the results do not provide information about early lung cancer, or give any indication of which test is most appropriate for the clinical evaluation of cancer patients. The available literature on lung cancer has recently been reviewed.[15]

The present study attempts to answer many of these questions. We have performed a battery of immunologic tests on uranium miners in whom various stages of preneoplastic and neoplastic changes have been identified by sputum cytologic exam as well as performing an immunoprofile on patients with known lung cancer. We have attempted to answer several important questions including: can changes in immunologic function be detected in individuals with preneoplastic lesions of the respiratory tract; and do these changes help identify those individuals most likely to progress and develop invasive lung cancer? Which immunologic test is most sensitive in detecting abnormalities in this high risk population group? Can one determine the relative contributions of the environmental carcinogens involved, i.e. radon exposure from uranium mining and tobacco smoking, to changes in immune function?

MATERIALS AND METHODS

Study Populations

The study populations comprised three general groups including healthy, age-and sex-matched controls; uranium miners; and patients with known lung cancers. The healthy controls were volunteers living in the same geographic area (western slope of Colorado) as the individuals in the other two groups, and with no history of uranium mining. The controls were further subdivided according to smoking history with non-smokers; light smokers (defined as less than 10 pack-years cigarettes); moderate smokers (10-20 pack-years cigarettes); and heavy smokers (greater than 20 pack-years cigarettes).

The uranium miner group consisted of individuals with at least six months of underground uranium mining experience. This group was further subdivided on the basis of sputum cytologic findings (see below) into a control uranium miner group (UGU) with normal or mildly atypical cytology; and groups with moderate atypia; moderate-marked atypia; marked atypia; and carcinoma in situ. The moderate-marked group included miners with changes in sputum cytology from moderate to marked or vice versa on the most recent cytologic exam, and may represent those individuals in transition to higher or lower degrees of respiratory tract abnormalities. The control UGU group was also subdivided on the basis of smoking history as was done for normal controls.

The lung cancer group consisted of patients with know lung cancer, diagnosed either histologically or by sputum cytology. The majority of these patients were studied during their initial hospitalization for diagnostic workup, and represented patients with localized disease without previous treatment. Patients with know metastatic disease or those undergoing treatment were excluded.

For all groups, individuals with recent histories of infection, or those on medications known to interfere with immune function were excluded.

SAMPLE COLLECTIONS

Sputum, Collection and Cytology

The sputum was collected and prepared by the Saccomanno method[16] and stained with Papanicolaou stain. Chart I shows these cell patterns in each category.

Peripheral Blood

Twenty milliliters of peripheral blood was collected by venipuncture into sterile, heparinized containers. For the uranium miner group, blood samples were obtained at the same time as sputum cytology specimens. A complete blood count including white blood cell count and differential were done on all samples.

IMMUNOLOGIC STUDIES

Lymphocyte Preparation

Lymphocytes were separated from whole blood by centrifugation through a Ficoll/Sodium Diatrizoate gradient (LSM medium, Litton Bionetics, Kensington, Md.) according to the method described by Boyum.[17] Lymphocytes recovered from the gradient interface were washed twice with Hanks' balanced salt solution (HBSS, Microbiological Associates, Bethesda, Md.), and divided into two equal portions. After a third wash, one lymphocyte sample was resuspended in HBSS supplemented with 20% fetal calt serum (FCS) at a concentration of 2×10^6 cells per milliliter. This sample was prepared for use in the rosette assays and rosette inhibition test (see below) by incubation with latex particles to identify contaminating phagocytic mononuclear cells. 0.1 ml of the lymphocyte suspension was added to 0.1 ml of 1% latex particles (0.801 micron diameter particles, 10%, Dow Chemical Co., Indianapolis, Ind.) and the mixture incubated at 37°C for 30 minutes. The mixture was layered on 1 ml FCS and centrifuged at 200 x g for five minutes to remove free latex particles.

METAPLASIA TO NEOPLASIA

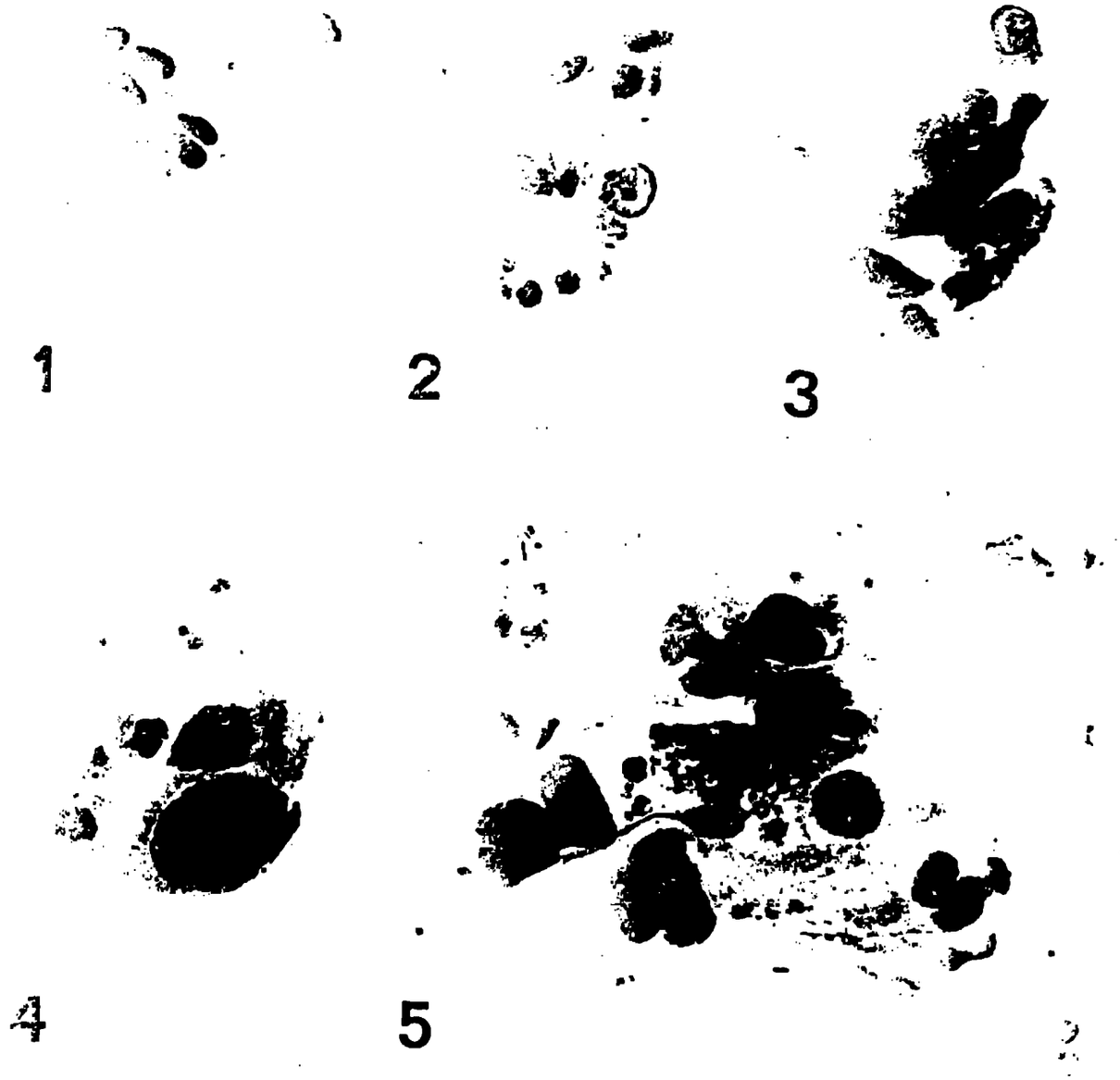


Chart I: Development of Epidermoid Carcinoma of the Lung

Progressive cytological atypia in development of cancer From: Figure 1 - mild; Figure 2 - moderate; Figure 3 - marked; Figure 4 - CIS, and Figure 5 - Invasive Carcinoma

The lymphocytes were washed once with HBSS and resuspended in HBSS to a final concentration of 1 x 10^6/ml.

The second aliquot of lymphocytes was resuspended in complete medium consisting of RPMI 1640 culture medium with 25 mM Hepes buffer (Microbiological Assoc., Bethesda, Md.) supplemented with 10% fetal calf serum (GIBCO, Grand Island, NY), 2 mM L-glutamine (GIBCO) and 100 micrograms penicillin, 1 microgram streptomycin, and 0.25 microgram fungizone per 100 ml (Microbiological Associates). A final concentration of 2 x 10^6 cells/ml in complete medium was made for use in the mitogen studies.

Total Lymphocyte Count

A white blood count and differential was performed on each blood sample.

T Cell Enumeration

T cells were identified by their ability to bind to sheep red blood cells (SRBC).[8,18] Fresh SRBC were obtained weekly and stored in Alsever's solution. Before use, they were washed three times in HBSS, counted and diluted to concentration of 2.5% and 0.5% in HBSS.

Total T Lymphocytes

0.1 ml of the lymphocyte suspension in HBSS was incubated for 5 min. at 37°C, then 0.1 ml of the 2.5% SRBC suspension added giving a SRBC: lymphocyte ratio of approximately 40:1. The mixture was centrifuged at 200xg for 5 minutes, then incubated 18 hours (overnight) at 4°C. The cell pellet was then gently resuspended and a sample placed on a hemocytometer. The proportion of rosette-forming cells (RFC) defined as any lymphocyte binding three or more SRBC was determined by counting 200 cells. Phagocytic cells containing latex particles were excluded. Lymphocyte viability was determined by new methylene blue dye uptake, and non-viable cells were excluded. Total T cells per mm^3 was calculated by multiplying the percentage of RFC by the total lymphocyte count.

Early Rosette-Forming Cells

By limiting the number of SRBC available for binding, and the amount of time lymphocytes are in contact with SRBC presumably one can identify more "active" rosette-forming cells.

The determination of the proportion of these "early" or active RFC may identify the subpopulation of T cells with high affinity receptors for SRBC. Early RFC were measured by the method described by Wybran and Fudenberg.[19] 0.1 ml of the lymphocyte suspension (HBSS) was preincubated at 37°C for 60 minutes, and then 0.1 ml of the 0.5% SRBC suspension was added to give a final SRBC lymphocyte ratio of 8:1. The mixture was centrifuged for 5 minutes at 200g, the cells gently resuspended and proportion of RFC counted immediately. Total early RFC/mm^3 peripheral blood was calculated by multiplying the percentage early RFC by the total lymphocyte count per mm^3.

B Cell Enumeration

B cells were determined using an EAC (erythrocyte-antibody-complement) technique which identifies cells with receptors for activated C3 component of complement. Antibody and complement coated SRBC were prepared by incubating 10ml of a 5% suspension of SRBC with an equal volume of 1:5000 dilution of rabbit IgM anti-SRBC antibody (Cappel Labs, Cochranville, MD.) for 30 minutes at 37°C. The antibody-coated SRBC (EA) were washed twice in HBSS and a 5% suspension in Veronal buffer (Microbiological Assoc.) made.

An equal volume of fresh guinea pig complement (Cappel Labs) diluted 1:10 in Veronal buffer was added and the mixture incubated for 45 minutes at 37°C. The antibody and complemented SRBC (EAC) were washed three times in HBSS and resuspended in HBSS to a 1% concentration. EAC rosette formation was determined by mixing 0.1 ml of the lymphocyte suspension (HBSS) with 0.1 ml of the 1% EAC, contrifuging 5 min at 200g incubating mixture for 20 minutes at 37°C. The cells were gently resuspended and %RFC counted as described above.

Total peripheral B cells was calculated by multiplying the percent EAC - RFC by the total lymphycyte count per mm^3.

Rosette Inhibition Test

Inhibition of T cell-SRBC rosette formation was determined using a horse antihuman thymocyte serum (ATS) kindly provided by Dr. Barbara Loughman, Upjohn Co., Kalamazoo, Michigan. Although the exact mechanism of action of ATS on T cells is unknown, the rosette inhibition test provides a measure of the strength of lymphocyte-SRBC interaction, presumably an indirect measure of T cell functional competence. The rosette inhibition test was performed by a modification of the technique previously described.[7,9] Serial double dilutions of ATS in HBSS, 1:100 to 1:12,800, were prepared and 0.1 ml of each dilution added to 12 x 75 mm culture tubes. 0.1 ml of the lymphocyte suspension in HBSS was added to each tube. 0.1 ml lymphocytes in 0.1 ml HBSS served as control tubes. All dilutions and controls were tested in duplicate. The ATS-lymphocyte mixtures were incubated at room temperature for 30 minutes, then 0.2 ml. fetal calf serum (previously absorbed with SRBC), and 0.1 ml of 2.5% SRBC were added. The cells were pelleted by centrifugation at 200 x g for 5 minutes, then incubated a further two hours at room temperature. After the two hour incubation, the cells were gently resuspended and the percentage of rosette-forming cells determined for each dilution as described above. The percent inhibition was then calculated for each dilution by dividing the percent RFC obtained in the ATS tube by the percent RFC in the control tubes. The 25% inhibitory titer was determined graphically by graphing % inhibition versus ATS titer. The result was expressed as the reciprocal of the dilution. In interpreting the results of the rosette inhibition test, the lower the dilution producing 25% inhibition, the more antibody required, thus the more functional the lymphocytes.

In Vitro Lymphocyte Transformation to Mitogens

Certain plant lectins are capable of inducing blastogenesis in lymphocytes, an event analogous to what occurs when sensitized lymphocytes encounter antigen and initiate an immune response. Though indirect, the ability of lymphocytes to undergo blastogenesis in response to mitogens has been utilized as a measure of functional competence of the cellular immune system. The degree of response to mitogen stimulation is measured by the amount of ^{3}H-thymidine incorporated into DNA by the stimulated lymphocytes.

The three mitogens used were phytohemagglutinin (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM). PHA and Con A are considered primarily T cell mitogens, although they apparently stimulate different subpopulations of T cells.[20] PWM, on the other hand, induces blastogenesis primarily in B cells. This response, however, depends on T cell helper function.[21]

Multiple dilutions of each mitogen were made in complete medium. Stock PHA solution (Burroughs Wellcome, Research Triangle Park, N. C.) was diluted 1:40, 1:80, 1:160, and 1:320 and these concentrations for testing. Con A (Sigma Chemical Co., St. Louis, Mo.) was diluted in complete medium to final concentrations of 2.5, 5.0, 10.0 and 25.0 micrograms per ml. for use. Dilutions of 1:10, 1:20, 1:50 and 1:100 of stock PWM solution (GIBCO) in complete medium were used.

All samples were cultured in triplicate in sterile flat bottom Microtest II microtiter plates (Falcon Plastics, Osnard, Calif.). To each culture 0.1 ml lymphocyte suspension (2 x 10^6 cells per ml) and 0.1 ml of the appropriate mitogen dilutions were added. Triplicate control or unstimulated cultures consisted of 0.1 ml of the lymphocyte suspension plus 0.1 ml of culture medium alone. Cultures were incubated for 72 hours at 37°C in a humidified atmosphere of 5% CO_2 in air. Eighteen hours prior to harvesting

each culture was pulsed with 1 μCi of methyl-^{3}H-Thymidine (Sp. act. 6.7 Ci/mM, New England Nuclear, Bedford, Mass.), and subsequently harvested onto glass fiber filters with a semi-automated multiple culture harvester (Otto Hiller Co., Madison, Wis.). The samples were dried, then placed in glass scintillation vials containing 10 ml of Aquasol-2 (New England Nuclear) and counted in a Packard Tri-Carb-Liquid Scintillation Spectrometer. Results are expressed as counts per minute (CPM), or as stimulation index (SI) which is calculated as:

$$SI = \frac{\text{CPM of stimulated cultures}}{\text{CPM of Unstimulated Cultures}}$$

Statistical analysis was performed using the Student's t test. For analysis of the rosette inhibition data, the log of the reciprocal titer was used because of the non-linearity of the test results.

RESULTS

Effect of Smoking on Immune Function

Fifty-seven normal controls were studied, of whom 32 were non-smokers and 25 were smokers. Results of the immunologic testing in this group are presented in Table 1. Total T cells and rosette inhibition titers were slightly elevated in the smoking controls compared to non-smokers, though these differences were not statistically significant. All other tests showed no differences between the two groups.

When the 25 smokers were further subdivided according to amount of smoking, an interesting pattern emerged. As can be seen in Table 2, there is no difference for any of the tests between the light and moderate smokers. However, in the heavy smoker group total T cells, total early % cells and total B cells are elevated compared to non-smokers, light and moderate smokers, with the differences almost reaching statistical significance ($p = 0.08$). Contrary to these observed increases in quantitative T and B cell numbers however, functional parameters including rosette inhibition titer, PHA, Con A and PWM

TABLE 2. IMMUNOLOGIC PARAMETERS IN NORMAL CONTROLS.

TEST	NON-SMOKERS N = 32	SMOKERS[a] 25	TOTAL 57
% T CELLS	65.3 ± 4.6	65.3 ± 7.1	65.3 ± 5.8
TOTAL T CELLS	1438 ± 382	1542 ± 483	1483 ± 429
% B CELLS	24.2 ± 3.3	23.1 ± 3.2	23.1 ± 3.0
TOTAL B CELLS	505 ± 138	536 ± 163	519 ± 149
% EARLY T CELLS	26.5 ± 2.9	25.3 ± 3.1	26.0 ± 3.0
TOTAL EARLY T CELLS	581 ± 151	591 ± 190	586 ± 168
R.I. TITER (RECIPROCAL)	325 ± 121	404 ± 226	361 ± 179
PHA RESPONSE, CPM	115,816 ± 18,535	112,132 ± 14,933	114,327 ± 17,017
S.I.	221 ± 65	217 ± 59	219 ± 62
CON A RESPONSE, CPM	67,878 ± 20,092	65,057 ± 20,933	66,669 ± 20,289
S.I.	124 ± 36	122 ± 39	123 ± 37
POKEWEED MITOGEN , CPM	37,264 ± 8881	37,147 ± 11,110	37,215 ± 9758
S.I.	70 ± 20	73 ± 29	71 ± 25

[a] No Significant Differences were noted between Smoking and Non-Smoking Groups for any of the tests.

TABLE 2. IMMUNOLOGIC PARAMETERS IN NORMAL CONTROLS ACCORDING TO SMOKING HISTORY

	SMOKING HISTORY			
	NON-SMOKERS	LIGHT	MODERATE	HEAVY
TEST N =	32	6	7	12
% T CELLS	65.3 ± 4.6	67.1 ± 7.3	66.6 ± 7.9	63.7 ± 6.9
TOTAL T CELLS/ mm^3	1438 ± 382	1342 ± 506	1413 ± 455	1717 ± 462
% EARLY T CELLS	26.5 ± 2.9	24.5 ± 2.1	26.6 ± 2.8	25.0 ± 3.7
TOTAL EARLY T CELLS / mm^3	581 ± 151	510 ± 179	561 ± 176	660 ± 193
R.I. TITER (RECIPROCAL)	325 ± 121	364 ± 166	361 ± 143	449 ± 289
PHYTOHEMAGGLUTININ RESPONSE				
CPM	115,816 ± 18,535	112,411 ± 13,667	116,904 ± 20,443	108,771 ± 12,205
POKEWEED MITOGEN RESPONSE				
CPM	37,264 ± 8,881	37,290 ± 10,817	34,222 ± 6,808	33,008 ± 3,682

responses are slightly depressed in the heavy smokers compared to non-smokers and light smokers. These differences were not statistically significant

Effect of Uranium Mining Exposure on Immune Function

Thirty-two individuals with at least six months history of uranium mining and normal sputum cytology were studied. Of these, 10 were non-smokers and 22 smokers. The results for this group are presented in Table 3. The effect of uranium mining exposure alone was evaluated by comparing non-smoking miners with non-smoking controls. Comparing these two groups, there were no observable differences for any of the tests. When the 10 non-smoking uranium miners were compared to the 22 miners who smoked, no differences in quantitative T and B cells were noted. Functionally though, slight abnormalities were noted with the rosette inhibition titer slightly increased and the PHA response slightly decreased in the smoker subgroup. When the amount of smoking is taken into account several suggestive changes are noted. (Table 4) Total T cells are decreased in the light, moderate and heavy smokers when compared to the corresponding groups in the normal controls. For the uranium miners, heavy smokers have elevated total T cells compared to non-smokers, light and moderate smokers, a similar pattern to that seen in the normal controls. However, the smoking effect is considerably blunted in the miner group, total T cells being $1526/mm^3$ in the heavy smoking miners compared to $1717/mm^3$ for heavy smoking normal controls. The same pattern though less pronounced was seen for total early T cells and total B cells.

Functional parameters including rosette inhibition titer and mitogen responses are slightly though not significantly depressed in the smoking miners compared to non-smoking miners. The abnormalities increase gradually as the amount of smoking increases. Comparing uranium miners to normal controls, for equivalent smoking histories -non smoker to heavy smoker- the rosette inhibition titer, PHA and Con A responses are abnormal though not significantly in the uranium miner groups.

TABLE 3. IMMUNOLOGIC PARAMETERS IN UNDERGROUND URANIUM MINERS WITH NORMAL RESPIRATORY CYTOLOGY

TEST	NON-SMOKERS	SMOKERS	TOTAL
N =	10	22	32
% T CELLS	62.5 ± 5.2	63.4 ± 4.8	63.1 ± 4.9
TOTAL T CELLS/mm^3	1400 ± 378	1378 ± 416	1385 ± 397
% EARLY T CELLS	25.1 ± 2.7	25.5 ± 2.7	25.4 ± 2.6
TOTAL EARLY T CELLS/mm^3	565 ± 110	548 ± 134	554 ± 125
% B CELLS	23.3 ± 3.2	23.7 ± 2.6	23.5 ± 2.8
TOTAL B CELLS/mm^3	513 ± 111	505 ± 126	508 ± 119
R.I. TITER (RECIPROCAL)	371 ± 73	441 ± 155	420 ± 138
PHA RESPONSE			
CPM	117,574 ± 17,160	109,606 ± 13,638	112,075 ± 15,021
S.I.	215 ± 39	186 ± 42	195 ± 43
CON A RESPONSE			
CPM	62,793 ± 22,443	63,007 ± 19,914	62,940 ± 20,369
S.I.	114 ± 16	109 ± 33	110 ± 29
POKEWEED MITOGEN			
CPM	37,563 ± 11,300	37,796 ± 10,432	37,723 ± 10,537
S.I.	67 ± 13	63 ± 20	65 ± 18

TABLE 4. IMMUNOLOGIC PARAMETERS IN URANIUM MINERS WITH NORMAL RESPIRATORY CYTOLOGY ACCORDING TO SMOKING HISTORY

		SMOKING HISTORY			
		NONE	LIGHT	MODERATE	HEAVY
TEST	N =	10	5	7	10
% T CELLS		62.5 ± 5.2	64.0 ± 3.9	60.7 ± 4.0	65.2 ± 5.3
TOTAL T CELLS/mm^3		1400 ± 378	1244 ± 274	1283 ± 363	1526 ± 502
% EARLY T CELLS		25.1 ± 2.7	26.8 ± 2.0	24.6 ± 3.1	25.6 ± 2.6
TOTAL EARLY T CELLS/mm^3		565 ± 110	534 ± 115	514 ± 129	582 ± 154
R.I. TITER (RECIPROCAL)		371 ± 73	420 ± 74	422 ± 151	487 ± 194
PHYTOHEMAGGLUTININ RESPONSE					
	CPM	117,574 ± 17,160	115,918 ± 7764	112,097 ± 11,804	104,571 ± 16,098
POKEWEED MITOGEN RESPONSE					
	CPM	37,563 ± 11,300	43,024 ± 9613	38,094 ± 11,076	34,973 ± 10,343

For all the tests discussed below statistical analysis was carried out comparing results for each atypia and carcinoma group to the control groups (normal and UGU) as a whole, and to the smoking subgroups of the control groups. The analysis is expressed as one p value unless discrepancies occurred.

Peripheral Blood T Cell Percentage

The percentage of peripheral blood lymphocytes identifiable as T cells for each group is presented in Table 5. As can be seen, there was no difference between the normal control and underground uranium miner control (UGU) groups. In individuals with atypical metaplastic changes, however, the percent T cells was significantly reduced. A progressive decrease in percent T cells was noted from control levels of 65.3 $\pm$ 5.8% and 63.1 $\pm$ 4.7% to 56.8 $\pm$ 9.4% for the moderate atypia group ($p < 0.02$), and 48.9 $\pm$ 6.9% for the moderate-marked atypia ($p < 0.001$). T cell levels for the marked atypias and carcinoma in situ groups were indistinguishable from the clinical lung cancer group ($p < 0.001$).

Total Peripheral Blood T Cells/mm^3

Total T cells were decreased in the moderate atypia group but not significantly compared to control groups. (Table 5) A marked reduction in total T cells was observed in the moderate-marked atypia group which was significantly different from controls ($p < 0.02$). Profound decreases were seen in the marked and carcinoma in situ groups to less than 50% of controls ($p < 0.001$), a level observed in the lung cancer group.

Peripheral Blood "Early" T Cell Percentage

No significant differences were observed for the percent early T cells in control, UGU and moderate atypia groups. (Table 6) Early T cell percentage was significantly decreased in the moderate atypia group at 21.0 $\pm$ 3.2% ($p < 0.005$). More marked depression was observed in the marked atypia and carcinoma in situ groups at 17.5 $\pm$ 2.6% and 18.0 $\pm$ 2.7% respectively ($p < 0.001$), a level identical to that seen in the invasive carcinoma group (17.6 $\pm$ 4.8%, $p < 0.001$).

TABLE 5. PERIPHERAL BLOOD T CELL LEVELS IN PULMONARY METAPLASIA/NEOPLASIA

PATIENTS

GROUP	N	PERCENT T CELLS ± S.D.	T CELLS/mm^3 ± S.D.
CONTROLS	57	65.3 ± 5.8	1494 ± 482
UCU	32	63.1 ± 4.9 [a]	1374 ± 381[a]
MODERATE ATYPIA	37	56.8 ± 9.4 [b]	1329 ± 431[a]
MODERATE-MARKED ATYPIA	11	48.9 ± 6.9 [c]	1135 ± 388[b]
MARKED ATYPIA	10	44.1 ± 8.0 [c]	674 ± 382[c]
CARCINOMA IN SITU	9	45.0 ± 9.1[c]	790 ± 138[c]
INVASIVE CARCINOMA	50	45.7 ± 11.9 [c]	609 ± 424[c]

[a] No Significant difference from controls.

[b] Significantly different from controls (smoking and non-smoking) with p less than 0.02.

[c] Significantly different from controls with p less than 0.001.

Total Early T Cells/mm^3 in Peripheral Blood

Less prominent changes were noted for total early T cells. No differences were observed between normal controls, UGU and moderate atypia groups. (Table 6) Total T cells were decreased in the moderate-marked atypia group, but the difference was not statistically significant. Total T cells were profoundly decreased in the marked atypia and carcinoma in situ groups to approximately 50% of control values ($p < 0.001$). A further decrease was observed in the invasive carcinoma group with only $237 \pm 160/mm^3$ compared to control levels of $596 \pm 186/mm^3$ ($p < 0.001$).

Peripheral Blood B Cell Percentage

No significant differences were noted for the percentage of peripheral blood lymphocytes identifiable as B cells (EAC rosettes) between controls, atypias and lung cancer groups. (Table 7)

Total Peripheral Blood B Cells/mm^3

Decreased total B cells were observed in the marked and carcinoma in situ groups, but the differences were not statistically significant compared to controls. Total B cells were significantly decreased in the invasive carcinoma group with ($p < 0.001$.) (Table 7)

Rosette Inhibition by Anti-Thymocyte Serum

Results for the rosette inhibition test are expressed as the reciprocal of the dilution of ATS resulting in 25% or greater inhibition of rosette formation. These results are presented in Table 8 and depicted graphically in Chart 2.

The mean reciprocal inhibition titer for 57 normal controls was 352 ± 161, with 56 of 57 having titers of 640 or less. The mean reciprocal inhibition titer for the UGU control group was slightly increased at 427 ± 133, though this difference was not significant statistically. The narrow range over which values for inhibition titers occur for these two controls groups is clearly

TABLE 6. "EARLY" T CELL PERCENTAGE IN PERIPHERAL BLOOD OF PULMONARY METAPLASIA/NEOPLASIA PATIENTS

GROUP	N	% EARLY T CELLS ± S.D.	TOTAL EARLY T CELLS/mm^3 ± S.D.
CONTROL	57	26.3 ± 3.0	596 ± 186
UGU	32	25.8 ± 2.6 [a]	559 ± 128[a]
MODERATE ATYPIA	37	24.1 ± 3.7 [a]	556 ± 159[a]
MODERATE-MARKED TYPIA	10	21.0 ± 3.2 [b]	473 ± 183[a]
MARKED ATYPIA	11	17.5 ± 2.6 [c]	310 ± 163[c]
CARCINOMA IN SITU	9	18.0 ± 2.7[c]	321 ± 67[c]
INVASIVE CARCINOMA	50	17.6 ± 4.8[c]	237 ± 160 [c]

[a] No significant difference from control.

[b] Significantly different from controls (smokers) with p less than 0.005.

[c] Significantly different from controls with p less than 0.001.

TABLE 7. PERCENTAGE AND TOTAL PERIPHERAL BLOOD B CELLS IN PULMONARY METAPLASIA/NEOPLASIA PATIENTS

GROUP	N	PERCENT B CELLS ± S.D.	TOTAL B CELLS/mm^3 ± S.D.
CONTROL	57	23.5 ± 3.1	526 ± 160
UGU	32	23.7 ± 2.9	509 ± 116
MODERATE ATYPIA	37	24.6 ± 3.5	581 ± 219
MODERATE-MARKED ATYPIA	10	23.1 ± 2.7	529 ± 165
MARKED ATYPIA	11	24.3 ± 2.7	440 ± 237
CARCINOMA IN SITU	9	25.0 ± 2.7	456 ± 144
INVASIVE CARCINOMA	50	24.2 ± 4.1	312 ± 186[a]

[a] Significantly different from controls with p less than 0.001. All other groups were not significantly different fron controls.

depicted in Chart 2. In the absence of respiratory cytopathology rosette inhibition titers occur in a tight distribution.

The mean inhibition titer for the 37 persons with moderate atypia was significantly increased compared to controls being 1024 $\pm$ 931 ($p < 0.001$). As can be seen in Chart 2 there was a wide scatter of values in this group compared to the control distribution. One of the striking features of this wide distribution was the large degree of overlap with the control population. This observation was used to evaluate the moderate atypia group as two distinct populations, a larger population with normal inhibition titers, and a smaller population with very abnormal inhibition titers. The division into two separate populations was done empirically from the scattergram (Chart 2) with an arbitrary division being made at a reciprocal titer of approximately 800 (UGU mean plus two standard deviations). As shown in Table 8, subgroup A consisted of 22 individuals with a mean inhibition titer of 517 $\pm$ 173. This was not significantly different from the control groups. Subgroup B consisted of 15 individuals with a mean inhibition titer of 1766 $\pm$ 1090 which was significantly increased compared to control ($p < 0.001$). As seen in Chart 2, subgroup B contained several individuals with markedly abnormal inhibition titers.

The mean inhibition titer for the 10 individuals in the moderate-marked atypia group was 1439 $\pm$ 1140 which was significantly increased compared to controls ($p < 0.001$). The large standard deviation for this group results primarily from several individuals with markedly abnormal inhibition titers (5 with titers of 1800 to 3931).

The mean inhibition titers for the 11 persons with marked atypia and 9 with carcinoma <u>in situ</u> were virtually identical at 1839 $\pm$ 706 and 1826 $\pm$ 580 respectively. These means were very highly statistically significant compared to control ($p < 0.0001$). Chart 2 demonstrates no overlap between these two groups and controls, with most individual values being markedly elevated over controls.

TABLE 8. ROSETTE INHIBITION BY ANTI-THYMOCYTE SERUM IN PULMONARY METAPLASIA/NEOPLASIA PATIENTS

GROUP	N	INHIBITION TITER (RECIPROCAL) ± S.D.	RANGE
CONTROL	57	352 ± 161	120-853
UGU	32	427 ± 133[a]	157-864
MODERATE ATYPIA TOTAL	37	1024 ± 931[b]	235-4400
A	22	517 ± 173[a]	235-827
B	15	1766 ± 1090[b]	933-4400
MODERATE-MARKED ATYPIA	10	1439 ± 1140[b]	306-3931
MARKED ATYPIA	17	1839 ± 706[c]	800-2950
CARCINOMA IN SITU	9	1826 ± 580[c]	960-2810
INVASIVE CARCINOMA	50	2808 ± 1741[c]	421-6826

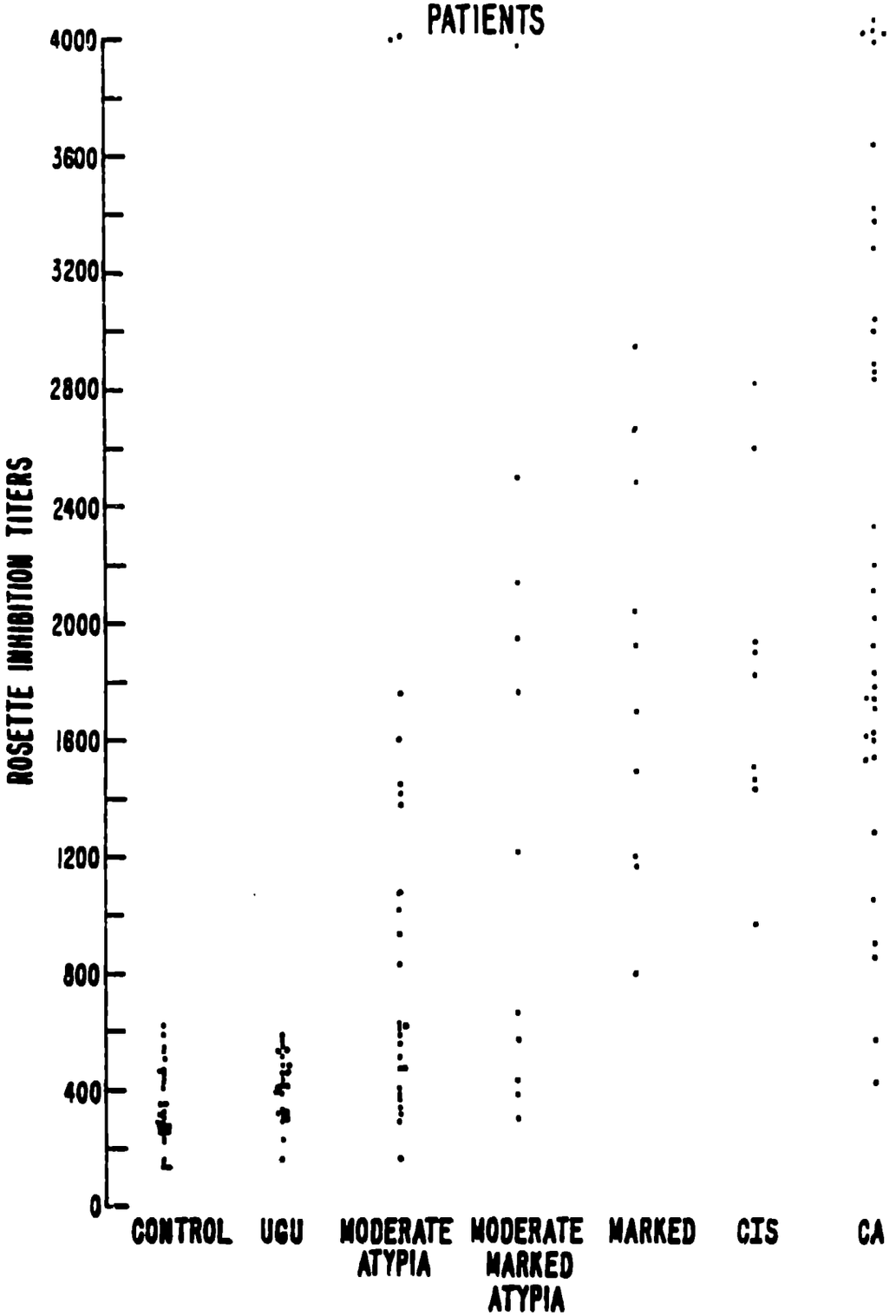
[a] No significant difference from controls.

[b] Significantly different from controls with p less than 0.001.

[c] Significantly different from controls with p less than 0.0001.

CHART 2: Reciprocal of the Rosette Inhibition Titers for Controls, Uranium Miners with Pulmonary Metaplasia, and Lung Cancer Patients. Each Point Represents One Individual.

ROSETTE INHIBITION TITERS IN PULMONARY METAPLASIA/NEOPLASIA PATIENTS



The mean inhibition titer for the 50 patients in the lung cancer group was markedly abnormal at 2808 ± 1741 compared to controls ($p < 0.0001$). Chart 2 shows that the large majority of individuals values were markedly abnormal, with only 5 of 50 with titers less than 1000.

Phytohemagglutinin Response of Peripheral Blood Lymphocytes

Lymphocyte transformation to PHA was measured by ^{3}H-Thymidine incorporation into DNA. The absolute response was expressed as counts per minute (CPM). Relative proliferative capacity was determined with the stimulation index which compares incorporation of stimulated cells with incorporation by resting cells. Results are presented in Table 9 and depicted graphically in Chart 3.

No significant difference was noted between normal controls and UGU. The mean CPM for the moderate atypia group was significantly depressed at 96,390 ± 13,822 compared to controls ($p < 0.05$). Further, progressive decreases were observed for the moderate-marked atypia, marked atypia and carcinoma in situ groups ($p < 0.005$). Though the mean values for these groups are very significantly different compared to controls, there is a wide range of values within each group as is reflected in the large standard deviations. This is graphically depicted in Chart 3, a scattergram of each individual PHA response. As can be seen, although the populations as a whole become progressively depressed compared to controls, individuals within each group overlap with the controls populations. This is particularly true for the moderate atypia group.

The PHA responses in the invasive cancer group are profoundly depressed at 43,302 ± 28,580 CPM compared to controls 115,816 ± 18,535 CPM ($p < 0.0001$). Chart 3 shows, however, that even in this group there is a wide range of responses with some overlap with controls.

The stimulation index follows a similar pattern to the absolute CPM with a progressive decline with progressive cytologic abnormalities.

TABLE 9. RESPONSE OF PERIPHERAL BLOOD LYMPHOCYTES TO PHYTOHEMAGGLUTININ AS MEASURED BY ^{3}H-THYMIDINE INCORPORATION INTO DNA IN PULMONARY METAPLASIA/NEOPLASIA PATIENTS

GROUP	N	COUNTS PER MINUTE, (CPM) ± S.D.	STIMULATION INDEX, (S.I.) ± S.D.
CONTROL	57	115,816 ± 18,535	221 ± 65
UGU	32	112,075 ± 15,021[a]	195 ± 43
MODERATE ATYPIA	37	96,390 ± 13,822[b]	176 ± 42
MODERATE-MARKED ATYPIA	10	76,219 ± 20,739[c]	138 ± 52
MARKED ATYPIA	11	63,911 ± 27,843[c]	112 ± 43
CARCINOMA IN SITU	9	62,010 ± 40,560[c]	84 ± 54
INVASIVE CARCINOMA	50	43,302 ± 28,580[d]	83 ± 52

[a] No significant difference from controls.

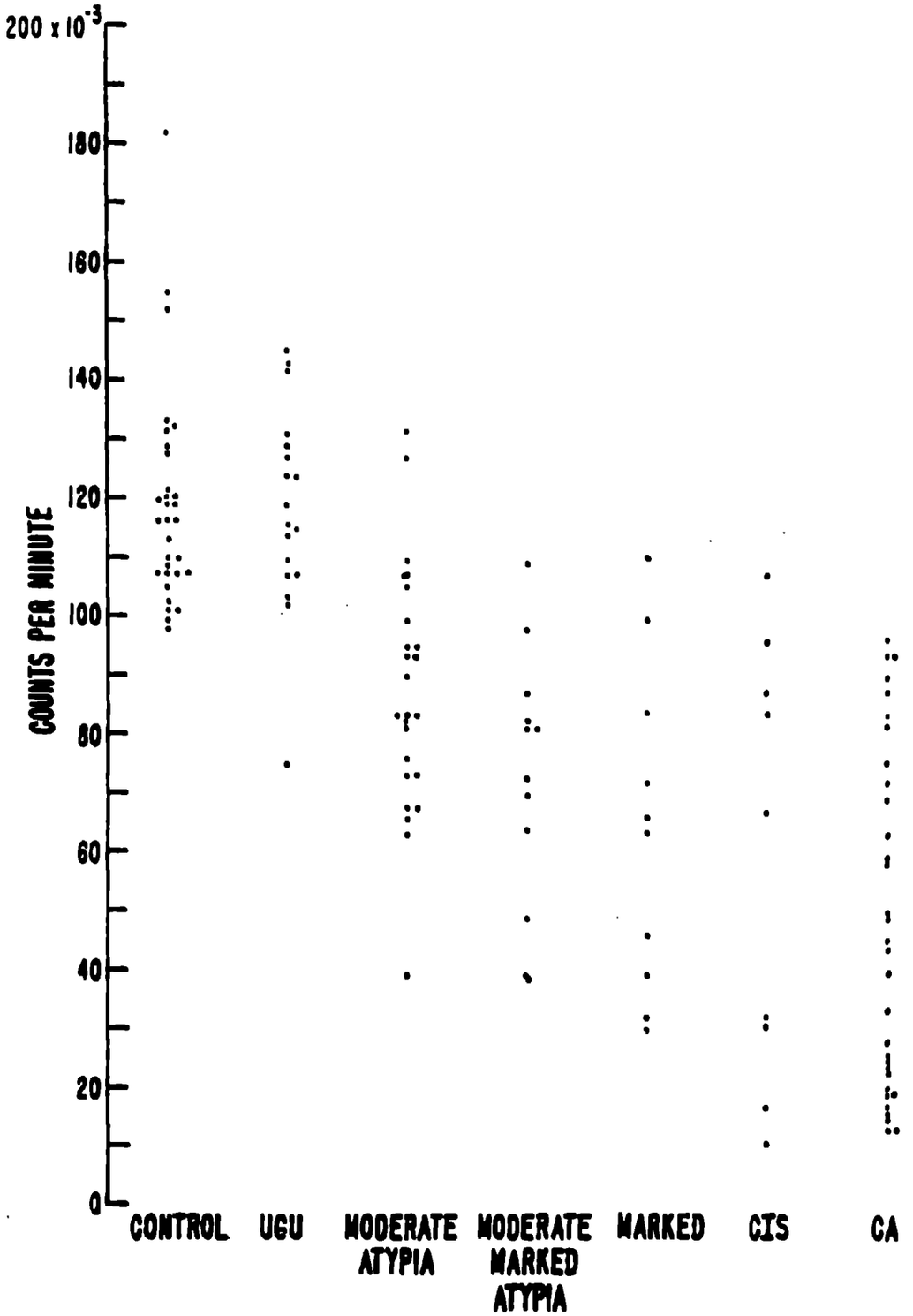
[b] Significantly different from controls with p less than 0.05.

[c] Significantly different from controls with p less than 0.005.

[d] Significantly different from controls with p less than 0.0001.

CHART 3: Phytohemagglutinin Response (CPM) in Controls, Uranium Miners with Metaplasia, and Patients with Lung Cancer. Each Point Represents One Individual.

PHA RESPONSE IN PULMONARY METAPLASIA/NEOPLASIA PATIENTS



Concanavalin A Response of Peripheral Blood Lymphocytes

As can be seen in Table 10, a similar progressive decline in Con A response occurred with increasing cytopathology. The moderate atypia group was significantly depressed at 52,739 ± 15,002 CPM compared to controls 66,660 ± 20,289 CPM ($p < 0.02$). A more marked depression to 36,284 ± 16,121 was observed for the moderate-marked atypia group ($p < 0.001$), a level similar to that observed for the marked atypia group. The Con A response was depressed to only 40% of control level in the carcinoma *in situ* group ($p < 0.001$), while the invasive carcinoma group mean response was less than one-third normal at 21,485 ± 16,539 CPM ($p < 0.001$).

The stimulation index followed a similar pattern with significant decrease observed in the moderate atypia group; more marked decreases in the moderate-marked and marked atypia groups; and the greatest depression in the carcinoma *in situ* and invasive carcinoma groups where the stimulation index was depressed equivalent amounts.

Pokeweed Mitogen Response of Peripheral Blood Lymphocytes

Abnormalities in PWM responsiveness can result from either abnormal B cell proliferative capacity, or from loss of T cell helper function, or a combination of both.

No differences were observed between normal controls, UGU and moderate atypia groups. (Table 11) A significant decrease was observed for the moderate-marked atypia group with a mean value of 25,571 ± 15,944 compared to controls 37,723 ± 10,527 ($p < 0.05$). A further decrease was noted for the marked atypia and carcinoma *in situ* groups, both being depressed to similar levels ($p < 0.02$). Marked depression of PWM response was observed for the invasive carcinoma group ($p < 0.001$).

The stimulation index changes paralleled those seen for absolute CPM with the exception that the indices for the carcinoma *in situ* and invasive carcinoma groups were closely similar.

TABLE 10. RESPONSE OF PERIPHERAL BLOOD LYMPHOCYTES TO CONCANAVALIN A AS MEASURED BY ^{3}H-THYMIDINE INCORPORATION INTO DNA IN PULMONARY METAPLASIA/NEOPLASIA PATIENTS

GROUP	N	COUNTS PER MINUTE (CPM) ± S.D.	STIMULATION INDEX, (S.I.) ± S.D.
CONTROL	57	66,660 ± 20,289	123 ± 37
UGU	32	62,940 ± 20,369[a]	110 ± 29
MODERATE ATYPIA	37	52,739 ± 15,002[b]	96 ± 25
MODERATE-MARKED ATYPIA	10	36,284 ± 16,121[c]	65 ± 28
MARKED ATYPIA	11	33,707 ± 22,903[c]	58 ± 30
CARCINOMA IN SITU	9	27,219 ± 19,054[c]	35 ± 21
INVASIVE CARCINOMA	50	21,485 ± 16,539[c]	41 ± 27

a No significant difference from controls.

b Significantly different from controls with p less than 0.02.

c Significantly different from controls with p less than 0.001.

TABLE 11. RESPONSE OF PERIPHERAL BLOOD LYMPHOCYTES FROM PULMONARY METAPLASIA/NEOPLASIA PATIENTS TO POKEWEED MITOGEN AS MEASURED BY INCORPORATION OF ^{3}H-THYMIDINE INTO DNA.

GROUP	N	COUNTS PER MINUTE, (CPM) ± S.D.	STIMULATION INDEX, (S.I.) ± S.D.
CONTROL	57	37,215 ± 9,758	70 ± 20
UGU	32	37,723 ± 10,527[a]	65 ± 18
MODERATE ATYPIA	37	36,304 ± 8,940[a]	67 ± 18
MODERATE-MARKED	10	25,571 ± 15,944[b]	45 ± 25
MARKED ATYPIA	11	20,981 ± 12,299[d]	38 ± 27
CARCINOMA IN SITU	9	21,963 ± 16,096[c]	28 ± 17
INVASIVE CARCINOMA	50	14,610 ± 11,517[d]	27 ± 18

a No significant difference from controls.

b Significantly different from controls with p less than 0.05.

c Significantly different from controls with p less than 0.02.

d Significantly different from controls with p less than 0.001.

Evaluation of Moderate Atypia Subgroups

Although the subdivision of the moderate atypia group into two subgroups was arbitrary based solely on rosette inhibition titers, the striking differences between the subgroups suggests that the division has validity. Table 12 presents all the immunologic data for the moderate atypia group as a whole and broken down into subgroups A and B. As can be seen in many instances mean values for a given test for the moderate group as a whole was not significantly different from controls. However, when subdivided into subgroups A and B, it is clear that 60% of the moderates (Subgroup A) did not differ from the controls, while 40% (Subgroup B) were significantly different. This was the case for total T cells, total early T cells and rosette inhibition titer.

Immune Function According to Histologic Type of Lung Cancer

Table 13 depicts results of immunologic testing in patients with lung cancer classifed according to histologic type. The predominant histologic type was squamous cell carcinoma accounting for 76% (38/50), with 14% being adenocarcinoma and 10% oat cell, or small cell undifferentiated, carcinoma. Statistical analysis was not performed because of the small numbers in the adenocarcinoma and oat cell groups.

For quantitative T and B cell parameters no difference was noted between the squamous and adenocarcinoma groups. Compared to these two groups, however, the oat cell group had decreased % and total T cells yet increased % and total early T cells. The small numbers involved do not permit any conclusions about the significance of this observation.

Although all functional parameters were markedly depressed for all histologic types compared to controls, the adenocarcinoma group had slightly better rosette inhibition and PHA responses than the squamous carcinoma group, while inhibition titer, PHA response and PWM response in the oat cell group were more abnormal than the squamous group.

TABLE 12. IMMUNOLOGIC PARAMETERS IN PATIENTS WITH MODERATE ATYPIA.

TEST	SUBGROUP A N = 22	SUBGROUP B 15	TOTAL 37
% T CELLS	59.8 ± 6.9	52.5 ± 11.0	56.8 ± 9.4
TOTAL T CELLS/mm^3	1467 ± 431	1126 ± 353	1329 ± 431
% EARLY T CELLS	24.8 ± 3.0	22.9 ± 4.3	24.1 ± 3.7
TOTAL EARLY T CELLS/mm^3	601 ± 170	497 ± 122	559 ± 159
% B CELLS	24.7 ± 3.6	24.4 ± 3.6	24.6 ± 3.5
TOTAL B CELLS/mm^3	616 ± 254	530 ± 149	581 ± 219
ROSETTE INHIBITION TITER	517 ± 173	1766 ± 1090	1024 ± 931
PHYTOHEMAGGLUTININ RESPONSE			
CPM	99,921 ± 9904	91,212 ± 17,198	96,390 ± 13,822
CONCANAVALIN A RESPONSE			
CPM	53,369 ± 12,061	51,813 ± 18,948	52,739 ± 15,000
POKEWEED RESPONSE			
CPM	36,397 ± 6659	36,161 ± 11,784	36,304 ± 8940

TABLE 13. IMMUNOLOGIC PARAMETERS IN LUNG CANCER PATIENTS ACCORDING TO HISTOLOGIC TYPE.

TEST	N =	TOTAL 50	SQUAMOUS 38	ADENO 7	OAT 5
% T CELLS		45.7 ± 11.9	46.5 ± 11.9	46.3 ± 11.0	38.4 ± 13.6
TOTAL T CELLS per mm^3		609 ± 424	604 ± 443	655 ± 362	576 ± 424
% EARLY T CELLS		17.6 ± 4.8	17.3 ± 4.3	17.4 ± 5.4	20.2 ± 7.2
TOTAL EARLY T CELLS/mm^3		237 ± 160	222 ± 149	264 ± 165	310 ± 242
R.I. TITER (RECIPROCAL)		2808 ± 1741	2637 ± 1591	2272 ± 1381	2916 ± 881
PHYTOHEMAGGLUTININ RESPONSE, CPM		43,302 ± 28,580	42,699 ± 27,905	57,085 ± 31,367	21,000 ± 15,108
POKEWEED MITOGEN RESPONSE, CPM		14,610 ± 11,517	15,106 ± 12,431	16,317 ± 8596	6891 ± 4395

Sensitivity of the Individual Immunologic Tests

To determine the relative sensitivity of the individual tests used in our immunoprofile, we determined the number of abnormal test responses in each group studied. An abnormal response was defined as any value differing from the control mean by more than 2 standard deviations of the control mean. These results are presented in Table 14.

In the control and UCU groups all test results fell within two standard deviations of the control mean with the exception of 1 abnormal % T cell value in each group and 1 rosette inhibition titer in the UCU group.

In the moderate atypia group the rosette inhibition titer was abnormal in 16 of 37, while the % T cell level was abnormal in 10 of 37. All other tests were only infrequently abnormal with 5 or fewer responses greater than two standard deviations from the control mean.

In the moderate-marked atypia group the rosette inhibition titer was again most frequently abnormal (7/10) with all other tests being abnormal in 5 or fewer individuals. In this group both % T cells and PHA response were abnormal in 5 of 10 patients.

In the marked atypia and carcinoma in situ groups the rosette inhibition titer was abnormal in all 20 individuals (11/11 marked atypia; 9/9 CIS). All other tests were considerably less sensitive. PHA response was abnormal in 60% of the individuals in these two groups (7/11 marked atypia; 5/9 CIS). Total T cells and total early T cells were abnormal in 11/20 each, while % T cells and Con A response were abnormal in half (10/20).

In the invasive carcinoma group, the rosette inhibition test was abnormal in 96% of patients tested (48/50). No other test approached this sensitivity. The PHA response was abnormal in 76% (38/50); Con A in 64% (32/50). Total T cells, total early T cells and PWM response were all abnormal in approximately 50% of the lung cancer patients. The least sensitive tests were % T cells with 38% abnormal; % early T cells with 28%; and total B cells at 24%.

TABLE 14. SENSITIVITY OF INDIVIDUAL IMMUNOLOGIC TESTS IN PULMONARY NETAPLASIA/NEOPLASIA PATIENTS

NUMBER OF PATIENTS WITH VALUES GREATER THAN TWO STANDARD DEVIATIONS FROM THE CONTROL MEAN

GROUP	N	% T	TOTAL T	% EARLY T	TOTAL T	TOTAL B	R.I. TITER	PHA, CPM	CON A, CPM	PWM, CPM
COBTROL	57	1	0	0	0	0	0	0	0	0
UGU	32	1	0	0	0	0	1	0	0	0
MODERATE ATYPIA	37	10	5	3	1	0	16	4	1	1
MODERATE-MARKED	10	5	4	2	3	0	9	5	3	-
MARKED ATYPIA	11	5	6	4	6	1	11	7	5	5
CARCINOMA IN SITU	9	5	5	4	5	0	9	5	5	4
INVASIVE CARCINOMA	50	19	25	14	24	12	48	38	32	26

If the marked atypia, carcinoma in situ and invasive carcinoma groups are considered together as the "neoplastic" groups, one notes that the rosette inhibition test was abnormal in 97% (68/70), compared to 71% for PHA response (50/70) the next most frequently abnormal parameter. Con A was abnormal in 60% (42/70), while total T cells, total early T cells and PWM response were all abnormal in 50% of cases. The remaining tests were relatively insensitive in detecting abnormalities in these individuals with neoplastic lesions, being abnormal in only 40% or fewer cases.

DISCUSSION

Many investigators have documented abnormal immune function in patients with carcinoma of the lung. [7,10-15] The results reported here however, represent the first attempt to apply immunologic assessment to individuals with apparent preneoplastic lesions of the respiratory tract. We feel that the results obtained clearly indicate that such an approach can be of great value diagnostically and predictively in high risk population groups.

In assessing the effect of smoking on immune function we found no significant reduction in immunocompetence between smoking and non-smoking controls. We did observe, however, increased total T cells, total early T cells and total B cells in the heavy smoking group. These increased quantitative values associated with heavy smoking may result from the chronic irritation and inflammation induced in the bronchial tree by cigarette smoke. Although increased numbers of T and B cells were observed, the function of these subpopulations as measured by rosette inhibition titer, PHA and PWM response was if anything slightly depressed compared to non-smokers. These qualitative changes were mild and not statistically significant however. These data provide no evidence that smoking per se impairs immune competence.

To assess the effect of isolated uranium mining exposure on immune function, we studied non-smoking uranium miners with known normal or mildly atypical sputum cytology. The carcinogenic promoter associated with uranium mining

is not conclusively known, though extensive epidemiologic studies have demonstrated a strong correlation between lung cancer and duration and amount of exposure in the mines to alpha radiation from radon daughters.[22] We found, however, that there were no observable differences in immune function between non-smoking miners and controls, regardless of the degree of exposure. This was true, of course, only for those individuals with normal or mildly atypical sputum cytologies. Minimal cellular changes, such as mildly atypical squamous metaplasia, are usually the result of inflammation and are reversible when the inflammatory agent is removed. The finding of normal immune function suggests that these mild changes are a local reaction to inflammation, while higher degrees of atypia represent carcinogenic changes associated with systemic as well as local effects. Uranium miners with normal cytologies who also smoked had immunologic profiles which did not differ significantly from controls or from non-smoking miners. However, when these miners were evaluated according to their smoking habits an interesting observation was made. Total T cells were moderately decreased and the rosette inhibition titer slightly elevated in the light and moderate smoking groups compared to controls and non-smoking miners. In the heavy smoking group of miners, total T cells were elevated compared to non-smoking miners, but this increase was considerably blunted compared to the increase observed in the control heavy smokers. Functionally, the rosette inhibition titer was elevated in all the smoking miner groups, with the greatest change observed in the heavy smokers. This difference almost reached statistical significance when compared to non-smoking controls. The quantitative increase in T and B cells is blunted in the uranium miners, while functional competence is decreased to a slightly greater extent than in smokers alone. Clearly more individuals in each category have to be studied before any conclusions can be reached. It is also

clear that the immune changes observed in the groups of uranium miners with pulmonary cytopathology were specific to the pathologic changes, with at most, only minor contributions from the exposure to carcinogens per se.

Immunologic abnormalities were detected in significant proportions in the groups with dysplastic or neoplastic changes on sputum cytologic exam. In the moderate atypia group immunologic abnormalities were noted in 20-40% of those studied depending on the test, with the most striking changes seen with the rosette inhibition test. Indeed, on the basis of this test we detected what we believe are two distinct subpopulations within the moderate atypia group. Approximately 40% had abnormal rosette inhibition titers associated with abnormalities in the other immunologic parameters. On the other hand, the 60% with normal inhibition titers resembled the control populations closely with all other immunologic parameters being normal. Interestingly, the general impression of Saccomanno and his colleagues [3,4] from data accumulated over many years and the examination of approximately 150,000 sputum samples is that mild atypical squamous cell metaplasia is a common product of pulmonary inflammatory disease of any origin, although it may represent the initital response to carcinogens. In a specific individual case it is impossible to determine whether inflammatory or carcinogenic agents induce these changes. Further, the analysis of many cases showing moderate atypical squamous cell changes revealed that both inflammatory and carcinogenic effects are involved. An estimated 70-80% of the moderate atypical changes are due to inflammatory effect and are probably reversible with no further progression of abnormality or disappearance of the abnormal cells from sputum samples and reversion to normal histology. In the remaining 20-30% the moderate atypia is probably due to carcinogenic effect and as such is not reversible. If followed sequentially, the great majority of this subgroup will develop progressively,

cytopathologist involved in this study that marked atypia actually represents an irreversible step in the progression to carcinoma and should be considered a neoplastic rather than a late dysplastic or premalignant lesion. The immunologic data strongly support this view and suggest that the cytologic criteria for differentiating premalignant from malignant cells is incomplete.

The findings in the lung cancer group confirm previous studies reporting marked abnormalities. Even in the presence of established cancer, however, there is considerable variability in the results of individual tests. Results of the rosette inhibition test were the most strikingly abnormal in the lung cancer patients, with titers greater than two standard deviations from the control mean in 48 of 50 patients (96%). This high degree of sensitivity far exceeded any other individual parameter and confirmed previous findings in an earlier study of localized lung cancer. Gross, et. al.[7] demonstrated markedly abnormal rosette inhibition tests in 28 of 29 (97%) patients with undiagnosed coin lesions of the lung on chest x-ray. At thoracotomy these patients were found to have lung cancer. In patients with normal rosette inhibition tests and coin lesions on x-ray, benign tumors were found at surgery. This earlier study and the one reported here considered together, provide the impressive finding that 76 of 79 patients with early, localized lung cancer had abnormal rosette inhibition tests. With this high degree of predictability in early cancer, it is not surprising that the test detects changes in patients with preclinical or even preneoplastic lung lesions. Indeed, in all 20 patients with marked atypia or carcinoma <u>in situ</u> the rosette inhibition test was abnormal.

We feel that these results provide compelling evidence that immunologic defects are present very early in the development of lung cancer. Abnormalities in the moderate atypia group suggest that either detectable immunologic changes occur at the very earliest stage of committed progression to cancer as a result of carcinogenic effect on the bronchial epithelium, or that underlying immunologic defects act as permissive changes selecting or predisposing

more abnormal sputum cytology with eventual development of lung cancer. With cytologic exam alone, moderate atypia is not recognizable as to whether it will develop into neoplasia. The results with the immunologic tests, in particular the rosette inhibition test, give the strong impression that they may be identifying individual cases in which the cellular changes are due to carcinogenic effect and which are committed to gradual progression to malignancy. If this finding is corroborated in further sequential study of these patients, this could represent a major argument for the inclusion of detailed immunologic assessment in any patient with moderate atypia on sputum exam. It also appears that the immunoprofile used remains normal when moderate atypia is due to inflammation alone regardless of cause. Further study is required to determine whether this conclusion is valid. It is tempting to suggest however that these immunologic tests become abnormal and thus predictive only when metaplastic changes have occurred which commit the abnormal cells to the path leading to neoplasia. In this regard, it is interesting that the mean rosette inhibition titer of the abnormal subgroup in the moderate atypias was equivalent to that observed for the marked atypia and carcinoma <u>in situ</u> groups.

Individuals with marked atypia all had markedly abnormal immunoprofiles. Of the tests used only % and total B cells were not significantly depressed in the group as a whole. In particular, the rosette inhibition test was markedly abnormal with no overlap in values with the control groups. 80-90% of those with marked atypias on sputum exam progress to develop lung carcinoma. Virtually all patients with cells characteristic of carcinoma <u>in situ</u> on sputum exam progress to invasive carcinoma. Immunologically all of these patients were markedly abnormal, especially in their rosette inhibition titers. For the rosette inhibition test we detected no difference between the marked atypia and carcinoma <u>in situ</u> groups. It has been the feeling of the

individuals exposed to carcinogens to irreversible progression to neoplasia. The answer to whether the immunologic changes are primary or secondary to the development of neoplastic cells awaits further prospective sequential studies of this uranium miner population with emphasis on the immunologic and cytologic study of those individuals currently with normal sputum cytology.

The conclusions regarding the presence of immunologic abnormalities in preneoplastic states and the usefulness of the rosette inhibition test were confirmed in another human cancer by La Via's group in Atlanta.[23] This group of investigators studied rosette formation and inhibition in patients with dysplasia and carcinoma in situ of the cervix. The rosette inhibition test was significantly depressed in the group with moderate dysplasia, a premalignant lesion. Similar to our findings, marked abnormalities were observed in those patients with severe dysplasia or carcinoma in situ, the two groups being indistinguishable from each other.

Clearly, the rosette inhibition test is capable of measuring changes associated with cells having undergone neoplastic transformation. It is not yet known what the rosette inhibition test measures, although it seems likely that either functional competence of T cells represented by high affinity or high density receptors for SRBC, or a subpopulation with some, as yet, undefined characteristic extremely sensitive to the presence of malignancy is being measured. With the potential clinical usefulness of this test, further investigation into the mechanisms of action involved is required. Until these mechanistic studies become available, the rosette inhibition test can be considered a very sensitive marker for malignancy whose exact significance is unknown.

With combined cytologic and immunologic evaluation we are capable of more precisely identifying those individuals at highest risk of progressing to lung cancer. With the known latency periods for progression from moderate atypia to invasive carcinoma (4.8 years),[3] this early identification could

conceivably result in ample time for the localization of lesions and institution of definitive therapy. If future results in this population group continue to be as encouraging and striking as at present, the role of early institution of immunotherapy should be evaluated. The idea that manipulation of the immune system during those early periods of neoplastic development might alter the progression of cytologic abnormalities and the ultimate expression of malignancy is exciting.

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