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Influence of Non-Specific Immunologic Factors on Prognosis in Advanced Bronchogenic Carcinoma

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Summary. *Fifty-nine evaluable patients with stage III bronchogenic carcinoma, participating in a randomized clinical trial evaluating the effect of adjuvant immunotherapy with levamisole or BCG in the treatment of clinically advanced lung cancer, were studied for their immunocompetence by in vitro and in vivo assays. Immunological tests consisted of measurements of natural killer (NK) cell and killer (K) cell cytotoxicity, skin testing reactivity to recall antigens, absolute lymphocyte count, and serum immunoglobulin (Ig) levels. Pretherapy K cell cytotoxic levels, skin test reactivity to trichophyton antigen, and increased IgA levels were predictive of the overall clinical course. Despite non-specific immunotherapy, progressive decline of NK and K cell cytotoxicity occurred during the course of the disease. These findings, however, were of limited clinical value. Initial performance status and disease extent significantly influenced time to progression and survival. Little further prognostic information was obtained from the immunological tests over those provided by clinical performance status and disease extent. No statistically significant differences were found in either time to progression or survival between controls and patients receiving either levamisole or BCG.*

Introduction

Defective cell-mediated immunity (CMI) has been described in patients with malignant disease [5, 8–10, 12, 13, 15, 16, 23–28]. In early and advanced stages of lung cancer, skin test reactivity and lymphoproliferative response to mitogens and antigens discriminated between patients with different prognoses [2–4, 8, 13, 15]. In patients with limited or extensive small cell carcinoma, increased NK cell cytotoxicity preceded and accompanied recurrent disease [11].

The present study of patients with bronchogenic carcinoma was designed to determine whether natural killer (NK) and killer (K) cell cytotoxicity and other immune parameters added prognostic information to those provided by host (performance status) and disease (cell type and tumor extent) characteristics known to affect the overall clinical course. Since these patients received BCG or levamisole, we also analyzed immunologic function and prognosis to determine whether they were altered by adding non-specific immunotherapy to chemotherapy with or without radiation therapy.

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Patients and Methods

Sixty-nine patients, who had biopsy-proven lung cancer of various histologic types either limited to one hemithorax and ipsilateral mediastinal nodes (limited disease, LD) or involving distant areas (extensive disease, ED) were treated with combination chemotherapy with or without radiation therapy and randomized to receive no further therapy, BCG, or levamisole.

Eligible patients had measurable or evaluable disease, had performance status of 0, 1, or 2 (ECOG scale), were less than 70 years of age, and had not received prior immunotherapy. Ten of the 69 patients initially randomized between levamisole, BCG, or no immunotherapy were excluded from the final analysis. Six of these patients did not satisfy the eligibility criteria, three patients were not evaluable because of major protocol violations, and one was excluded because of death from myocardial infarction 2 days after entry into the study. The ten exclusions were evenly distributed among the randomized arms. Of the final 59 evaluable patients, 16 had limited and 43 extensive disease. Nine patients with small cell carcinoma (SCC) all had extensive disease. The numbers of patients of performance status 0, 1, and 2 were 18, 32, and 9, respectively; 25 patients were randomized to receive no immunotherapy, 20 patients levamisole, and 14 patients BCG.

Immunological pretreatment assessment consisted of evaluation of NK and K cell cytotoxicity, measurement of absolute lymphocyte count, evaluation of delayed cutaneous hypersensitivity to recall antigens, and determination of serum immunoglobulin (Ig) levels. NK and K cell functions were re-evaluated after the completion of radiation therapy in patients with LD, at the end of the first cycle of chemotherapy in patients with ED, and at the time of progression of disease. These cytotoxic functions were measured in a 5-h ^{51}Chromium-release assay on frozen and stored lymphocytes as previously described [6]. Frozen peripheral mononuclear blood cells were obtained from each individual donor prior to and during therapy. All samples from an individual patient were tested in parallel in a single assay for NK and K cell cytotoxicity. NK cell activity was measured against the human tumor myeloid cell line K562 and against the human bronchogenic cell line A549. K cell activity was assayed using Chang cells (originated from normal human hepatocytes) as targets. Rabbit anti-Chang cell serum, kindly provided by Dr Steven Shore, was used at the final dilution of 1 : 10,000. Antigens used for skin tests, candida (Hollister-Stier, Downers

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Grove, IL, USA), mumps (Eli Lilly, Indianapolis, IN, USA), streptokinase-streptodornase (Lederle), trichophyton (Hollister-Stier, Downers Grove, IL, USA), and purified protein derivative of tuberculin (Merck, Darmstadt, FRG; Sharp & Dome, West Point, PA, USA), were administered as previously described [14]. Induration of diameter > 5 mm recorded at either of two measurements (24 and 48 h) was scored as a positive response. Reactivity to at least one of the antigens administered was considered a non-anergic response. Serum immunoglobulin levels (IgG, IgA, IgM) were measured by radioimmunodiffusion using the Mancini method.

Patients were randomized by balanced block design with stratification for disease extent to receive levamisole, BCG, or no immunotherapy with chemotherapy. The chemotherapy regimen (CCM) given in 8-week cycles consisted of CCNU (70 mg/m^2) PO on day 1, and cyclophosphamide (500 mg/m^2) IV with methotrexate (15 mg/m^2) IV on days 1, 8, 29, and 36. Patients with LD received radiation therapy in addition to chemotherapy. A total dose of 5,500 rads was given on a 'split course'. Following a 3-week rest period, chemotherapy was started. Two-thirds of the calculated drug doses were given on the first cycle following radiation therapy. Levamisole (100 mg/m^2 PO) was administered for 3 consecutive days every 2 weeks during radiation therapy and then on days 10, 11, 17, 18, 24, 25, 38, 39, 45, 46, 52, and 53 of each chemotherapy cycle. BCG (Tice stain 0.3 ml of a preparation with 2×10^8 colony-forming units/ml) was given by the tine technique every 2 weeks during radiation therapy and then on days 15, 22, 43, and 50 of each cycle of chemotherapy. The maximum duration of immunotherapy with either levamisole or BCG was 6 months. Time to progression and survival was measured from the onset of therapy.

Estimates of the time to progression and time of survival were calculated by the Kaplan-Meier method. For tests of time to progression and duration of survival, the log-rank test [19] was used for two groups, a Jonckheere-type log-rank test [7] was used to test for trend in performance status, and Spearman's rank correlation coefficient [17], adjusted for censored data in the manner of Prentice [22], was used to test for association with variables measured on a continuous scale (NK and K cell cytotoxic levels, absolute lymphocyte count, serum Ig value). For contingency table analysis, Fisher's exact test was employed.

Immunologic functions were tested with the Wilcoxon signed-rank test [17] for paired data, the Mann-Withney test [17] for unpaired data, and the rank correlation coefficient test for association of continuous data. Unless otherwise noted, only P-values corresponding to tests between therapy groups were adjusted by stratifying subjects by PS and disease extent. All P-values correspond to two-sided tests.

Results

Relationship of Immune Reactivity with Disease Extent and Patient Prognosis

Pretreatment absolute lymphocyte count failed to show any correlation with tumor extent and patient prognosis ($P > 0.15$ for the comparisons tested). There were equal proportions of positive responses to PPD, candida, mumps, and SK-SD in the group of patients with ED and in the group with LD. The two groups were also equally represented by non-anergic patients ($P > 0.2$ for all the comparisons tested). A significant decrease in the number of patients reactive to trichophyton antigen occurred in patients with ED as against those with LD ($P < 0.05$). Reactivity to trichophyton skin test antigen was also predictive of time to progression ($P < 0.10$) and survival ($P < 0.05$). Median time to progression and median survival time were 70 and 140 days, respectively, for patients anergic to trichophyton, and 140 and 410 days for reactive patients. The other antigens, either as a group or singly, were not predictive of time to progression or time of survival ($P > 0.2$ for all the combinations of patients compared).

Serum immunoglobulins were determined in 26 patients. Increased levels of IgA (> 330 mg/100 ml, normal range 67–330 mg/100 ml) were observed in 18 patients. To see whether these high IgA values were characteristic of other types of patients, two concurrent control groups of 26 age-matched patients were used for comparisons. One was of patients hospitalized for benign disease (11 patients with increased levels) and a second was of patients hospitalized for malignancies other than lung cancer (10 patients with elevated levels). Median values of IgA were 370 (range 150–930), 290 (40–630), and 280 (60–770) mg/100 ml for lung cancer patients, patients with malignancy other than lung cancer, and patients with benign disease, respectively. IgA levels in patients with bronchogenic carcinoma were higher than those in the other two groups ($P < 0.01$ for comparison of bronchogenic carcinoma with non-lung malignancy; $P < 0.1$ for bronchogenic carcinoma against benign disease), suggesting a possible association between this abnormality and lung cancer. High values of IgA in the lung cancer patients were associated with a longer time to progression ($r = 0.4$; $P < 0.01$) but not with longer survival. These findings were not associated with quantitative abnormalities of the other two classes of Ig measured (25 and 21 of these 26 patients had IgG and IgM within the normal ranges, respectively), nor did correlation exist between overall patient prognosis and IgG or IgM levels ($P > 0.2$ for all the comparisons tested).

A wide range of basal NK and K cell cytotoxic levels was observed on pretherapy samples obtained from 50 of the 59 evaluable patients. Overall values of the two cytolytic functions were significantly correlated with each other ($r = 0.4$ and $P = 0.01$ for all the combinations tested). A trend towards higher cytotoxic levels against K562 cells in patients with more advanced disease was observed (Table 1). No relationship existed between tumor extent and pretherapy NK cell cytotoxicity against A549 cells or K cell activity against Chang cells. A significant correlation was found between K cell activity and length of survival (Table 1). However, pretherapy NK cell activity for K562 or A549 cells did not reflect or predict the clinical course.

To further investigate the relationship between cell mediated cytotoxicity (CMC) and the evolution of disease, NK cell and K cell cytotoxicities were measured after completion of radiation therapy or at the end of the first chemotherapy cycle and at the time of disease progression. Because of low basal values of cytotoxic activity against A549 cells, variations of this function during the course of the disease were not considered representative of real modifications and were not statistically analyzed. Progressive decreases in NK and K cell cytotoxic activities compared with pretreatment values occurred during the course of disease (Fig. 1). This occurred in patients with progressive disease as well as in patients with stable disease. Prior to the second measurement of NK and K cell activities, all patients had received radiotherapy or chemotherapy. Thus the decline in CMC probably in part resulted from these therapies.

Table 1. Pretherapy NK and K cell cytotoxic levels[a] according to tumor extent

Assay	% Specific ^{51}Cr release	Disease extent[b]				Median survival[c]	
		Limited		Extensive			
NK cell cytotoxicity – K562 target cells	< 5.5	5		9		140	
	5.5–14	8	$P < 0.1$	12		260	$P > 0.4$
	> 14	2		14		130	
NK cell cytotoxicity – A549 target cells	< 2	4		11		340	
	2–5	5	$P > 0.6$	13		100	$P > 0.2$
	> 5	6		11		250	
K cell cytotoxicity – Chang target cells	< 8	6		10		130	
	8–14	4	$P > 0.4$	12		180	$P < 0.05$
	> 14	5		13		280	

[a] The median and range values of pretherapy NK and K cell cytotoxicity (expressed as percentage of ^{51}Cr release) measured in 50 patients were: 8% (−1% to 64%) for NK cell cytotoxicity for K562 target cells; 3% (−7% to 27%) for NK cell cytotoxicity for A549 target cells; 11% (−1% to 50%) for K cell cytotoxicity for Chang target cells. Effector cells (frozen and stored comparably to those from the patients) from two normal donors were tested in parallel with patient effector cells in the cytotoxic assays. The median and range percent ^{51}Cr release values for these two normal donors were: NK cell cytotoxicity against K562 cells 27% (14%–62%) and 18% (12%–53%); NK cell cytotoxicity against A549 cells 5% (−2%–25%) and 15% (7%–24%); K cell cytotoxicity against Chang cells 9% (5%–29%) and 35% (24%–59%)

[b] Compared by Mann-Whitney test

[c] Compared by Spearman's rank correlation test

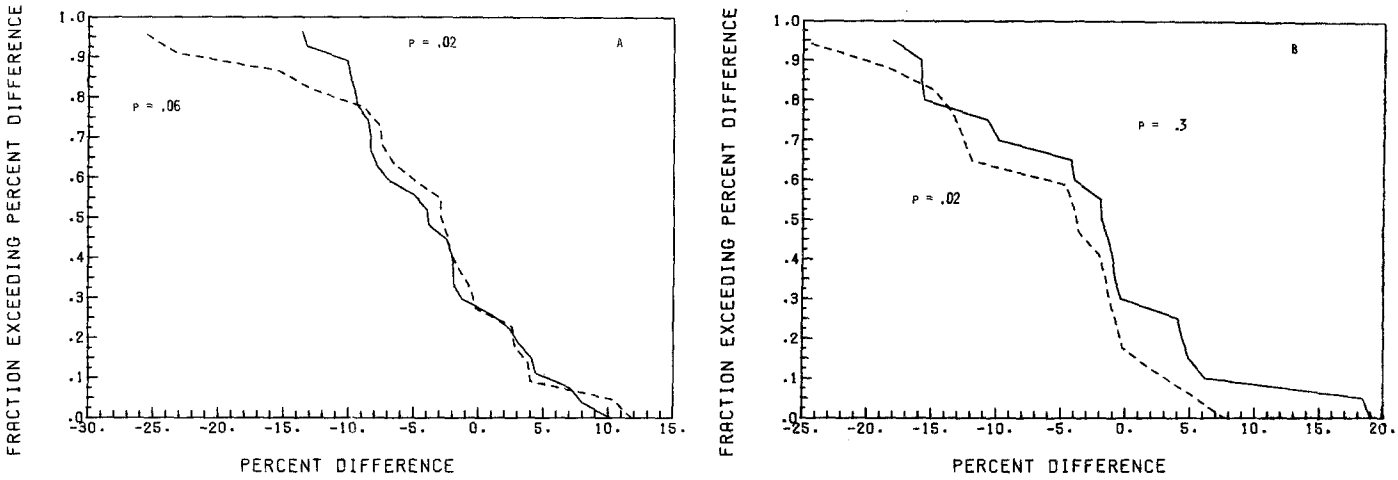

Fig. 1. A and B Distributions of differences in NK and K cell cytotoxicities during the course of the disease. Differences between the pretherapy cytotoxic values and those observed after completion of radiation therapy or at the end of the first chemotherapy cycle for patients without evidence of progressive disease (– – –), and at the time of progression (———). All differences are arithmetic (posttherapy % ^{51}Cr release minus pretherapy % ^{51}Cr release). Pretreatment compared with first posttreatment measurement for K562 NK cell activity, $P = 0.06$; Pretreatment compared with progression measurement for K562 NK cell activity, $P < 0.025$; Pretreatment compared with first posttreatment measurement for Chang K cell activity, $P < 0.025$; Pretreatment compared with progression measurement for Chang K cell activity, $P < 0.3$

Time to Progression an Survival According to Clinical Prognostic Factors and Immunotherapy Regimens

The influence of cell type, disease extent, and performance status on time to progression and survival was examined. Histology did not influence patient prognosis. Patients with LD had a longer time to disease progression (180 vs 65 days for MPT, $P < 0.01$) and survived longer (310 vs 150 days for MST, $P < 0.01$) than those with ED (Fig. 2A). Similarly, performance status (ECOG 0, 1, or 2) was predictive of time to progression (140 vs 80 vs 30 days for MPT, $P < 0.001$) and survival (260 vs 160 vs 70 days for MST, $P < 0.005$) (Fig. 2B).

Time to progression and survival were somewhat longer for patients receiving either levamisole or BCG than for patients not receiving immunotherapy. Patients receiving no immunotherapy had MPT and MST of 65 and 150 days (Fig. 3). Those patients receiving levamisole had MPT and MST of 80 ($P = 0.25$ compared with those not receiving immunotherapy) and 210 ($P = 0.31$) days, respectively, those receiving BCG, 100 ($P = 0.16$) and 260 ($P = 0.09$) days. The length of time to progression and survival in this study was comparable to that of other studies involving patients with advanced bronchogenic carcinoma [18, 21]. Similar results were obtained when these analyses were repeated separately for the groups of patients with LD and ED. The exclusion of the nine patients with SCC did not change the overall analysis. When sites of disease progression (failure in the primary lesion, clinical metastases, or appearance of new metastatic sites) were compared among patients randomized to receive

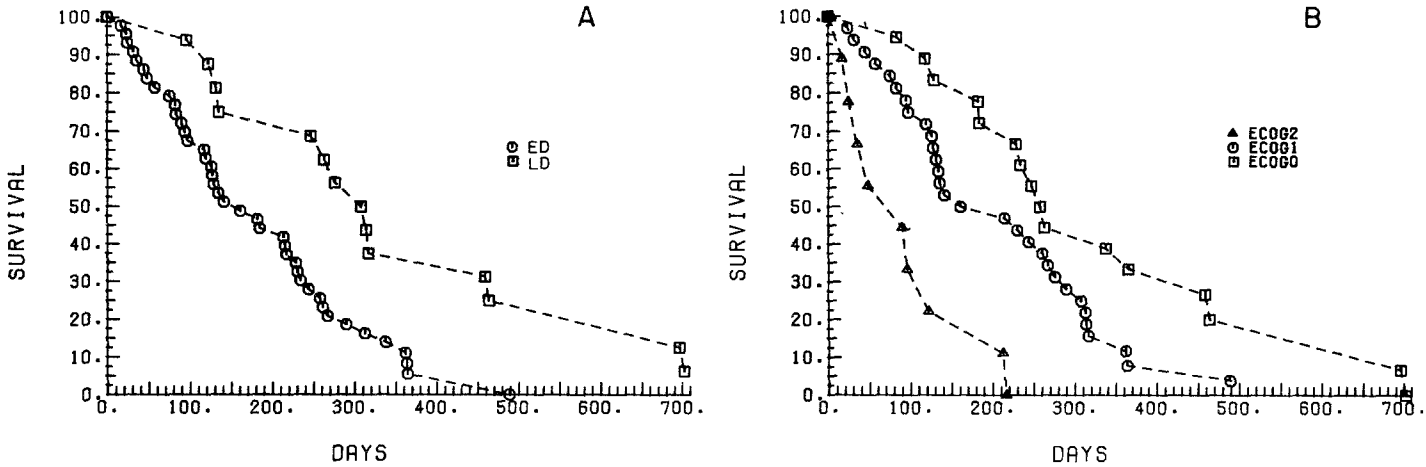

Fig. 2. A and B Survival curves according to tumor extent (**A**) and performance status (**B**). The numbers of patients with limited and extensive disease in the therapy arms were, respectively, 6 and 19 (no immunotherapy), 5 and 15 (levamisole), 5 and 9 (BCG). The number of patients in the various performance status categories and with SCC were evenly distributed among the randomization arms. **A)** ○———○ extensive disease; □———□ limited disease; **(B)** ECOG performance status: (△———△) 2; (○———○) 1; (□———□) 0

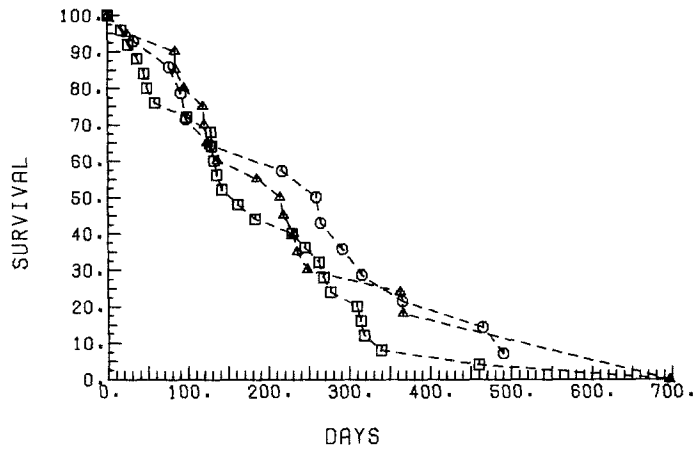

Fig. 3. Survival by randomized treatment. (□———□) no immunotherapy; (△———△) levamisole; (○———○) BCG, Log-rank comparisons of survival: BCG vs no immunotherapy, $P < 0.1$; levamisole vs no immunotherapy, $P < 0.35$

the different treatments, no significant differences existed between the treatment groups.

Side-effects of CCM chemotherapy were myelosuppression and gastrointestinal toxicity. Leukopenia was observed in 29 patients, thrombocytopenia in eight patients and gastrointestinal toxicity in 30 patients (four patients with LD who progressed while receiving radiation therapy were excluded from the analysis of toxicity from chemotherapy). Comparisons of hematologic and gastrointestinal toxicity rates among controls and patients receiving adjuvant immunotherapy with either BCG or levamisole did not show any statistically significant difference, with the exception of a higher incidence of thrombocytopenia in patients receiving BCG ($P < 0.05$). Toxicity from levamisole was limited to nausea and vomiting in eight patients. In two of these, the severity of symptoms required discontinuation of the drug. Nine patients receiving BCG had local reactions (consisting of erythema, induration, and pruritus at sites of BCG vaccination). One patient developed secondary infections at sites of BCG inoculations and a second developed erythema multiforme necessitating discontinuation of BCG.

Discussion

Antibody-dependent CMC (K cell cytotoxicity), serum IgA, and reactivity to trichophyton antigen on skin testing were predictive of overall clinical course in a group of patients with bronchogenic carcinoma. However, the differences found in time to progression and survival between groups of patients defined by increased K cell activity and trichophyton reactivity were not significant after adjusting for imbalances in clinical performance status and disease extent among groups of patients. Increased IgA, while predictive of time to progression after adjustment for performance status and disease extent, did not predict survival. Thus, these pretreatment immunologic function tests were of limited clinical value in predicting prognosis.

A decline of NK cell and K cell activity was observed during the course of the disease. This probably resulted from the immunosuppressive effect of chemotherapy and progressive tumor. This decline in immunologic reactivity occurred in all the randomized treatment arms. It thus was not prevented by BCG or levamisole.

Immunological parameters examined were not correlated with tumor extent. The lack of relationship between immune reactivity and tumor burden possibly reflected the narrow difference between groups of patients with advanced disease. All the patients included in the present study had, in fact, stage III lung cancer. Greater differences in immunoreactivity might be expected if patients with more limited disease were compared to advanced disease.

Although not statistically significant, both immunotherapy regimens produced a prolongation of time to progression and survival. Evidence for the role of host response on the clinical course of patients with lung cancer is provided by the beneficial effects obtained with a nonspecific immunotherapy in patients with resectable tumors [1, 20]. Thus, further assessment of immunomodulation in combination with more effective regimens in advanced lung cancer patients may prove benefical.

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