Cellular Immunity in Breast Cancer Patients Completing Taxane Treatment

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ABSTRACT

Purpose: **A field study of postchemotherapy immune functioning relative to the use of taxanes is reported. Immune responses in breast cancer patients were analyzed as a function of whether patients received taxane as part of their adjuvant chemotherapy.**

Experimental Design: **Immune levels of 227 stage II/III breast cancer patients were measured immediately after surgery prior to chemotherapy and again 12 months later when all chemotherapies had been completed. T-cell blastogenesis and natural killer (NK) cell lysis levels of patients receiving taxanes ($n = 55$) were compared with levels of patients not receiving taxanes ($n = 172$).**

Results: **Regression analyses were conducted. The administration of taxane as part of combination chemotherapy predicted increased T-cell blastogenesis and NK cell cytotoxicity after the conclusion of all chemotherapies. For the Taxane group, average phytohemagglutinin-induced blastogenesis was 37% higher and NK cell cytotoxicity was 39% higher than the values for the No-Taxane group.**

Conclusions: **Data from group comparisons with appropriate controls in a sizable clinical sample contravene traditional wisdom that taxanes suppress patients' immune cell functions. Problems in generalizing direct-contact laboratory models to the field of cancer treatment are highlighted.**

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INTRODUCTION

The taxanes represent a class of chemotherapeutic agents that have shown promise in a variety of human neoplasms, most noticeably in breast cancer patients requiring adjuvant therapy or treatment for metastatic disease. Paclitaxel (Taxol) and docetaxel (Taxotere) are the two taxanes used most frequently in the clinical setting. Both exhibit significant activity as single agents; however, they have shown greatest efficacy when used in combination with other cytotoxic compounds (1). A frequently used adjuvant regimen in women with node-positive tumors is the administration of four cycles of doxorubicin in combination with cyclophosphamide followed by four cycles of paclitaxel (2). Paclitaxel was initially identified in the bark of the Pacific yew tree *Taxus brevifola*, and docetaxel was generated from the needles of the European yew *Taxus baccata* via a semisynthetic approach. These antineoplastic agents inhibit mitosis via their ability to prevent the depolymerization of cytoskeletal microtubules to free tubulin. Consistent with this proposed mechanism of action is the finding that paclitaxel has a distinct binding site on the β-tubulin protein, thus allowing it to stabilize microtubule structure and arrest the cell cycle at the G_2-M mitotic interface (3).

In general, administration of cytotoxic chemotherapy is characterized by bone marrow suppression that manifests as reduced numbers of peripheral blood immune effectors. These effects are reversible and do not lead to permanent alterations in immune function. The taxanes appear to be no different in this regard. However, there are numerous reports that these drugs may exert unique immunomodulatory effects on immune cells, some of which may lead to activation of innate and specific immune effectors (4). Paclitaxel is able to bind to macrophages and stimulate responses that are similar to those induced by lipopolysaccharide, one of the major components of bacterial endotoxin. Stimulation of macrophages with paclitaxel leads to the induction of important pathways involved in the elimination of microbes (*e.g.*, inducible nitric oxide synthesis) as well as the production of proinflammatory cytokines such as interleukin (IL)-1β, tumor necrosis factor α, and IL-8 (5). Furthermore, paclitaxel-induced production of IL-12 by tumor-associated macrophages may be important in alleviating the T-cell suppression that is observed in the tumor-bearing host (6). *In vitro* studies of the direct effects of paclitaxel on the cytotoxic activity of T cells and natural killer (NK) cells have generally revealed an inhibitory effect, but some studies suggest a more complex effect of taxanes on the function of these lymphocyte populations *in vivo*. Mason *et al.* (7) reported that 40% of murine mammary carcinomas responding to docetaxel showed heavy infiltration with T-helper lymphocytes and NK cells. Similarly, a clinical study indicated that the development of a lymphocytic infiltrate after chemotherapy correlated with a positive clinical response to neoadjuvant paclitaxel therapy (8).

Few studies have systematically examined the overall im-

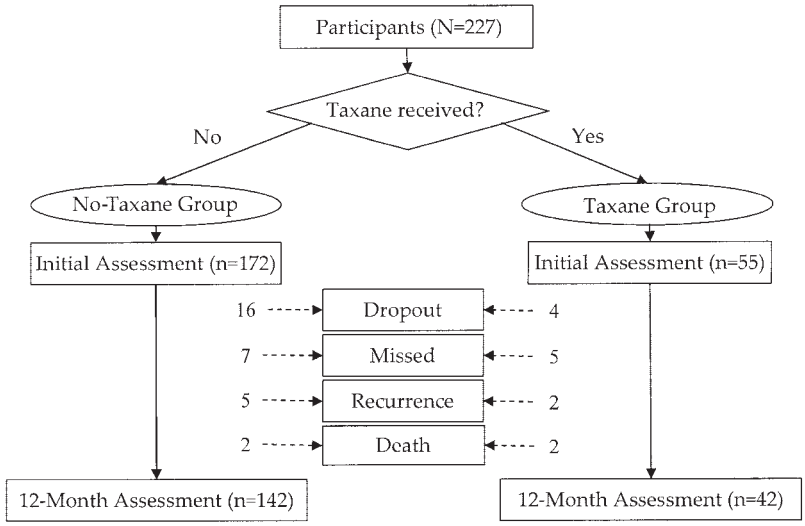

Fig. 1 Flow diagram of research design comparing Taxane and No-Taxane groups. Groups were determined retrospectively based on whether patients received taxane as part of their adjuvant treatment.

mune effects of taxane administration in the setting of chemotherapy for breast carcinoma, and none to date have examined the long-term effects of these drugs in homogeneous populations. Tong *et al.* (9) studied lymphocyte subsets, levels of circulating cytokines, T-cell mitogenic function, and NK cell function in 10 patients with advanced cancer who received paclitaxel or docetaxel. They found that patients exhibiting a partial or complete response to therapy demonstrated higher than normal CD4:CD8 T-cell ratios and increased T-cell proliferation in response to phytohemagglutinin (PHA) at 4 weeks posttreatment. T-cell activation during the course of paclitaxel combination chemotherapy has also been described (10). These findings suggest that taxanes may have complex and long-lasting effects on host immunity that could possibly impact the ability of a cancer patient to respond to infectious complications and the recurrence of malignant disease.

We have conducted a randomized Phase III clinical trial in patients with stage II and III breast cancer, investigating the effects of a stress reduction intervention on patient behavior and immune function. As a part of this study, patients underwent an extensive analysis of NK cell and T-cell function immediately after enrollment (*i.e.*, shortly before the start of chemotherapy) and at regular intervals thereafter. During the accrual period, a taxane-containing adjuvant chemotherapy regimen came into widespread usage in patients with nodal disease. We used this change as an opportunity to identify two patient groups that differed in their exposure to taxane-based chemotherapy and to test for differential immune outcomes.

PATIENTS AND METHODS

Research Design and Procedures

A clinical trial assessing the effects of a psychosocial intervention on stress and immunity [reported elsewhere (11)] was conducted. Eligible patients were surgically treated for stage II or III breast cancer and were awaiting adjuvant therapy. Exclusion criteria included the following: age <20 or >85 years; residence >90 miles from the research site; prior cancer diagnosis or refusal of any cancer treatment; diagnoses of mental retardation, severe or untreated psychopathology (*e.g.*, schizophrenia), neurological disorder, dementia, chronic fatigue syndrome, or immunological condition/disease (*e.g.*, rheumatoid arthritis). Patients were consecutive cases at a university-affiliated National Cancer Institute-designated Comprehensive Cancer Center or referrals from community physicians accrued between May, 1994, and May, 2000. All were provided, in person, with oral and written informed consent in keeping with institutional guidelines and in accordance with an assurance filed with and approved by the United States Department of Health and Human Services. After an initial assessment prior to the start of adjuvant chemotherapy, patients were randomized to psychological Intervention and Assessment or Assessment-only study arms. The initial assessment included behavioral measures relevant to the trial and a 60-ml drawing of peripheral blood.

For the present study (see Fig. 1), two groups (Taxane *versus* No-Taxane) were subsequently defined on the basis of whether or not patients had received any taxane (either paclitaxel or docetaxel) as part of their adjuvant treatment. The study design compares these two groups using two observations in time, with the initial assessment prior to the start of chemotherapy and a follow-up assessment at 12 months, after patients had completed adjuvant treatment.

Immune Assays

Procedures. Peripheral blood leukocytes (PBLs) were isolated from 60 ml of venous blood by using Ficoll density gradient centrifugation (Pharmacia Biotech, Inc., Piscataway, NJ). The isolated leukocytes were washed in calcium- and magnesium-free PBS and counted on a Coulter counter (Coulter Corp., Miami, FL). Aliquots of 6×10^6 isolated PBLs were suspended again in 0.6 ml of RPMI 1640 supplemented with 10% fetal bovine serum (HyClone, Logan, UT), 2 mercaptoethanol (BME; Sigma, St. Louis, MO), and 100× antibiotic-antimycotic stock, HEPES, sodium bicarbonate, and L-glutamine (all from Life Technologies, Inc., Grand Island, NY).

Numeration of Total T-lymphocyte Counts, T-Cell Subsets, and NK Cells. PBLs were labeled with florescent-conjugated monoclonal antibodies specific for the following cell surface markers: total T lymphocytes (CD3, FITC), T4 subsets (CD4, rhodamine), T8 subset (CD8, FITC), and NK cells (CD56, rhodamine). Monoclonal antibodies were purchased from Beckman Coulter Corp. Briefly, an aliquot of PBLs (2.5×10^6 cells) was treated with Erythrocyte Lysis Buffer (154 mM NH_4Cl, 10 mM $KHCO_3$, 0.082 mM EDTA-Na), resuspended in Dulbecco's phosphate-buffered saline, and centrifuged for 5 min at 3300 rpm. Cells (0.5×10^6) were incubated with the appropriate monoclonal antibodies for 15 min in the dark on ice. After the incubation, the labeled cells were washed with Dulbecco's phosphate-buffered saline and were fixed with 2% formaldehyde (made using 10% ultrapure formaldehyde). Dual-labeled IgG was used to determine nonspecific immunofluorescence binding. Samples were analyzed with a Coulter EPICS XL-MCL flow cytometer.

NK Cell Cytotoxicity. Briefly, PBLs were resuspended in complete medium at a density of 2.5×10^6 cells/ml and were seeded into 96-well V-bottomed microtiter plates in a volume sufficient to provide an E:T cell ratio of 100:1, 50:1, 25:1, 12.5:1, 6.25:1, or 3.13:1 (triplicate wells). Complete medium was added to each well to give a total volume of 200 μl. The NK-sensitive human myeloid K562 cell line was used as the target in a ^{51}Cr assay (12, 13). K562 cells were harvested from culture, labeled with ^{51}Cr, and washed. Then, 5×10^3 K562 target cells were added to each well in a volume of 50 μl. Plates were centrifuged at $300 \times g$ for 5 min and were incubated for 5 h in an atmosphere of 5% CO_2 at 37°C. After this incubation, the plates were again centrifuged at $300 \times g$ for 5 min, and 100 μl of supernatant were harvested and counted using a Beckman 5500 gamma counter. Minimum and maximum ^{51}Cr release was determined using target cells that had been incubated in complete medium or 5% SDS detergent solution, respectively. Cytotoxicity was calculated using the following equation (12, 13):

$$\text{Experimental } {}^{51}\text{Cr release} - \frac{\text{Minimum release}}{\text{Maximum release}} - \text{Minimum release}$$

Blastogenic Response to PHA. Isolated PBLs, resuspended in supplemental RPMI without phenol red, were seeded in triplicate at 0.5×10^5 cells/well in the presence of PHA (2.5, 5.0, and 10.0 μg/ml) in sterile 96-well flat-bottomed plate and were incubated for 68–72 h at 37°C, in an atmosphere of 5% CO_2. Wells were pulsed for the final 4 h with MTS, *i.e.,* 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (Promega Corp., Madison, WI) and phenazine methosulfate (PMS), an electron coupling reagent, to measure proliferative response. The MTS assay is a nonradioactive calorimetric procedure that labels metabolically active cells via reduction of a colored substrate. The amount of proliferation was determined via absorbance readings of the suspension in the well compared with cells and media alone, using an HTS7000 Bioassay microplate reader (Perkin-Elmer) at a determination wavelength of 492 nm and a reference wavelength of 690 nm, as has been noted previously (14, 15).

Statistics

Accrual and Retention. The overall accrual rate was 52% and retention at 12 months, excluding subjects who had recurrent disease or died, was 85%. The accrual rate is comparable and retention is higher than that typically found in randomized clinical trials of behavioral interventions (16, 17).

Analysis Plan. Descriptive analyses compared the Taxane and No-Taxane groups using χ^2 or ANOVA as appropriate. Patients' baseline characteristics and other variables that might affect 12-month immune outcomes were also examined. These included immune level at initial assessment, age, study arm (intervention *versus* assessment), menopausal status, disease characteristics (hormone receptor status, number of nodes), treatment (surgery type, receipt of radiation, hormonal therapy recommended, receipt of hormonal therapy at 12 months, days elapsed at 12 months because of completion of chemotherapy/radiation), and cell counts (*e.g.,* NK cell counts for NK cell cytotoxicity, T-cell counts for T-cell blastogenesis). Variables that were significantly correlated with an immune outcome were identified as control variables for the multiple regression analyses described below.

Nested ordinary least squares linear regression models were used for each of the two functional immune outcomes, NK cell cytotoxicity and the PBL blastogenic response to PHA, at 12 months. NK cell cytotoxicity was expressed as the mean of standardized scores (*i.e.,* Z-scores, differences in SD units from the overall mean) from three E:T ratios (12.5:1, 6.25:1, and 3.13:1). These ratios were selected based on previous observations in the investigators' laboratory that they were the most sensitive to chemotherapy effects. PHA blastogenesis was expressed as the mean of standardized scores from the three dilutions (2.5, 5.0, and 10.0 μg/ml). The first step in each nested linear regression procedure entered the control variables as predictors. The second step added a binary variable representing the administration of taxane (0 = no, 1 = yes) as a predictor. This second step tests whether taxane explains significant variance ($P < 0.05$) in an immune outcome beyond that explained by control variables.

Before conducting the ordinary least squares regression analyses, we checked major assumptions of this model: error variance normality and constancy (homoscedasticity) and the appropriateness of the linear model. Residual plots and formal tests indicated that the above assumptions were not violated. Analyses detected outliers, but follow-up analyses indicated that the outliers did not significantly influence the fit of the regression function. In sum, linear ordinary least squares regression was an appropriate model for the present analyses.

RESULTS

Description of the Total Sample and the Groups. Table 1 provides descriptive data. With the exception of four variables, the Taxane and No-Taxane groups were initially equivalent on demographic, prognostic, treatment, and chemotherapy variables. Regarding the differences, the Taxane group was significantly more likely to have positive nodes (89 *versus* 59%), to have received a higher dose of doxorubicin (36.71 *versus* 32.67 mg/m^2/week), to have fewer days lapse since the treatment (251 *versus* 317), and to be on hormonal therapy at 12

Table 1 Initial equivalence of Taxane and No-Taxane groups on demographic, prognostic, treatment, and chemotherapies received
Percentages or means and SDs in the sample are provided.

Variables	Taxane			No Taxane			Total		
	n	Mean or %	SD	*n*	Mean or %	SD	*n*	Mean or %	SD
Demographic and prognostic									
Age (yr)	55	49.00	9.62	172	51.40	11.07	227	50.82	10.76
Karnofsky performance status	55	83.64	6.77	172	85.58	8.25	227	85.11	7.95
Tumor size (cm)	55	3.24	2.03	172	2.94	1.67	227	3.02	1.77
Days since treatment (either chemo- or radiation therapy)[a]	42	250.50	69.43	122	316.61	111.54	164	299.68	106.23
Stage (n, % stage II)	48	87.3%		157	91.3%		205	90.3%	
Nodes (n, % positive)[a]	49	89.1%		101	58.7%		150	66.1%	
ER[b]/PR (n, % positive)	37	67.3%		118	68.6%		155	68.3%	
Menopausal status (n, % postmenopausal)	24	43.6%		81	47.1%		105	46.3%	
Treatment									
Surgery (n, % mastectomy)	35	63.6%		95	55.2%		130	57.3%	
Radiation therapy (n, % yes)	31	56.4%		92	53.5%		123	54.2%	
Hormonal therapy (n, % yes)	41	74.5%		130	75.6%		171	75.3%	
Chemotherapy (n, % yes)	55	100%		136	79.1		191	84.1%	
Psychological intervention (n, % yes)	28	50.9%		86	50.0%		114	50.2%	
Hormonal therapy status at 12 months (n, % yes)[a]	42	61.9%		142	35.5%		184	47.3%	
Chemotherapy									
Doxorubicin									
Received (mg/m^2/wk)[a]	55	36.71	9.28	113	32.67	9.66	168	34.00	9.70
Relative dose intensity (%)		92.84	7.50		90.20	18.12		91.07	15.49
Cyclophosphamide									
Received (mg/m^2/wk)	49	355.80	82.29	138	450.75	387.30	187	425.90	337.6
Relative dose intensity (%)[a]		94.25	8.33		87.40	20.87		89.20	18.65
Methotrexate									
Received (mg/m^2/wk)	0			27	28.23	15.59	27	28.23	15.59
Relative dose intensity (%)					78.58	25.69		78.58	25.69
5-Fluorouracil									
Received (mg/m^2/wk)	0			41	353.90	129.30	41	353.90	129.30
Relative dose intensity (%)					88.30	21.85		88.30	21.85
Paclitaxel[c,d]									
Received (mg/m^2/wk)	46	110.0	23.15	2	0.00	0.00	48	105.40	31.73
Relative dose intensity (%)		92.66	12.80		0.00	0.00		92.66	12.80
Docetaxel									
Received (mg/m^2/wk)	10	44.43	13.47	0			10	44.43	13.47
Relative dose intensity (%)		89.06	9.90					89.06	9.90

[a] $P < 0.05$.
[b] ER, estrogen receptor; PR, progesterone receptor.
[c] Two patients originally prescribed taxane did not actually receive the drug.
[d] One patient reacted adversely to docetaxel and thereafter received paclitaxel instead.

months (62 *versus* 36%) than the No-Taxane group (all *P*s $< .05$).

Chemotherapy regimens were as follows: most taxane recipients received paclitaxel in combination with doxorubicin and cyclophosphamide ($n = 45$). The remaining taxane recipients received docetaxel in combination with doxorubicin ($n = 5$), or docetaxel, doxorubicin, and cyclophosphamide ($n = 5$). Finally, one participant reacted adversely to her first cycle of docetaxel and, thereafter, received paclitaxel in combination with doxorubicin. Among the No-Taxane group, the most common chemotherapy regimen was doxorubicin with cyclophosphamide ($n = 95$), followed by cyclophosphamide, methotrexate, and 5-fluorouracil ($n = 25$), cyclophosphamide, doxorubicin, and 5-fluorouracil ($n = 13$), and other miscellaneous protocols ($n = 3$). Thirty-six of the No-Taxane participants received no chemotherapy.

Table 2 provides mean and SD values of the immune measures at initial (baseline) and at 12 months. Across all of the immune variables, there were no significant differences between the groups at the initial assessment (all *P*s > 0.06). However, the groups differed significantly at 12 months. As shown in Fig. 2, patients in the Taxane group exhibited greater 12-month PHA-induced blastogenesis and NK cell cytotoxicity than patients in the No-Taxane group across the dilutions and E:T ratios. These differences were generally significant for the NK cytotoxicity assays ($P = 0.004$, 0.0003, and 0.0003, for the 12.5:1, 6.25:1, and 3.125:1 E:T ratios, respectively) as well as for the PHA blastogenesis assays ($P = 0.071$, 0.004, and 0.001 for the 10, 5, and 2.5 μg/ml dilutions, respectively). Specifically, average PHA blastogenesis levels at the 10, 5, and 2.5 μg/ml dilutions were 25, 41, and 45% (mean, 37%) higher in the Taxane group than in the No-Taxane group. Similarly, NK cell cytotoxicity levels were 27, 39, and 50% (mean, 39%) higher in the Taxane group for the 12.5:1, 6.25:1, and 3.125:1 E:T ratios, respectively.

Table 2 Description of initial and 12-month immune values for the Taxane and No-Taxane groups and the total sample

Means and SDs in the sample are provided.

Standardized average scores were computed by first calculating a Z-score for each participant (dividing their difference from the overall mean by the sample SD) on each raw variable (*i.e.*, PHA at 10, 5, and 2.5 μg/ml; NK lysis at 12.5:1, 6.25:1, and 3.13:1 E:T), then averaging these Z-scores across all three dilutions or E:T ratios.

Immune variable Time of assessment	Taxane			No Taxane			Total		
	n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
T-cell count									
Initial									
$CD3^+$ (k/μl)[a]	50	1.183	0.503	156	1.315	0.613	206	1.283	0.590
$CD4^+$ (k/μl)	50	0.893	0.405	156	0.897	0.444	206	0.896	0.434
$CD8^+$ (k/μl)	50	0.398	0.185	156	0.435	0.236	206	0.426	0.224
12-month									
$CD3^+$ (k/μl)	38	0.861	0.367	125	1.007	0.482	206	0.973	0.461
$CD4^+$ (k/μl)	38	0.633	0.290	125	0.650	0.347	206	0.646	0.334
$CD8^+$ (k/μl)	38	0.329	0.153	125	0.400	0.236	206	0.384	0.221
PHA[b] blastogenesis									
Initial									
Absorbance									
10 (μg/ml)	53	0.325	0.153	160	0.304	0.153	213	0.309	0.153
5 (μg/ml)	53	0.312	0.160	160	0.291	0.145	213	0.297	0.149
2.5 (μg/ml)	53	0.282	0.166	160	0.279	0.145	213	0.280	0.151
Standardized average	53	0.074	1.044	160	−0.025	0.968	213	0.000	0.986
12-month									
Absorbance									
10 (μg/ml)	33	0.291	0.165	125	0.233	0.162	158	0.246	0.164
5 (μg/ml)	33	0.321	0.173	125	0.227	0.158	158	0.247	0.165
2.5 (μg/ml)	33	0.308	0.145	125	0.212	0.149	158	0.232	0.153
Standardized average	33	0.407	0.961	125	−0.107	0.955	158	0.000	0.976
NK Cell									
Initial									
Count (k/μl)	50	0.228	0.116	156	0.213	0.144	206	0.217	0.137
% lysis									
(E:T) 100:1	54	52.09	17.18	162	57.81	19.84	216	56.38	19.33
50:1	54	46.59	17.85	162	48.41	19.38	216	47.95	18.98
25:1	54	36.37	17.01	162	34.22	14.84	216	34.76	15.39
12.5:1	54	24.76	13.01	162	23.05	11.94	216	23.48	12.20
6.25:1	54	15.45	8.84	162	13.94	8.21	216	14.32	8.37
3.125:1	54	7.62	4.84	162	7.18	4.86	216	7.29	4.85
Standardized average	54	0.10	1.02	162	−0.03	0.96	216	0.00	0.98
12-month									
Count (k/μl)	38	0.176	0.083	125	0.181	0.112	206	0.180	0.106
Percent Lysis									
(E:T) 100:1	37	59.68	19.01	134	60.88	18.17	171	60.62	18.31
50:1	37	55.55	18.64	134	53.26	17.55	171	53.76	17.76
25:1	37	46.43	18.66	134	40.97	15.98	171	42.15	16.69
12.5:1	37	35.48	15.76	134	28.05	12.94	171	29.65	13.90
6.25:1	37	23.80	11.32	134	17.14	9.28	171	18.58	10.10
3.125:1	37	13.08	7.86	134	8.73	5.84	171	9.67	6.55
Standardized average	37	0.49	1.12	134	−0.13	0.89	171	0.00	0.98

[a] k/μl, thousands per microliter.

[b] PHA, phytohemagglutinin; NK, natural killer.

Regression Analyses. As noted above, variables that were significantly correlated with immune levels at 12 months were identified as control variables. The controls identified for PHA-induced blastogenesis were study arm (Intervention *versus* Assessment), surgery type (segmental mastectomy *versus* other), days since completion of chemotherapy and/or radiation, receipt of hormonal therapy at 12 months, and CD8 count, in addition to initial (baseline) levels of blastogenesis. Days since completion of chemotherapy and/or radiation was used to control for possible suppressive effects of either treatment on immune function. (Alternatively, using either "days since completion of chemotherapy" or "days since completion of radiation" as separate single controls yielded the same pattern of results.)

As shown in Table 3, the Controls-Only model explained 13% of the variance in PHA blastogenesis at 12 months. Consideration of the Taxane group in the second step significantly improved the model ($P = 0.007$). Taxane administration predicted PHA blastogenesis even after controlling for baseline PHA blastogenesis values and other variables such as extent of surgery and study arm. Although study arm was a significant predictor of greater PHA blastogenesis, follow-up analyses indicated that the impact was equivalent in the Taxane and No-

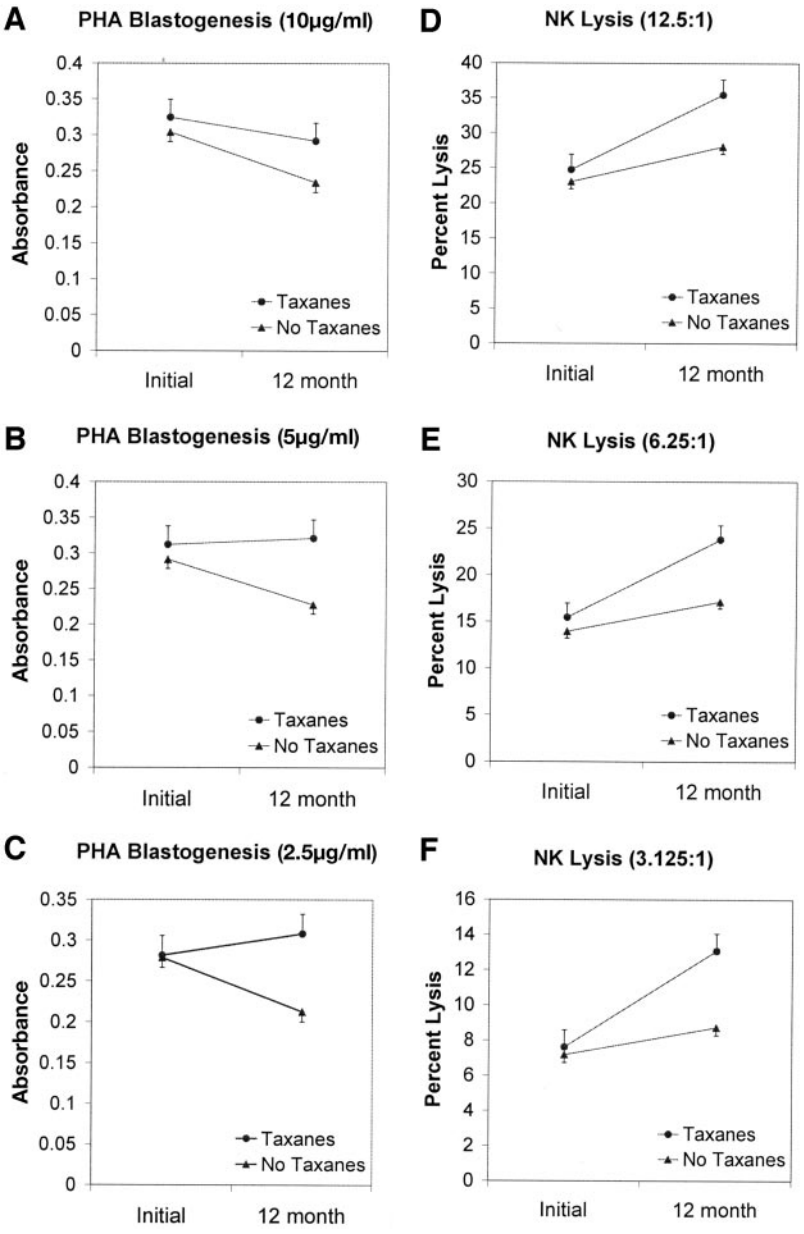

Fig. 2 Mean and SEs of immune levels for Taxane and No-Taxane groups at initial and 12-month assessments. *A–C,* phytohemagglutinin (*PHA*) blastogenesis values for 10.0, 5.0, and 2.5 dilutions, respectively. *D–F,* natural killer (*NK*) cell lysis 12.5 (*12.5:1*), 6.25 (*6.25:1*), and 3.125 (*3.125:1*) E:T cell ratios, respectively.

Taxane groups. Graphs *A* through *C* in Fig. 2 provide a pictorial representation of changes in blastogenesis over time per dilution.

The findings were similar for the NK cell cytotoxicity analyses. For these analyses, the controls were age, hormone receptor status, receipt of hormonal therapy at 12 months, CD8 count, NK cell count, and initial NK cell lysis levels. Study arm was not used as a control because it had no effect on NK cell lysis at 12 months. Table 4 shows that the control variables explained 32% of the variance in NK cell cytotoxicity. Again, the addition of the Taxane group significantly increased the variance explained ($P = 0.004$), because the receipt of taxane predicted greater NK cell cytotoxicity beyond that explained by the control variables alone. Follow-up analyses of study arm indicated that the psychological intervention had no overall effect on the Taxane or No-Taxane groups with respect to NK cell lysis. Graphs *D* through *F* in Fig. 2 provide a pictorial representation of changes in NK cell cytotoxicity over time per E:T ratio.

The effect of taxane on the immune outcomes appears robust. The effect appeared across assays and in the context of heterogeneous chemotherapy regimens. As noted above, taxane was administered with some adjuvant therapies (*e.g.*, doxorubicin), but not others (methotrexate, 5-fluorouracil). To further test the reliability of the findings, additional analyses were conducted in homogeneous subsets of the sample. Two sets of analyses were conducted. For the first, we included only patients receiving a doxorubicin/cyclophosphamide combination, either

Table 3 Multiple regression findings for phytohemagglutinin (PHA) blastogenesis at 12 months

Results show that beyond the contribution of control values, taxane administration predicted higher levels of blastogenesis at 12 months. This effect is replicated across three dilutions (10.0, 5.0, and 2.5 μg/ml).

Outcome PHA blastogenesis	Models				
	Controls only[a]	Controls + taxane[b]			
	Variance explained	Variance explained	β[c]	*t* (*df*, 137)	*P*
Standardized average[d]	0.136	0.184	0.237	2.752	0.007
2.5 μg/ml	0.130	0.191	0.268	3.139	0.002
5.0 μg/ml	0.147	0.199	0.248	2.910	0.004
10.0 μg/ml	0.124	0.150	0.176	1.999	0.048

[a] Model includes control variables only: initial levels of PHA blastogenesis, study arm, surgery type, days since completion of adjuvant treatment, receipt of hormonal therapy at 12 months, and CD8 count.
[b] Controls-only model plus taxane administration (yes *versus* no).
[c] Standardized β weight for taxane administration after controlling for other variables.
[d] Standard scores per concentration, averaged across the three dilutions.

with Taxane (n = 55) or with No-Taxane (n = 95). These analyses yielded identical findings for both PHA blastogenesis and NK cell lysis, indicating that differences in chemotherapies other than the taxanes cannot account for the primary results. The majority [n = 45 (81%)] of taxane recipients received paclitaxel and not docetaxel. For the second set of additional regressions we restricted the subgroups further by excluding the 10 docetaxel recipients. Thus, we included only patients receiving doxorubicin and cyclophosphamide, with paclitaxel (n = 45) or without paclitaxel (n = 95). Again, the pattern of results for PHA and NK was unchanged. In summary, the observed effects of the taxanes on immunity appear to be robust with the addition of other standard chemotherapies to the regimen and with variation in the type of taxane used (paclitaxel or docetaxel).

DISCUSSION

These data demonstrate that the administration of taxane-based adjuvant chemotherapy to women surgically treated for regional breast cancer was associated with higher T-cell blastogenesis and NK cell lytic activity relative to a comparison group at long-term follow-up. The patients were participating in an ongoing clinical trial testing the efficacy of a psychological intervention that incorporated monitoring of behavioral and immune parameters. The current investigation pooled patients from either study arm together and then split them into two comparison groups differing in the receipt of adjuvant taxane. Patients in the two groups were similar with respect to demographics, stage of disease, participation in the investigational arm of the protocol, and initial immune function. Regression models indicated that the group receiving taxanes had significantly higher posttreatment levels of PHA-induced blastogenesis and NK cell cytotoxicity than the group not receiving taxanes. Follow-up analyses revealed that the psychological intervention also predicted greater PHA-induced blastogenesis (an effect that was equivalent between the Taxane and the No-Taxane groups), but had no effect on NK cell cytotoxicity. This study represents the first instance of long-term immune benefits in response to taxane administration, a finding that runs counter to currently held beliefs pertaining to the effects of cytotoxic agents on bone marrow-derived lymphocyte populations.

Previous work suggests that the taxanes exert primarily inhibitory effects on immune effectors of the lymphocyte compartment. Indeed, short-term exposure of lymphocytes to Taxol leads to significant inhibition of the T-cell proliferative re-

Table 4 Multiple regression findings for the prediction of natural killer (NK) cell cytotoxicity at 12 months

Results show that beyond the contribution of control values, taxane administration predicted higher levels of NK cell lysis. This effect is replicated across three E:T ratios (12.5, 6.25, and 3.125).

Outcome NK cell cytotoxicity	Models				
	Controls only[a]	Controls + taxane[b]			
	Variance explained	Variance explained	β[b]	*t* (*df*, 154)	*P*
Standardized average[d]	0.324	0.362	0.201	2.955	0.004
(E:T) 3.125:1	0.287	0.334	0.222	3.191	0.002
6.25:1	0.319	0.360	0.209	3.069	0.003
12.5:1	0.324	0.348	0.158	2.304	0.023

[a] Model includes control variables only: initial levels of NK cell cytotoxicity, hormone receptor status, age at initial, NK count, receipt of hormonal therapy at 12 months, and CD8 count.
[b] Controls-only model plus taxane administration (yes *versus* no).
[c] Standardized β weight for taxane administration after controlling for other variables.
[d] Standard scores per concentration, averaged across the three E:T ratios.

sponse. In one set of experiments, splenic CD4+ T cells from tumor-bearing mice were exposed to Taxol for 4 h and then were tested for their proliferative response to Concanavalin A. Importantly, there was a significant reduction in the T-cell response to this mitogen (18). This effect was not due to a direct toxic effect, because the lymphocytes remained viable for the duration of the assay. Similarly, both paclitaxel and docetaxel cause a significant suppression of lymphocyte activation and growth in response to stimulatory concentrations of IL-2 [*e.g.*, 10 and 50 μg/ml (19)]. *In vitro* studies of T-cell and NK cell cytotoxic activity after taxane exposure reveal an overall inhibitory effect for this class of agents (20). The most commonly documented immune response in patients receiving paclitaxel is the development of myelosuppression in the weeks after the administration of this cytotoxic agent (21). However, an analysis of leukocyte subsets in patients receiving taxane therapy revealed no alterations in T-cell or B-cell frequency or CD4:CD8 ratio (9). Other reports purporting a stimulatory effect of paclitaxel have focused on its ability to activate immune effectors such as monocytes/macrophages that have receptors for lipopolysaccharides and other foreign compounds that can cross-react with the paclitaxel molecule (22). These effects are limited primarily to monocyte production of pro-inflammatory cytokines such as tumor necrosis factor α, IL-1β, and inducible nitric oxide synthesis (5).

There appears to be precedence for our observations. Krainer *et al.* (23) examined 15 patients who had received BEP chemotherapy (bleomycin, etoposide, and cisplatin) for testicular cancer for evidence of immune modulation over a 1-year period. They examined the prevalence of CD3+, CD4+, and CD8+ T cells in these patients as well as the blastogenic response to a standard panel of mitogens. Interestingly, this group found that polychemotherapy led to a significant increase in the T-cell mitogenic response at 1 year, but not at the 5-year time point, despite no change in the prevalence of CD3+ lymphocytes. Thus, the positive effects of chemotherapy on immune functioning may not be limited to Taxol-based regimens.

As of this writing, there is no clear mechanism for the long-term-immunostimulatory effects of taxane that were observed in the present study. A careful analysis of T-cell subpopulations in our study patients revealed no changes in the overall prevalence of CD4+ helper T cells or CD8+ cytotoxic T cells. Neither did NK cell numbers appear to be affected in the long term by taxane administration. Therefore, the positive effects of taxane on T-cell proliferation and NK cytolysis did not appear to be simply the result of increased numbers of effector cells. It is possible that the effects of taxane on T cells and NK cells in this study reflect an alteration in the makeup of these populations (24). It is also possible that the changes induced in the T-cell and NK cell populations are quite subtle and not easily detected with the immune assays used in this study. Indeed, we have determined that stress and anxiety after the breast cancer diagnosis can lead to altered expression of NK cell adhesion molecules and killer-inhibitory receptors that are not normally measured in clinical studies of immune function (25). Alternatively, taxane chemotherapy might have caused changes in the peripheral and/or bone marrow microenvironment that led to alterations in the maturation rate or activation state of T cells and NK cells. Importantly, these two cell populations exhibit a number of similarities at the level of cell surface adhesion molecules and signaling intermediates (26). Hence, one might expect any type of chemotherapeutic effect to have similar influences on both cell compartments.

The functional significance of the observed immune changes is not yet known, and clarification of this issue will require further follow-up in the two patient subsets. Of prime concern is the potential for cancer recurrence in patients who have experienced alterations in the functional capacity of their T-cell and NK cell compartments. Whether patients receiving taxane will exhibit fewer tumor recurrences depends on the contribution of the measured parameters to the elimination of malignant cells. These issues have not yet been resolved in large prospective clinical trials, and it is not clear whether we currently possess effective measures of antitumor immunity as they relate to the postoperative patient. Certainly, one would predict that robust T-cell blastogenesis would favor the development of an antitumor immune response, and it is even possible that patients might respond more favorably to micrometastatic autologous tumor cells after taxane chemotherapy. Of note, one study has shown a correlation between the development of tumor-infiltrating lymphocytes in breast cancer metastases after neoadjuvant paclitaxel chemotherapy and complete clinical response (27).

In summary, group comparisons with appropriate controls in a sizable cancer sample contravene conventional wisdom that taxane chemotherapy leads to a suppression of patients' immune cell function. Follow-up will be required to determine any effect of the immune alterations on immune surveillance and disease recurrence. Additional immune analyses are under way to determine the scope and nature of the changes that have occurred in the T-cell and NK cell populations.

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