Indomethacin Sensitive Suppressor-Cell Activity in Head and Neck Cancer Patients

The Role of the Adherent Mononuclear Cell

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Head and neck cancer (H&N CA) patients have known depression of cell-mediated immunity. There is suggestive evidence that prostaglandin (PGE_2)-secreting cells may be a major factor. The authors have sought to determine the role of PGE_2-releasing monocytes–macrophages in this immune depression by determining the effects of adherent cell depletion and by measuring the effects of indomethacin, a PGE_2 synthetase inhibitor, on selected tests of lymphocyte function. Lymphocyte stimulation with phytohemagglutinin (PHA) (T-cell stimulant) and Staph phage lysate (SPL) (B-cell stimulant) was done in the presence of varying concentrations of indomethacin; the effect of adherent cell depletion also was determined. The study population included 45 patients with localized or locoregional squamous CA of the H&N and 40 controls. Results included the following: (1) lymphocyte stimulation responses to PHA and SPL were generally depressed in the CA patients *versus* controls; (2) incubation with indomethacin produced bivalent effects in both controls and CA patients, depending on the concentration of indomethacin and lymphocyte stimulant; incubation with optimum concentrations of indomethacin generally produced augmented responses in both study groups whereas high concentrations of indomethacin were suppressive; (3) the immune potentiating effects were not observed in older patients with advanced disease; and (4) removal of adherent leukocytes (mainly monocytes) also restored depressed lymphocyte responses. Although other factors also are operative, our data suggest that PGE_2-secreting monocytes–macrophages may have a major role in the immune depression of H&N CA patients. Age and host effects of the cancer and the malnutrition common to these patients probably are involved also, although their singular contribution has not been measured. This depression is largely reversible by a PGE_2 synthesis inhibitor, indomethacin, which suggests the potential value of *in vivo* administration of indomethacin to H&N CA patients as an adjunct.

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PATIENTS WITH head and neck cancer have known depression of cell-mediated immunity.[1-8] In part, this is thought to be due to various circulating suppressive factors such as suppressor T-cells[9,10] and suppressor macrophages (MO),[11] to various serum factors such as immune complexes,[3] and, possibly, to excess prostaglandin production by the tumor.[12] Adherent MO are known to have suppressive effects on lymphocyte function, which may be mediated, in part, by prostaglandins (PGE_2).[13-21] A prostaglandin E_2 synthetase inhibitor, indomethacin, may permit indirect measurement of reversible suppressor cell activity on selected lymphocyte function.[17,19,22] The assay consists of comparing the lymphocyte blastogenic response to mitogens or antigens under various culture conditions with and without indomethacin. Dose–response curves to these stimulating agents can be generated, and the effect of indomethacin calculated. Several authors have used variations of this assay to demonstrate indomethacin-sensitive suppressor-cell activity in cancer patients. Goodwin and colleagues demonstrated that the depressed peripheral blood mitogen response in patients with Hodgkin's disease was due to overproduction of PGE_2 by peripheral blood monocytes in these patients.[23] This defect was reversed by adding a prostaglandin synthetase inhibitor.[23] Han and Takita have demonstrated the presence

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of indomethacin-sensitive suppressor cells in lung cancer patients,[17] and Maca and Panje have demonstrated similarly responding cells in head and neck cancer patients.[18,19] Panje has carried these studies even further and has treated head and neck cancer patients with indomethacin; he elicited apparent regression of measurable tumor in five of seven patients.[24]

This study determines the role of indomethacin-sensitive suppressor cells, presumably adherent monocytes, in the immune depression which frequently exists in head and neck cancer patients.

Patients and Methods

There were 45 patients with squamous cancer of the head and neck, including 36 men and seven women, whose average age was 61 years (range, 28–81). Their disease stages, according to the American Joint Committee for Clinical Staging, were as follows: Stage I, six patients; Stage II, six patients; Stage III, 15 patients; and Stage IV, 18 patients. Thirty-seven patients had primary cancers and eight had recurrent disease. All of the latter were listed as Stage IV. Primary sites included the oral cavity in 17 patients; the pharynx in eight patients; and the larynx in 13 patients (all were squamous cancers). In addition, there were seven patients with other types of head and neck cancer. Included in the latter group were two cancers of the parotid gland (mucoepidermoid), one of the maxillary sinus, one of the nasal cavity (both adenocystic), one of the orbit, and one of the upper esophagus (both epidermoid cancer). Previous radiation had been given to ten patients; one patient had received previous chemotherapy and was tested between chemotherapy cycles. Most of the patients were operative candidates and had good performance status (Karnofsky status ≥80%). About one third of the advanced disease group required nutritional support. Although strict nutritional assessment was not done, most of the Stage III patients and essentially all of the Stage IV patients had nutritional impairment (8%–15% less body weight). In general, patients were tested before the initiation of treatment, with the exception of those receiving preoperative radiation. The normal controls were 28 hospital personnel, 13 men and 15 women, who had an average age of 35 years (range, 20–60). To assess the effects of age, an additional set of 12 controls (ten women, two men) was studied. This included six young individuals whose average age was 29 years (range, 20–42) and six older individuals whose average age was 58 years (range, 48–77).

Lymphocyte Function and Indomethacin Assay

The lymphocytes of controls and patients were extracted from heparinized whole blood (40–60 ml/person) using the Ficoll-Hypaque (Sigma Chemical Co., St. Louis, MO) method.[25] Blood was diluted approximately 1:1 with Earle's Balanced Salt Solution (EBSS) without calcium (Ca^{++}) or magnesium (Mg^{++}) (GIBCO, Grand Island, NY), and 35 ml of diluted blood was gently layered onto 15 ml of Ficoll-Hypaque and centrifuged for 25 to 30 minutes at approximately 400 × *g* in an IEC Model V centrifuge (International Equipment, Boston, MA) at room temperature (23°C). The density layer of mononuclear cells was carefully removed and placed in another 50 ml polypropylene centrifuge tube (Corning, General Scientific, Corning, NY), the volume of which was adjusted with EBSS to 50 ml. The tube was centrifuged for 10 to 15 minutes at 400 × *g*. The supernatant was aspirated and the cell pellet resuspended in 2 ml of EBSS. After the pellet was resuspended, the volume in the tube was adjusted to 50 ml with EBSS, and the tube was spun for 10 minutes at 400 × *g*. The supernatant was aspirated, and the pellet was resuspended in 1 ml of Roswell Park Memorial Institute (RPMI)-1640 (GIBCO) 10% heat-inactivated human AB serum (GIBCO), and 10 μl were added to 190 μl of a 0.5% crystal violet 10% acetic acid solution and counted in a hemacytometer. The cell concentration was then adjusted to 5×10^5 cells/ml based on this count. The percentage of viability was generally in the 98% to 99% range.

The indomethacin (Sigma Chemical Co.) solutions were prepared by first dissolving 10 mg of indomethacin in 2 ml of 95% ethanol (ETOH). After the crystals dissolved, 8 ml of RPMI-1640 was added to give a stock solution. The concentrations used were serial dilutions of this stock solution. Twenty-five microliters of the appropriate dilutions of indomethacin were added to the appropriate wells of a 96-well Costar tissue culture plate (M. A. Bioproducts, Silver Spring, MD).

The indomethacin dose–response curves in each test were constructed from indomethacin concentrations ranging from 40.0 μg/ml (generally suppressive) to more dilute concentrations, 0.8 to 0.04 μg/ml, which were considered to be in the immunoregulatory range. To the same plate, 25 μl of medium RPMI-1640 or phytohemagglutinin-M (PHA-M; GIBCO) or Staph phage lysate (SPL) were added. The stock concentrations of PHA-M were 1000 μg/ml, 5000 μg/ml, or 10,000 μg/ml. The respective concentrations in the well were 100 μg/ml, 500 μg/ml, and 1000 μg/ml. Staph phate lysate types I and III (Delmont Labs, Swarthmore, PA) were used undiluted, to a final dilution of 1:10 in the well. The above concentrations previously were determined to give the best sensitivity to differences between normals and cancer patients in this laboratory.[26] Two hundred microliters of 5×10^5 cells/ml in RPMI-1640 with 10% human type AB sera (ABS) were added to all wells, and the plates were placed in a 37°C, 5% carbon dioxide

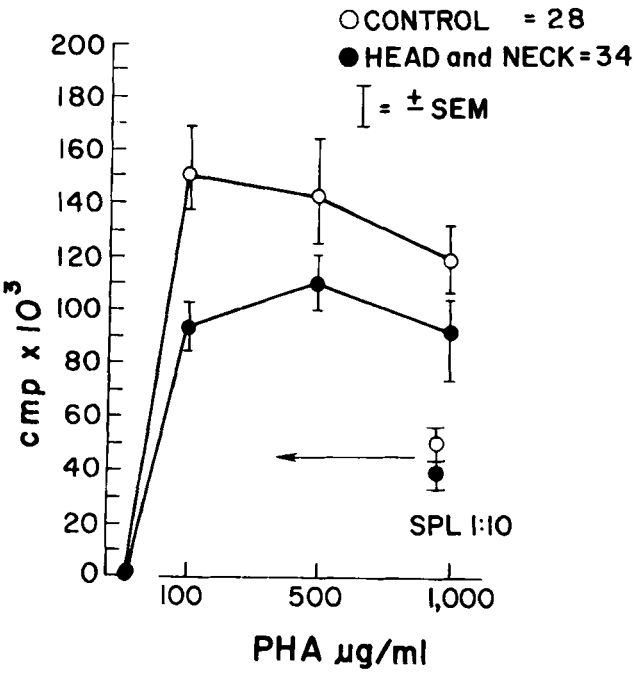


FIG. 1. The mean absolute lymphocyte blastogenic response to three different concentrations of PHA-M and Staph phage lysate is shown. There is a significant difference in response between the two populations to PHA (100 µg/ml) but not to PHA 500 µg/ml or 1000 µg/ml, or to SPL.

(CO_2), 88% humidified incubator for 3 days. On the third day, 2 µCi/50 µl of ^{3}H-thymidine (SA 6.7 Ci/mmoles; New England Nuclear, Boston, MA) were added to each well, and the plates were replaced in the incubator for another 24 hours.

The plates were removed and placed in a cold room (4°C) until they were collected, using an Otto-Hiller harvester (Otto Hiller, Madison, WI) on Whatman glass fiber paper (Whatman #934AH, Whatman Laboratory Products, Clifton, NJ). The paper circles were removed, placed in 2 ml of Beckman Ready-Solv HP counting cocktail (Beckman Instruments, Fullerton, CA), allowed to stand for at least 3 hours, and then counted in a LS-8000 liquid scintillation counter for 1 minute.

The data were calculated in the following manner:

$$\text{percent change} = \frac{X - Y}{Y} \times 100\%,$$

where X is the cpm of sample containing medium, mitogen, or antigen and a known concentration of indomethacin, and Y is the cpm of sample containing medium, mitogen, or antigen with no indomethacin.

Statistical analysis included chi-square; two-tailed, two-sample *t* test; linear regression; and Wilcoxon matched pairs signed-rank test. In the tables and figures, which show the mean percent change, only numbers which showed 10% change were listed (10% was approximately the standard error of all the test data). Lesser changes were recorded as no response.

Adherent Cell Depletion Experiments

To determine the effects of depleting adherent cells on lymphocyte stimulation by PHA and whether this action modified the indomethacin effect, experiments were carried out in an additional 25 controls and 27 head and neck cancer patients. Adherent cells were depleted by incubating 5 ml of 2×10^6/ml Ficoll-Hypaque separated cells in RPMI-1640 containing 10% ABS on 60 × 15-mm petri dishes (General Scientific, Richmond, VA) for 2 hours at 37°C in 5% CO_2. After 2 hours, the petri dishes were agitated by swirling, were washed twice with 1 × EBSS, and all cell-containing media were collected. The cells were spun at 400 × *g* for 10 minutes, and the cell pellet was resuspended in 1 ml of RPMI-1640 with 10% AB serum. The cells were counted, and the count was adjusted to 5×10^5 cells/ml. The resulting cell suspension generally contained less than 5% to 8% esterase-positive cells.

Results

The overall mean mitogen and antigen responses are shown in Figure 1. The mean lymphocyte stimulation

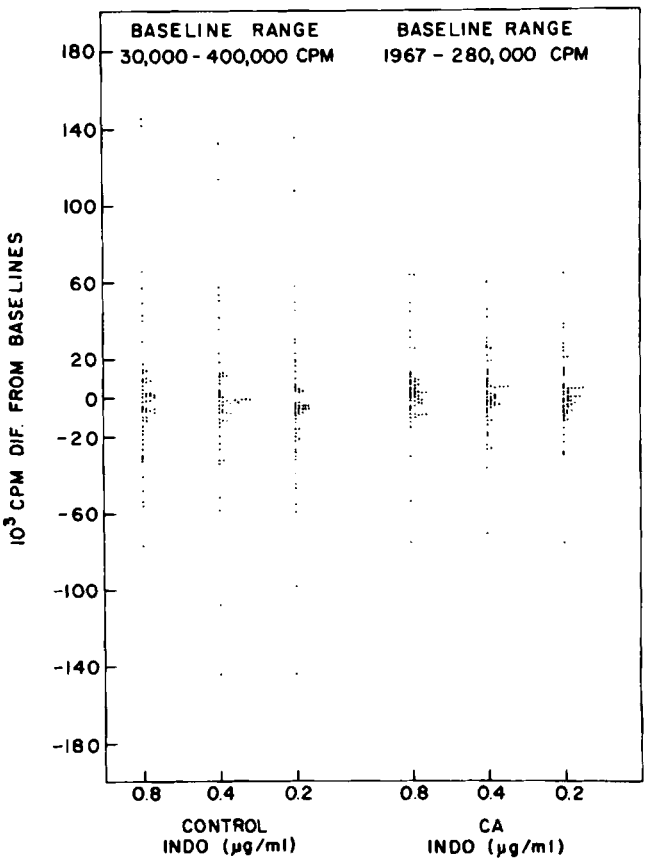


FIG. 2. The scattergram depicts the incremental changes in PHA response induced by lymphocyte incubation with indomethacin at three different concentrations at a PHA concentration of 100 mg/ml.

responses were higher in the controls at the three concentrations of PHA tested, with the greatest difference occurring at the lowest concentration (PHA 100 μg/ml). The effects of incubating with different concentrations of indomethacin on the lymphocyte responses at three different concentrations of PHA and at one dilution of SPL are shown in Figures 2 through 6A and 6B and Figures 7A and 7B, respectively. Indomethacin concentrations of 40 μg were universally suppressive (probably toxic), whereas doses of 0.8 to 0.04 μg/ml were considered biologically active. The indomethacin effects on lymphocytes stimulated by PHA 100 μg/ml is shown in Figures 2 and 3. The mean effects of indomethacin at concentrations of 0.2 to 0.8 μg/ml produced an increase over baseline of 33% to 46% in controls and 53% to 60% in cancer patients ($P < 0.005$) (Figs. 2 and 3). The data for the indomethacin dose response curves is displayed to include a scattergram showing the change in individual responses (Fig. 2).

A similar number of cancer patients and controls (14%–16%) had increase in PHA response of greater than 20,000 CPM. Approximately 17% to 24% of the tests in the controls, and 6% to 9% of the tests in the cancer patients, showed indomethacin-induced depression, >20,000 cpm, at this PHA dose level. Overall, there were no differences between cancer patients and controls. The greatest responses occurred in the intermediate PHA range (500 μg/ml) where there was an overall increase in the number of subjects who showed greater than 10% change from the baseline response at indomethacin concentrations of 0.8 to 0.04 μg/ml. The individual responses to indomethacin are shown in the scattergram, Figure 4, and the mean percent change and percentage of subjects showing change is shown in Figures 5A and 5B. Indomethacin concentrations of 0.8 to 0.04 μg/ml generally augmented the response (the 40.0 μg dose is suppressive). The overall effect of indomethacin at three dose levels was to increase the pHA response from baseline in 54% of controls and 57% of the cancer patients. Increases of >20,000 cpm (about 20%–30% of baseline) occurred in 28% of controls and 23% of cancer patients. Depressed responses, >20,000 cpm, occurred in <10% of the controls and cancer patients. The greatest responses to indomethacin occurred at PHA concentrations of 1000 μg/ml (Figs. 6A and 6B). The controls showed a greater augmentation than did the cancer patients. Indomethacin effects on lymphocyte stimulation with SPL (Figs. 7A and 7B) showed more uniform

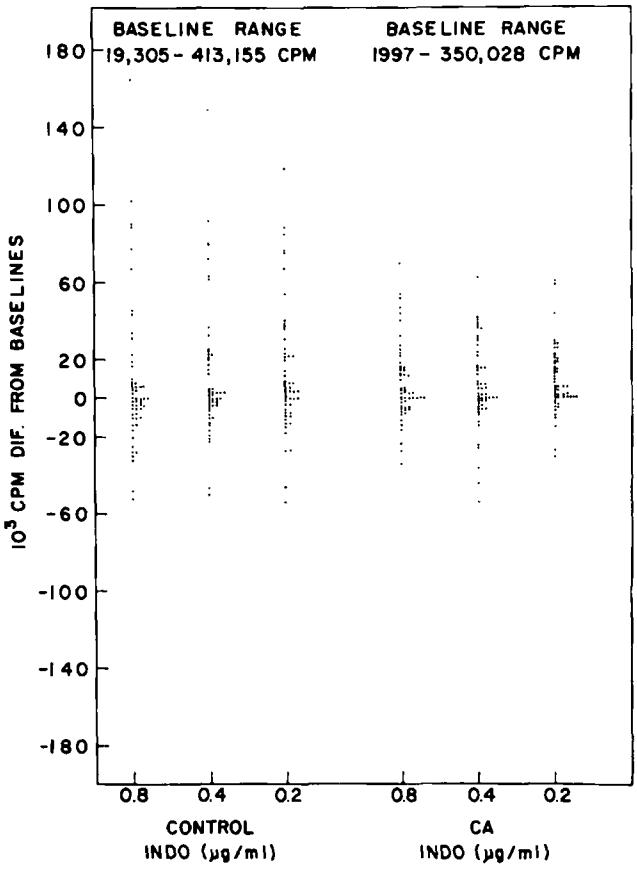


FIG. 4. The scattergram depicts the incremental changes in PHA response induced by lymphocyte incubation with indomethacin at three different concentrations and a PHA concentration of 500 μg/ml. The percent of test subjects showing incremental increases above baseline of >20,000 cpm (>10% increase) was 28% for controls and 23% for the cancer patients. Less than 9% of controls and 6% of cancer patients showed significant depression (negative decrement of 20,000 cpm).

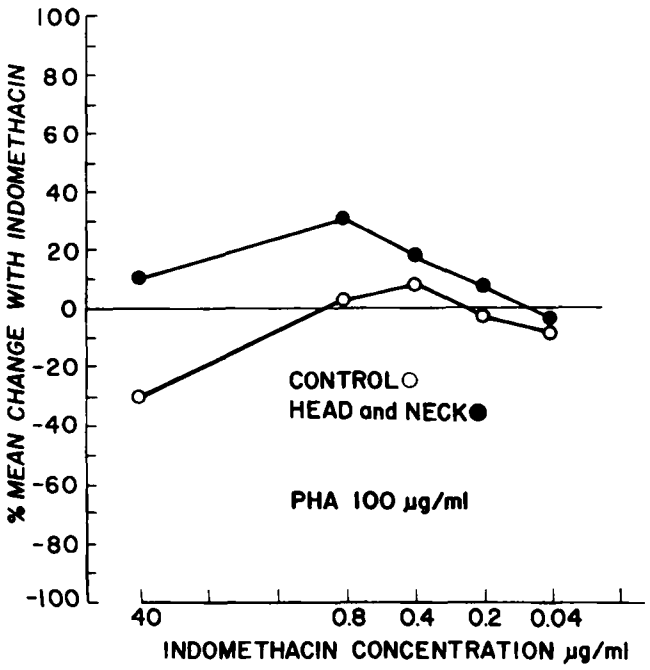
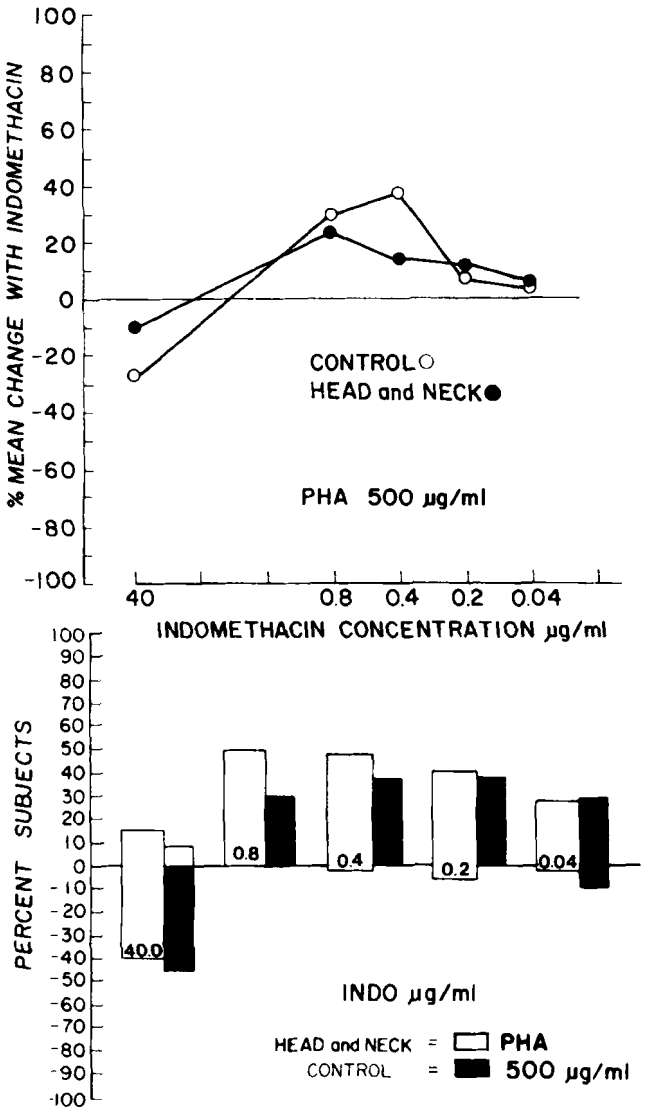


FIG. 3. This figure shows the mean percent change in PHA response as modified by coculturing of lymphocytes with different indomethacin concentrations. A typical dose–response curve results ranging from marked suppression at the highest indomethacin concentration, peak, and then demonstrated responses with increasing dilution of drug.

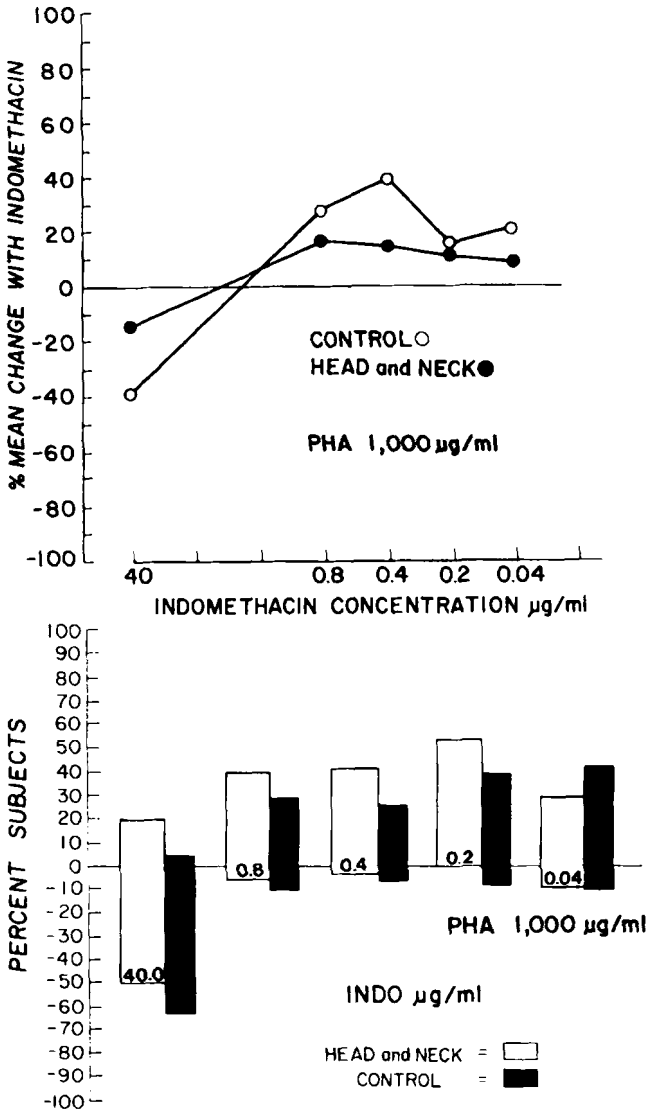


FIGS. 5A AND 5B. (A, top) The dose-response curve for the lymphocyte response to PHA at 500 µg/ml after coculturing with indomethacin is shown. (B, bottom) The percentage of subjects showing >10% change from baseline is displayed. There is no difference between controls and the cancer patients.

increases than those seen with any of the PHA concentrations. Again, indomethacin elicited a classic dose-response curve (with marked suppression) at concentrations of 40 µg/ml, an elevated response at concentrations of 0.8 to 0.2 µg/ml, and a lesser response at indomethacin concentration of 0.04 µg/ml. The mean peak response was 30% to 40% (Fig. 7A), and 50% to 60% of test subjects showed augmented responses.

Relation of Indomethacin Response to Stage of Disease

Comparison of responses according to stage of disease is shown in Table 1. Presented in this table are the indomethacin effects on the lymphocyte response to PHA at a highest concentration (1000 µg/ml) and to SPL at 1:10 dilution. The frequencies of changes in response in the cancer patients were compared to that in the controls using the Wilcoxon matched-pairs test. Positive or negative responses were those >10% of the mean baseline response to the stimulant (done in triplicate). The responses with indomethacin 40.0 µg/ml were depressed in both patients and controls (presumably on a toxic basis due to the drug or alcohol concentration). The alcohol concentration of 0.4% in this indomethacin preparation produced a modest depression of itself. The alcohol in the more dilute preparations had no effect. The cancer patients generally showed increased responses within each stage after culturing with indo-

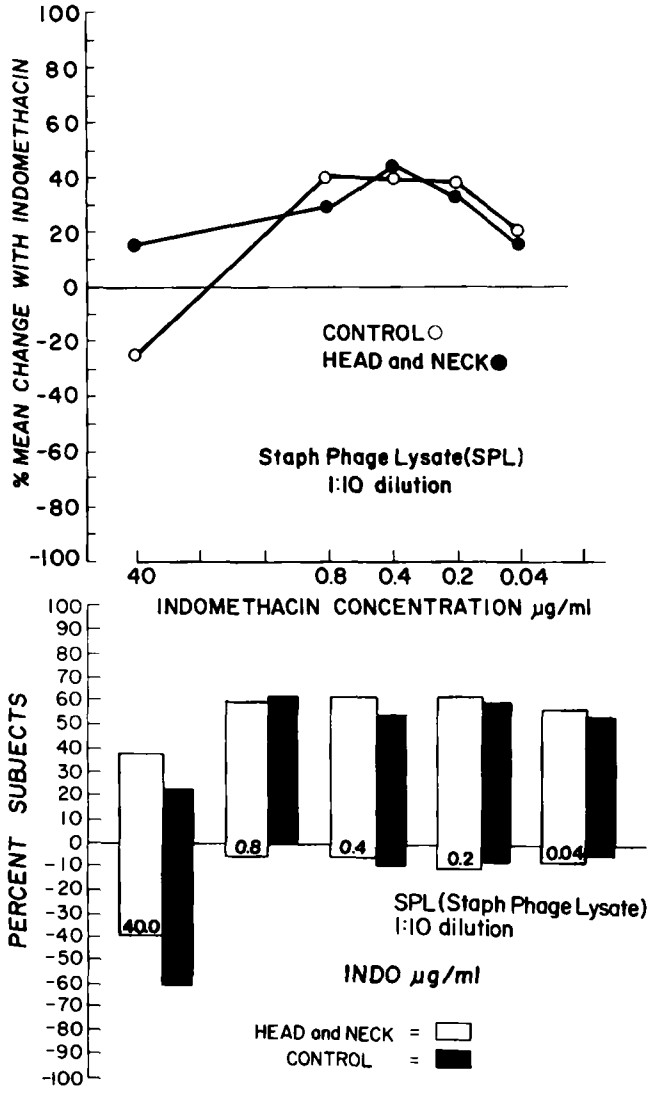


FIGS. 6A AND 6B. (A, top) The dose-response curves (mean percent change) for PHA stimulated lymphocytes after coculturing with indomethacin is shown (PHA at 1000 µg/ml). (B, bottom) The figure shows the percent of subjects whose PHA lymphocyte responses are modified by indomethacin and produce >10% change from baseline.

methacin 0.8 to 0.04 μg/ml. Approximately 52% to 60% of cancer patients had increased responses to PHA and 71% to 73% showed increased responses to SPL. These responses in the cancer patients were statistically significant in comparison to controls only in patients with Stage III (considering the PHA response) and Stages III and IV (considering the response to SPL).

Relation of Indomethacin Effects to Baseline Lymphocyte Responses

There appeared to be a relationship between the baseline response to suboptimal concentrations of PHA and the ability of selected concentrations of indomethacin to augment that response (Table 2). Among the patients who had low response rates (less than 50,000 cpm) to a suboptimal PHA concentration of 100 μg/ml, 63% showed augmentation in comparison to 31% in the group with baseline PHA responses more than 50,000 cpm ($P < 0.005$ by chi-square). However, there were no differences at the two higher PHA concentrations. In controls, only at PHA 500 μg/ml was a significant difference noted between the PHA baseline responses less than 50,000 cpm and those greater than 50,000 cpm. Here, 85% of the group with low baseline responses were augmented by indomethacin compared to 54% in the group whose baseline responses were normal (>50,000 cpm) ($P < 0.05$). Examination of the correlation coefficients (Pearson) between the PHA response and the augmentation effects of indomethacin did not show a significant correlation between the absolute level of the PHA response and the percentage of indomethacin-induced augmentation or depression. Thus, at PHA concentrations of 100 μg/ml, the values at the most responsive concentration of indomethacin (0.8 μg/ml) were 0.385 in cancer patients and 0.084 in controls (P = not significant [NS]).



FIGS. 7A AND 7B. (A, top) The indomethacin effects on the lymphocyte response to staph phage lysate are shown. (B, bottom) The percent of test subjects whose lymphocyte responses to SPL are modified >10% from baseline by indomethacin are shown.

Effect of Age

Normal persons: A separate study was done to examine the effect of age on the indomethacin response in 12 normal persons who were studied during the same time period Table 3. There were six younger persons, with a mean age of 29 years (range, 20–42), and a group of six older persons, with a mean age of 58 years (range, 48–77). We compared the effect of indomethacin on the whole leukocyte population and on the adherent cell-depleted lymphocyte population. Both younger and older subjects showed increased responses by all indomethacin concentrations, except for the largest indomethacin concentration (40.0 μg/ml), which produced depression. Although the mean increment increase ranged from 9% to 23% in both age groups, these differences were not statistically significant. Only indomethacin at 40 μg/ml produced significant changes (depression, in this case). Comparison of the mean responses between younger and older controls showed modest differences in incremental increases at certain indomethacin concentrations. Indomethacin at 0.4 μg/ml was associated with greater responses in younger persons, and indomethacin 0.04 μg/ml produced greater responses in older individuals ($P = 0.05$). If one includes all of the subsets studied with different indomethacin concentrations, there does not appear to be a difference between the younger and older patients.

Cancer patients: The effects of age on the indomethacin modulation (0.2 μg/ml) of the PHA (100 μg/ml) response in head and neck cancer patients and controls is shown in Figure 8. Age 50 was used as a cut-off point

TABLE 1. Effect of Indomethacin on Lymphocyte Responses of Head and Neck Cancer Patients According to Stage of Disease

Lymphocyte stimulant	Stage 1 (N = 6)		Stage 2 (N = 6)		Stage 3 (N = 15)		Stage 4 (N = 18)	
PHA 1000 μg/ml	(%↑)	(↓%)	(%↑)	(↓%)	(%↑)	(↓%)	(%↑)	(↓%)
Indomethacin								
40.0 μg/ml	17	67	40	60	20	73	17	72
0.8	17	0	40	20	60	13	56	22
0.4	56% 67	17	60% 60	0	60% 53†	0	52% 44	22
0.2	83	0	80	20	67†	7	56‡	17
0.04	100	0	40	20	50†	7	33	33
SPL 1:10								
Indomethacin								
40.0 μg/ml	33	67	20	60	73	13	31	69
0.8	67	0	100	0	73§	13	63‡	19
0.4	72% 83	0	73% 60	20	73% 80§	0	71% 75‡	13
0.2	67	0	60	0	67§	20	75‡	13
0.04	67	17	80	0	71§	14	44	19

• %↑ or ↓% refers to percentage of patients in whom there was >10% change induced by indomethacin. The mean (%) change of three optimum indomethacin concentrations (0.8 to 0.2 μg/ml) is shown.

The above values are compared to the responses in the controls at the respective indomethacin concentrations. Differences: †$P < 0.05$; ‡$P < 0.02$; §$P < 0.01$ by Wilcoxon matched pairs signed rank test.

PHA: phytohemagglutinin; SPL: staph phage lysate.

in view of the above study in normal persons, which generally showed no difference with this cut-off point. Responses were categorized as either positive (showing augmentation by indomethacin) or negative (no response, or decreased response). The number and percentage of positive responses in the head and neck cancer patients younger than 50 years *versus* the controls younger than 50 years was four of five (80%) *versus* 31 of 49 (63%) (P = NS). Among the study subjects older than age 50 years, the number of positive responses was 32 of 48 (67%) in the head and neck cancer patients *versus* nine of 11 (82%) in the controls (P = NS). If one examined the head and neck cancer patients within the same stage (IV, generally the most depressed group), the patients younger than 60 years of age showed a generally greater number of augmented responses with indomethacin than in the older patients, especially at PHA concentrations of 500 μg/ml and 1000 μg/ml. Most of these differences were significant ($P < 0.005$) (Table 4).

Effect of Depletion of Adherent Cells

The effect of depleting the adherent cells on the lymphocyte stimulation response and its modulation by indomethacin was examined in the cancer patients and controls. Plastic adherence of Ficoll-Hypaque separated leukocytes produced about 25% lymphocyte enrichment. This was analyzed in six patients and seven controls by use of the nonspecific esterase stain[27] and the Giemsa and Wright's stains. The percentages of lymphocytes (±SD) after Ficoll-Hypaque separation were 70.1 ± 6.4% in controls and 79.6 ± 11.2% in the cancer patients (by nonspecific esterase stain). A single-step plastic adherence for 1 hour changed this to 90.4 ± 10%

TABLE 2. Relation Between the Baseline PHA Response and the Augmentation Induced by Indomethacin (0.8 μg/ml)

		Patients' baseline PHA response		Controls' baseline PHA response	
PHA concentration	Indomethacin effect	<50,000 cpm	>50,000 cpm	<50,000 cpm	>50,000 cpm
100 μg/ml	+	25† (63%)	29 (31%)	7 (47%)	34 (38%)
	−	15	65	8	55
500 μg/ml	+	25 (63%)	60 (67%)	11* (85%)	38 (54%)
	−	15	29	2	32
1000 μg/ml	+	45 (75%)	40 (66%)	21 (81%)	53 (77%)
	−	15	21	5	24

* $P < 0.05$.

† $P < 0.005$.

Notice: PHA at 100 μg/ml shows the greatest discrimination in the responses between cancer patients and controls (Fig. 1).

±: Indomethacin at a concentration of 0.8 μg/ml produced augmentation (+), or depression, or no change (−); PHA: phytohemagglutinin.

in controls and 93.9 ± 4% in cancer patients. If the step is repeated, the percentages of lymphocytes are further increased to 97.5 ± 1.6% and 96.3 ± 2%, respectively. This amount of adherent cell depletion with the two-step maneuver resulted in a loss of lymphocyte responsiveness to mitogens, and thus only the one-step method was used.

The effect of depletion of adherent cells on the lymphocyte response to PHA 100 μg/ml in control persons and cancer patients is shown in Figures 9 and 10. Depleting adherent cells resulted in a significant increase in the mean lymphocyte responses to PHA in the cancer patients, but not in the controls. Despite a marked increase in responses in the cancer patients, the values achieved were still significantly less than the responses in the controls. The effects of indomethacin are best shown by registering the number of patients showing change from their baseline (Fig. 11), rather than by measuring changes in the means of the group studied (Figs. 9 and 10), because the broad standard error involved nullifies the observed changes. Indomethacin produced the greatest increase in the PHA responses of the total lymphocyte population and much more modest changes in the lymphocytes that had been depleted of adherent cells. Of the controls, an equal number showed an increase or a decrease in the PHA responses of the total lymphocytes and of the lymphocytes depleted of adherent cells. The effect of age on adherent cell depletion in a selected group of normal persons (older or younger than 50 years) is shown in Table 3. Adherent cell depletion increased the total lymphocyte response to PHA (100 μg/ml) from a mean of 3.04×10^5 to 3.35×10^5 cpm (18.8% increase) in the younger persons and from 2.34×10^5 cpm to 3.04×10^5 cpm (29.9% increase) in the older group ($P < 0.05$). Indomethacin produced only slight augmentation in the adherent-cell depleted lymphocyte response to PHA in younger persons (about 6%–10%) and essentially had no effect in the older persons (1%–3% increase). None of these changes were significant.

Discussion

Although there are many factors involved in the depression of cell-mediated immunity in head and neck cancer patients, this and other studies suggest that indomethacin-sensitive suppressor cells may have an important role in this phenomenon. Incubation with selected concentrations of indomethacin produced bidirectional effects on the lymphocytes of cancer patients and controls. Lymphocyte stimulation response to PHA was increased in over 50% of cancer patients and control normal persons by optimum concentrations of indomethacin. There were corresponding depressive effects in both study groups as well depending on the concentration of

TABLE 3. Effect of Age on the Modulation of PHA Lymphocyte Blastogenesis by Indomethacin (Culture Conditions: PHA 100 μg/ml)

	Total cell			Adherent cell-depleted		
	Young (N = 6)	Old (N = 6)	Significance	Young (N = 6)	Old (N = 6)	Significance
Baseline	334990 ± 39009	233612 ± 53901		397790 ± 74940	303612 ± 57172	
Indo 40 μg/ml	212127 ± 29851* (↓37%)	124405 ± 35033 (↓47%)	$P < 0.05$	219560 ± 37270 (↓45%)	135039 ± 26228 (↓56%)	$P < 0.05$
Indo 0.8 μg/ml	381824 ± 44765 (↑14%)	253556 ± 60938 (↑9%)	$P = NS$	431147 ± 68054 (↑8%)	293034 ± 50422 (↑3%)	$P = NS$
Indo 0.4 μg/ml	391006 ± 40791 (↑17%)	260206 ± 54925 (↑11%)	$P < 0.05$	431575 ± 71351 (↑9%)	306760 ± 56703 (↑1%)	$P = NS$
Indo 0.2 μg/ml	398275 ± 53610 (↑19%)	261380 ± 60322 (↑12%)	$P = NS$	435389 ± 66710 (↑10%)	293044 ± 52048 (↑3%)	$P = NS$
Indo 0.04 μg/ml	387906 ± 40995 (↑16%)	286859 ± 56225 (↑23%)	$P < 0.05$	420154 ± 65411 (↑6%)	300745 ± 54755 (↑1%)	$P = NS$

Mean values young versus old

1. Mean values, young *versus* old: Significant difference, $P < 0.05$ at Indo concentrations of 40, 0.4, and 0.04 μg/ml
2. Increment change from baseline; *young *versus* old Indo 40 μg/ml $P < 0.05$

1. Mean values young *versus* old: No difference at any Indo concentration
2. Increment change from baseline: no difference

Nos: mean cpm ± SE; young: mean age 29 yr (20–42 yr); old: mean age 58 yr (48–77 yr); PHA: phytohemagglutinin; Indo: indomethacin; NS: not significant.

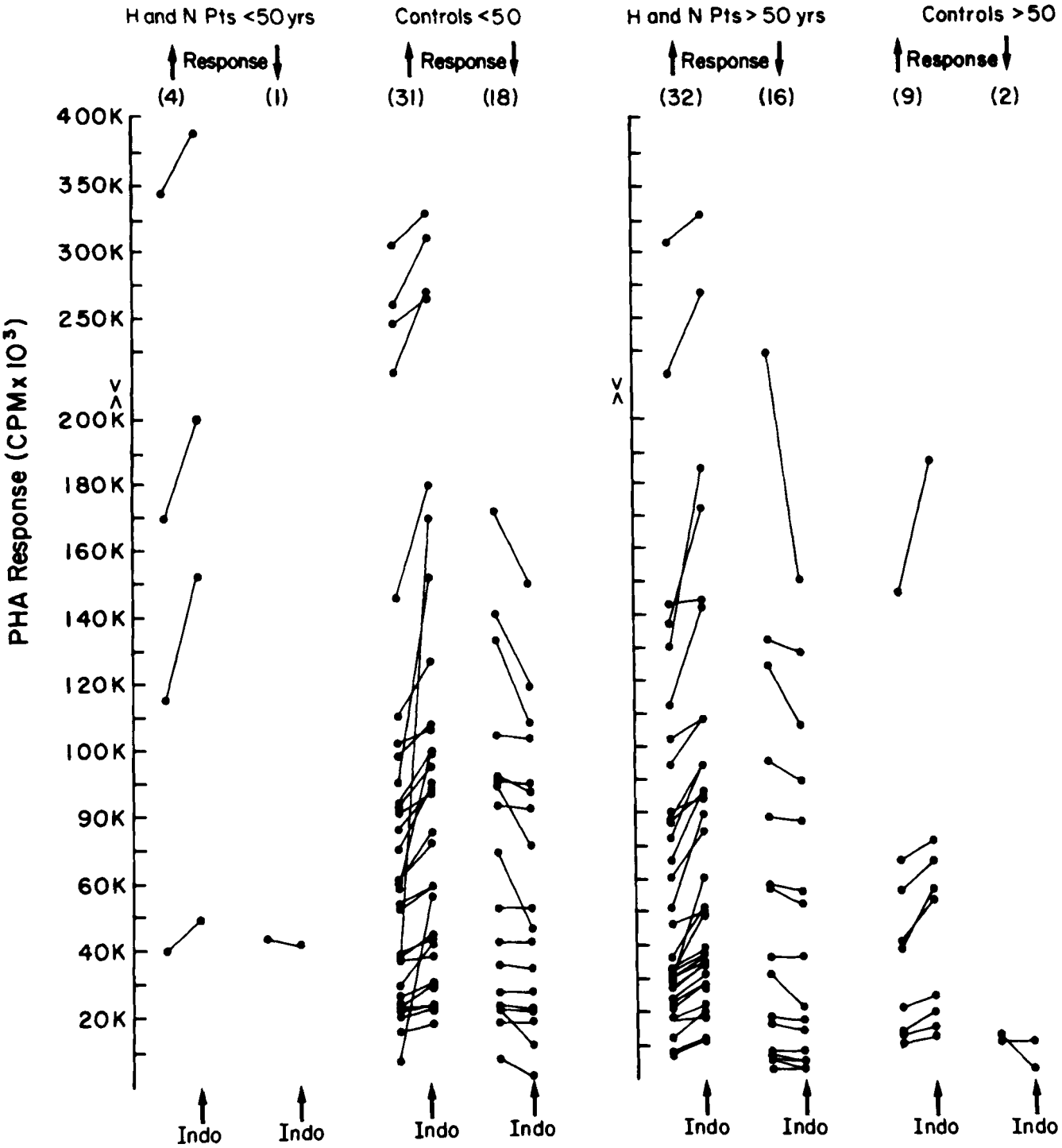


FIG. 8. The effect of age on the modulating effect of indomethacin (0.2 μg/ml) on the response to PHA (100 μg/ml) is shown in individual subjects using age 50 as the cut-off. The positive response rate in cancer patients *versus* controls was 80% *versus* 63%, respectively, in the >50-year-old group (P = NS); in the <50-year-old group, the response rate was 67% *versus* 82%, respectively (P = NS).

PHA used; usually, less than 10% of test subjects showed any suppression. A more consistent augmenting effect was observed where the lymphocyte stimulant was an antigen (Staph Phage Lysate). This may be more biologically relevant to the *in vivo* situation in man, in whom the immune system is continually bombarded by a variety of antigens, ranging from bacteria and parasites to tumor cells.

It would appear that a regulation defect is operative here. Perhaps continued T-cell stimulation has been off-

TABLE 4. Effect of Age on Indomethacin Response of Patients With Stage IV Head and Neck Cancer

	< 60 yr old, n = 8			>60 yr old, n = 12		
	Indo Conc (μg/ml)	%↑	%↓	%↑	%↓	chi-square
PHA 100 μg/ml	40.0	0	63	0	92	NS
	0.8	(33%) 50	12.5	(25%) 25	17	$P < 0.10$
	0.4	(33%) 25	25	(25%) 33	33	NS
	0.2	(33%) 25	25	(25%) 17	33	NS
	0.04	12.5	38	8.3	33	NS
PHA 500 μg/ml	40.0	25	50	0	100	$P < 0.005$
	0.8	(58%) 50	0	(28%) 25	25	$P < 0.005$
	0.4	(58%) 75	12.5	(28%) 17	25	$P < 0.005$
	0.2	(58%) 50	12.5	(28%) 42	17	NS
	0.04	50	0	25	17	$P < 0.005$
PHA 1000 μg/ml	40.0	12.5	75	0	100	$P < 0.005$
	0.8	(79%) 88	0	(42%) 50	8.3	$P < 0.005$
	0.4	(79%) 75	0	(42%) 33	25	$P < 0.005$
	0.2	(79%) 75	0	(42%) 42	17	$P < 0.005$
	0.04	63	12.5	50	25	NS

Indo: indomethacin; Conc: concentration; NS: not significant.

(%): Mean percent change induced by indomethacin concentrations of 0.2, 0.4, and 0.8 μg/ml.

set by an over-exuberant macrophage response with overproduction of PGE_2 with a corresponding depression of some aspects of T-cell function. Although we have not measured macrophage production of PGE_2 in these patients, the indomethacin effects suggest that the monocyte/macrophage is involved. The augmentation

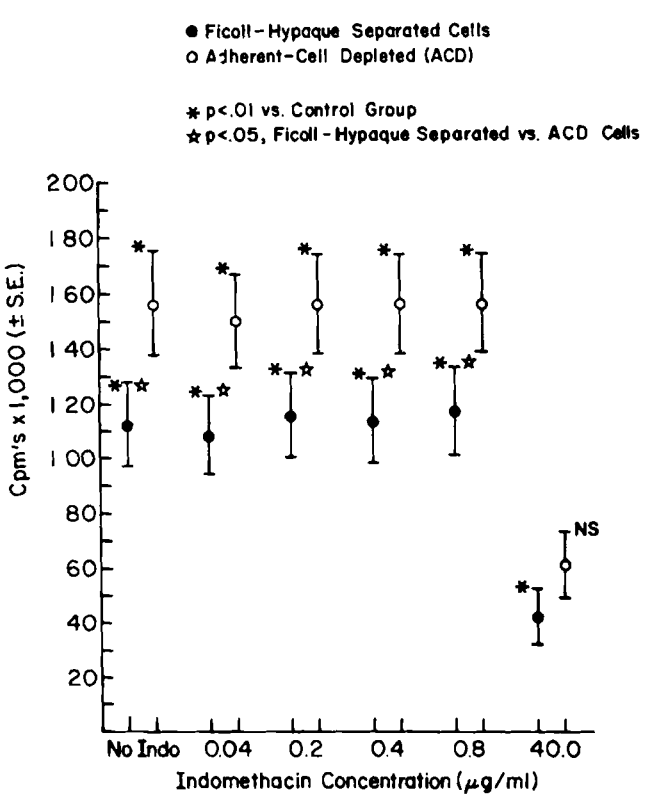


FIG. 9. Response to PHA (100 μg/ml) + indomethacin in cancer patients, n = 27. Removal of adherent cells in the cancer patients resulted in a restoration of the baseline response (no indomethacin group). There was no further augmentation by adding indomethacin to the adherent cell-depleted lymphocytes in the cancer patients.

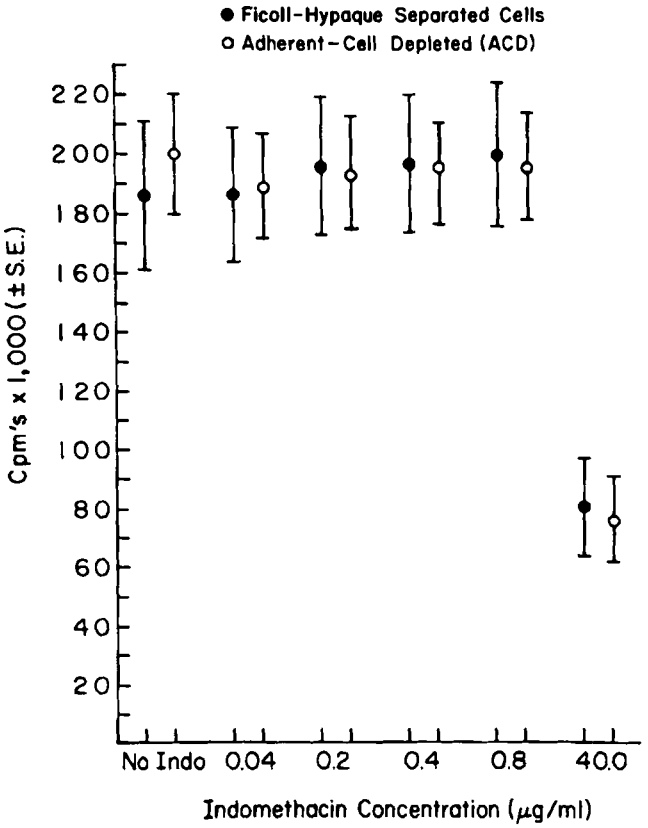


FIG. 10. Response to PHA (100 μg/ml) + indomethacin in control group, n = 25. Removal of adherent cells in the controls did not significantly alter the PHA response (no indomethacin group); nor did the addition of indomethacin alter the responses.

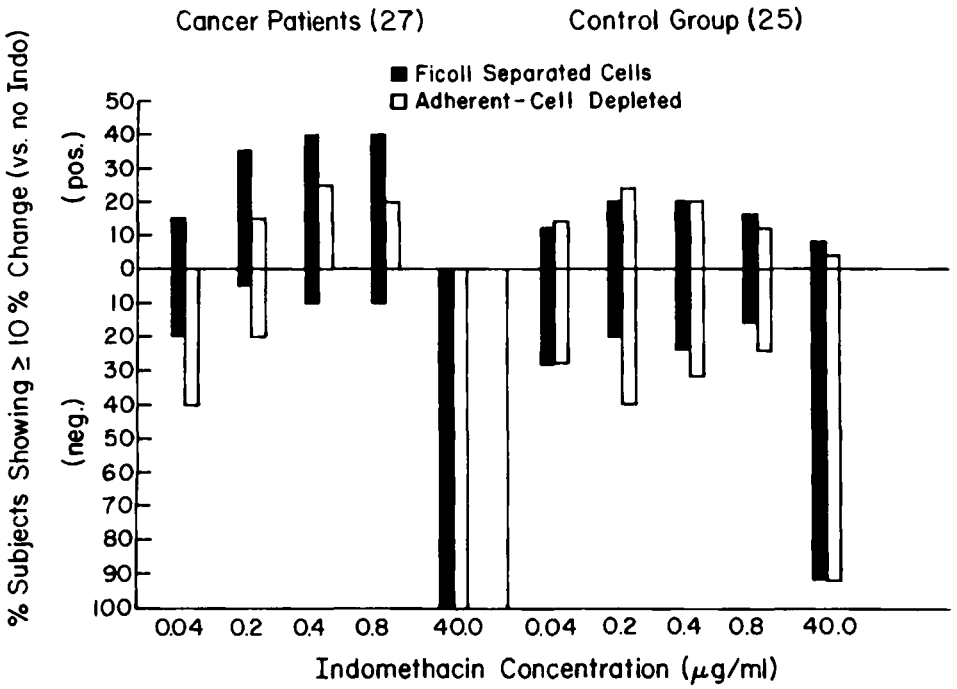


FIG. 11. The individual effects of indomethacin on the PHA (100 μg/ml) responses of the total mononuclear population (Ficoll-Hypaque separated) compared to the adherent cell-depleted cells is shown. A greater number of the cancer patients than controls showed indomethacin-induced augmentation in studies of the total mononuclear population (at indomethacin concentrations of 0.04 to 0.8 μg/ml) ($P = 0.05$). There were no significant differences in the adherent cell-depleted group.

of the lymphocyte response by indomethacin would appear to be due largely to the inhibition of PGE_2 release by adherent mononuclear cells. Similar effects also were obtained by simple removal of a large nonspecific esterase-positive adherent cells using plastic adherence. This suggests that the adherent mononuclear cells in the circulation may be functioning as suppressor cells and as such may have a major role in the depressed lymphocyte responses to mitogens and antigens seen in the head and neck patients. Although the exact nature of these cells was not exhaustively defined, it appears that most of them are of a monocyte–macrophage origin; however, some adherent T-cells also could be involved.

The findings reported here regarding head and neck cancer patients support what was demonstrated by Goodwin and colleagues[23] as occurring in patients with Hodgkin's disease. They identified a glass-adherent, prostaglandin-producing suppressor cell as being responsible for the hyporesponsiveness to phytohemagglutinin commonly reported in Hodgkin's patients. Other causes of cell-mediated suppression also could be operative in the head and neck cancer patients.[28–30] Rice and co-workers have evaluated three suppressor systems in human blood that can inhibit T-cell proliferation[28]: (1) a prostaglandin-releasing adherent-cell system (as mentioned above); (2) suppressive non-PGE_2-releasing adherent cells (acting *via* cell-to-cell contact); and (3) suppressor T-cells that are induced by mitogens.

The prostaglandin synthetase inhibition would be expected to exert its major effect *via* the adherent monocyte, as this would appear to be the major producer of PGE_2 among the immunocytes.[29] Suppressor T-cells also may participate in the cell-mediated suppression of the head and neck cancer patients,[30] but the extent of the suppression that is reversible by a prostaglandin synthetase inhibitor suggests that T-cell suppression was not a major factor, at least in this study. Prostaglandins are not thought to be a major participant in the activity of suppressor T-cells.[28] The role of suppressor T-cell activity in the lymphocyte hyporesponsiveness of head and neck cancer patients is not clear. One study has shown an impaired ability to generate suppressor T-cells by the concanavalin A method in head and neck cancer patients, but the basal suppressor T-cell function was not completely assessed.[9] It must be remembered, however, that although the prostaglandin-producing cells in human peripheral blood are thought to be glass-adherent monocytes,[28,29] because the techniques of monocyte separation all employ some method of removing adherent cells, it is not possible to rule out a prostaglandin-producing, glass-adherent T-cell.[31]

The prostaglandin-producing suppressor cell also exerts a more dominant role with aging and is considered to be a major contributor to the depression of cellular immune function seen in patients older than 70 years.[31,32] In the study by Rao and co-workers, there were no significant differences in the indomethacin effects between subjects in the 23-year-old to 48-year-old

group and those in the 65-year-old to 74-year-old group.[33] Goodwin and Webb observed that age became a factor only when the patient was older than 70 years.[34] Many patients with head and neck cancer are in the intermediate or older age groups; therefore, we may suspect that the aging process also is involved. There may be additional factors that affect immune regulation in this group, however. In the cancer patients with advanced Stage IV disease who were older than 60 years, indomethacin was less effective in restoring depressed lymphocyte responses than observed in the younger patients with the same stage of disease. Thus, here the immune regulatory imbalance induced by senescence (and presumably responsive to indomethacin) was no longer responsive to indomethacin inhibition of PGE_2 production, suggesting that the system has been further subverted by the disease state (*i.e.*, host effects from the cancer or perhaps malnutrition).

The mechanism for the apparent prostaglandin-mediated immune suppression in head and neck cancer patients is not clear. Malignancy itself can be involved as a contributing agent. Plescia and co-workers have shown reversal of tumor-mediated immunosuppression *in vitro* with PG synthetase inhibition.[35] The same investigators have described a PGE_2-secreting, chemically induced tumor that can inhibit the antibody response to sheep red blood cells (SRCB) by mouse splenocytes.[36] Osheroff and Webb have shown in the mouse that upon stimulation by PGE_2, glass-adherent lymphocytes can release a factor that activates adherent suppressor cells, which in turn can inhibit the lymphocyte response to PHA.[37] Other tumor products of various types also might be involved, such as released antigens and immune complexes, which could activate suppressor activity in circulating mononuclear cells. Not to be forgotten is the underlying history of smoking and chronic alcoholism (common in these patients), which could activate the suppressor cell systems. Alcohol is a known inducer of acute immune suppression.[38] Whether this suppression is mediated by prostaglandins is not known, however.

The use of an inhibitor of prostaglandin synthesis has provided a useful probe to examine the mechanism of immune depression in patients with head and neck cancer. A corollary to these observations is the need to examine the *in vivo* effects of indomethacin as a potential immune modulating agent in these patients.

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