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

RICHARD D. MACA, MD* AND WILLIAM R. PANJE, MD†

The effects of the addition of indomethacin to PHA or Con A stimulated lymphocytes from patients with untreated squamous cell carcinoma of the head and neck or from patients with the disease who have just finished irradiation therapy from the disease was quantitated and compared to those of the control group. Lymphocytes from eight of 26 patients with untreated carcinoma were significantly augmented by the addition of indomethacin. The remaining eighteen patients were equal to the controls. For all 17 patients who had just finished extensive field irradiation therapy, significant enhancement of PHA and Con A reactivity by indomethacin was found, which did not appear to be solely a function of low baseline mitogen reactivity. In additional studies, stimulated lymphocytes of irradiated patients were tested for their sensitivity to the inhibitory effect of PGE_2. The mitogen treated lymphocytes from all patients that had just finished irradiation therapy were found to be significantly more sensitive to the inhibition by PGE_2 as compared to the normal lymphocyte response. This effect was also found not to be related merely to a low PHA or Con A reactivity of the lymphocytes. In both patient groups there was a striking correlation between the percent augmentation of indomethacin and the percent inhibition of PGE_2 in that when the percent augmentation values were low so were percent inhibition values and when the degree of augmentation by indomethacin was elevated so was the inhibition by PGE_2. This data suggests that increase sensitivity of stimulated lymphocytes to PGE_2 may be responsible, at least in part, for the depressed mitogen response and the significant augmentation of this immune response by indomethacin in about ⅓ of the untreated patients with advanced head and neck carcinoma and in those patients who have just finished irradiation therapy. The results of this study support the hypothesis that perhaps patients receiving irradiation therapy may benefit by the oral administration of indomethacin, an approach that needs further consideration.

Cancer **50:483–489, 1982.**

MANY PATIENTS with head and neck carcinoma have impaired cell mediated immunity as demonstrated in both *in vivo*[1-4] and *in vitro*[5-9] methods. T-lymphocyte levels have been shown to be depressed as reported by Zighelboim *et al.*,[4] Papenhausen *et al.*,[5] and others.[7] In regard to lymphocyte function tests, the lymphocyte response to various mitogens, such as Concanavalin A (Con A) or phytohemaglutinin (PHA), or allogeneic lymphocytes has also been demonstrated to be depressed, even in patients with early disease such as Stage I or II.[4,6-9] In addition, other investigators have found that progressive suppression of these tests was associated with advancing disease.[7,9] Recently Ryan *et al.*[10] reported that pretreatment PHA and Con A induced blastogenesis was of predictive value in that pretreatment reactivity was greater in patients who remained tumor-free and often depressed in patients in whom tumor recurred.[10]

Radiation therapy has also been shown to be immunosuppressive in patients with head and neck carcinoma. T-lymphocyte levels decline progressively during therapy.[11,12] Mixed lymphocyte reactions and PHA responses were also shown to be depressed in this treated patient group[12,13] and this suppressed immune competence may persist for prolonged, disease-free periods of time.[14]

Although the etiologic cause for depressed PHA response may be multiple, one mechanism for this hypoactive state is the existence of a suppressor cell in the mononuclear cell fraction. Such regulatory cells have already been described in patients with different tumor

From the Department of Medicine and Otolaryngology, University of Iowa College of Medicine, Iowa City, Iowa.

This work was supported in part by an American Cancer Society Institutional Research Grant.

* Associate Professor of Medicine, Veterans Administration Medical Center and Louisiana State University, Shreveport, Louisiana.

† Director, Division of Head and Neck Surgery, Department of Otolaryngology and Maxillofacial Surgery, University of Iowa Hospitals, Iowa 52242.

Address for reprints: William R. Panje, MD.

The authors thank Ms. Gretchen Rosenburger for her very capable technical assistance.

Accepted for publication June 1, 1981.

0008-543X/82/0801/u483 $0.85

types.[4,6,15-18] These suppressor cells appear to be monocytes and the mechanism by which they exert their suppressor effect may be several. In Hodgkin's disease, this suppressor action appears to be mediated by the synthesis and release of prostaglandins of the E series (PGE_2) and thus is sensitive to the effects of indomethacin, a prostaglandin synthesis inhibitor.[15] In patients with head and neck carcinomas, Berlinger *et al.*[6] have described adherent suppressor cells that display a suppressor effect in the mixed lymphocyte reaction. More recently, Zighelboim *et al.*[4] have shown that a population of phagocytic cells were capable of suppressing PHA responses in these patients. However, neither group presented data to indicate whether these suppressor cells were mediating their effects by the production of prostaglandins. Thus the main objective of this study was to determine whether the depressed PHA reactivity of lymphocytes from patients with head and neck carcinoma could be significantly improved or completely corrected by the addition of indomethacin to the culture medium. Both patients with untreated disease and patients who have just finished irradiation for therapy of their disease were studied. The identification of such an indomethacin sensitive suppressor cell (ISSC) in this patient group would obviously have important clinical implication in the management and in designing specific treatment programs for patients with head and neck carcinoma.

Methods and Materials

Control and patient groups

Two control and two patient groups were studied. One control group consisted of 16 normal blood donors average age of about 30 years. The second control group referred to as ENT control group consisted of 12 patients with nonmalignant, noninfectious ear-nose-throat (ENT) problems, such as dizziness. The average age of this group of patients was 56, which was similar to the age group of the two patient groups. One patient group consisted of newly diagnosed, untreated Stage III or IV head and neck squamous cell carcinoma. The other patient group consisted of patients with this disease who have just finished radiation therapy for treatment of their disease. The irradiation therapy consisted of two forms, wide field and medium size field. Wide or extensive field irradiation indicates that the patient received 10 MeV X-ray or Cobalt 60 to right and left lateral opposing 12 × 13 cm fields and an anterior yoke 11 × 28 cm field to a midplane tumor dose of 4950 rad, usually given over 5 to 5½ weeks. 175–200 rad per day was given in five daily doses with a two-day rest period. A tumor dose boost to 7 × 6 cm lateral opposing fields was then completed to a total midplane tumor dose of 1980 rad, giving an overall midplane tumor dose of 6930 rad. Medium field irradiation usually indicated that the patient received 10 MeV x-ray to 15 × 13 cm lateral opposing fields to a midplane tumor dose of 5000 rad with a subsequent reduction in field size to 6 × 6 cm and further delivery of a midplane tumor dose of 2000 rad. This was usually given over a seven-week period.

Preparation of the peripheral blood lymphocytes (PBL)

PBL were obtained from heparinized blood from either patients or controls by Ficoll-Hypaque density gradient centrification.[19]

The interface cells were washed and resuspended at a density of 10^6/ml in RPMI 1640 medium containing 10% fetal calf serum (FCS), 15 mM HEPES buffer and Neomycin (100 U/ml) (GIBCO, Grand Island, N. Y.) for use in the various assay systems.

Quantitation of Indomethacin Augmentation of PHA and Con A Stimulated PBL

One-tenth milliliter of cell suspension containing 100,000 PBL was added to each flat bottom well of a 96 well microtiter plate (#3040-Falcon Corporation, Oxnard, CA). The cells were stimulated with either various concentrations of Con A, 5–20 μg/ml (Sigma Chemical Co., St. Louis, Mo.) or PHA, either 0.5, 1.0 and 2.0 μg/ml (Burroughs-Wellcome, Greenville, NC) to insure adding the optimal stimulatory amount. The PBL were also treated either with 2 μg/ml indomethacin (Sigma Chemical Co.) or equal volumes of ethanol (solvent for the indomethacin). The cultures were then incubated at 37°C in a humidified incubator with 5% CO_2 atmosphere for 72 hours. After this time the cultures were pulsed for six hours with 1 μCi ^{3}H-TdR (tritiated thymidine) (New England Nuclear, Boston, Massachusetts, Specific Activity, 6.7 Ci/mM) and harvested on to glass fiber filters with a multiple sample harvester (Brandel Co., Gaithersburg, MD). The radioactivity of each disc was counted in a liquid scintillation counter and the degree of augmentation was calculated as follows:

$$\% = 100 \times \left[\frac{\text{Average cpm of indomethacin treated cultures}}{\text{Average cpm of control (ETOH) cultures}}\right] - 1.0$$

The percent augmentation by indomethacin of normal lymphocytes increased when suboptimal concentrations of mitogens were used. Therefore, the percent augmentation values were always calculated using the CPM value for cultures that were stimulated maximally with the optimal mitogen dosage. It was not uncommon for

TABLE 1. The Enhancement of PHA and CON A Reactivity by Indomethacin in Patients with Head and Neck Cancer Either Untreated or After Radiation Therapy

			Addition of Indomethacin (2 μg/ml)			
			PHA* Treated PBL		CON A* Treated PBL	
Study group	Age (yr)	No. of expts.	Cpm × 10^3 ± S.E.	% Augmentation ± S.E.	Cpm × 10^3† ± S.E.	% Augmentation ± S.E.
Normal controls	30	16	95.4 ± 4.6	12.8 ± 2.0	76.1 ± 5.8	10.1 ± 9.5
ENT Controls	56	12	78.8 ± 8.6	6.8 ± 2.3	52.9 ± 7.4	12.2 ± 7.4
Cancer patients with normal individual augmentation value	59	18	73.1 ± 3.5	6.3 ± 1.5	56.8 ± 3.3	14.6 ± 2.8
Cancer patients with elevated individual augmentation values	67	8	49.1 ± 6.5	48.4 ± 4.2	41.7 ± 8.3	56.9 ± 16.6
Cancer patients finishing *extensive field irradiation*	59	17	20.6 ± 2.6	88.0 ± 9.7	20.3 ± 2.8	76.4 ± 11.6
Cancer patients finishing *medium field irradiation*	69	5	55.0 ± 12.1	36.6 ± 11.3	40.8 ± 12.9	43.8 ± 9.2

* Maximal Stimulatory Conc., ranging from 1-2 μg/ml for PHA and 5-20 μg/ml for Con A.

† CPM of Control (ETOH) mitogens stimulated cultures.

PBL from cancer patients to require more mitogen than for normal lymphocytes. All cultures were performed in triplicate and the significance determined by the Student's *t* test and a *P* value of <0.05 was considered significant.

The Depletion of Lymphocytes From the PBL Preparation

One-tenth ml of PBL (10^6/ml) were added to wells of microtiter plate. After incubation at 37° for one hour the nonadhering cells were collected, pooled, recounted, and adjusted to 1×10^5, 2×10^5 and 10×10^5 cells/ml. The adherent cells layer was washed × 3 to remove any remaining nonadherent lymphocytes, after which one of the three dilutions of the nonadherent lymphocyte population was added to the wells containing the monocyte monolayer. Lymphocytes were then treated with PHA and indomethacin as previously described. With this approach the number of reactive lymphocytes was markedly decreased without having to decrease the number of monocytes per well, which provided an opportunity to test the effect of indomethacin on PHA blastogenesis in light of low mitogen reactivity.

Quantitation of PGE_2 Sensitivity of PHA Stimulated PBL

PBL (10^6/ml) were treated with indomethacin (2 μg/ml) and then with PHA at an optimal stimulatory concentration of either 1 or 2 μg/ml. Then 0.1 ml of the cell suspension was added to each well after which various concentrations of PGE_2 varying from 0.001 to 1.0 ng/ml was then added. The cultures were then incubated for 72 hours at 37°, pulsed with 1 μCi ^{3}H-TdR for six hours, harvested and radioactivity quantitated as previously described. The percent inhibition was calculated as follows:

$$\% = 1.0 - \left[\frac{\text{Average cpm of } PGE_2 \text{ treated cultures}}{\text{Average cpm of control (ETOH) cultures}}\right] \times 100$$

Drugs

Prostaglandin E_2 was obtained from Upjohn Co., Kalamazoo, Michigan, and the indomethacin from Sigma Chemical Co. Both drugs were dissolved in 95% ethanol at a concentration of 10 mg/ml and diluted with culture medium until the final concentration was obtained.

Results

Response and Effect of the Addition of Indomethacin

The average PHA response and the percent augmentation by indomethacin of this response for the normal controls averaged $95.4 \times 10^3 \pm 4.6$ cpm (counts per minute)/well and 12.8 ± 2.0%, respectively, as compared to $78.8 \times 10^3 \pm 8.6$ cpm/well and 6.8 ± 2.3%, respectively, for the ENT control group (Table 1). These augmentation values were not significantly different even though a difference in age and in baseline reactivity is apparent. The results using Con A were similar. For the patient group consisting of untreated Stage III and IV patients with squamous cell carcinoma of the head and neck, the average PHA reactivity and percent augmentation for the PHA response averaged 65.7×10^3 cpm/well and 19.3%, respectively. However, when the individual values for both the control and patient group were plotted individually on a scattered

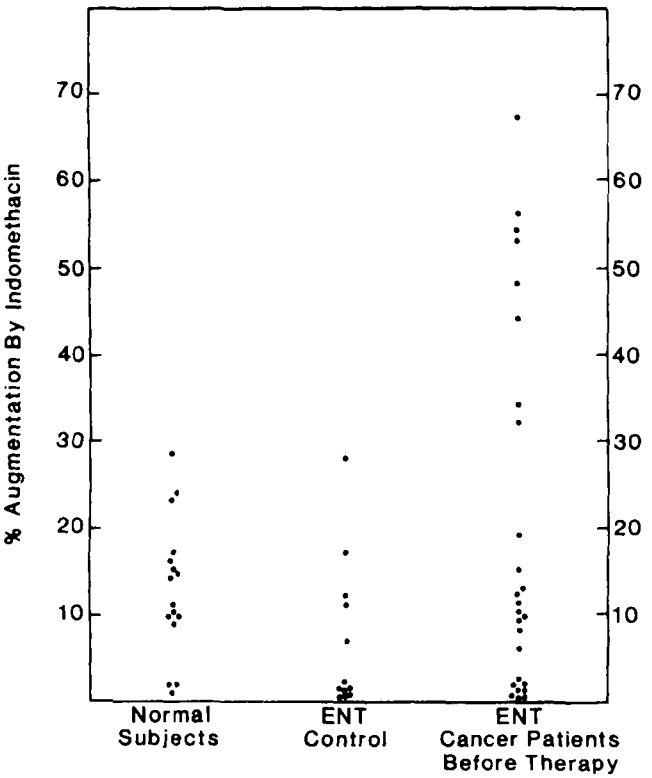

FIG. 1. The percent augmentation of the PHA response by indomethacin of peripheral blood lymphocytes from either normal subjects, control patients or patients with untreated carcinoma of the head and neck.

diagram one can see, from Figure 1, that eight of the 26 values (36%) lie outside the normal range (>30% augmentation by indomethacin) and each of these values were greater than the normal, average mean + 3 S.E., being 16.8% and 13.7% for the normal and ENT control groups, respectively. Since these values were significantly different than for the controls, this group was separated from the other patients with normal individual values and the two groups analyzed separately, the results of which are seen in Table 1. As would be expected, the baseline mitogenic response and the percent augmentation of those responses from the ENT patients whose individual values were within normal range were not different from those of the controls. However, for those patients whose individual values were significantly different from the mean, the average percent augmentation values were significantly different from the control values for both the PHA and Con A responses ($P < 0.01$). This data indicates that mitogenic response of lymphocytes from patients with untreated Stage III and IV head and neck carcinoma can be significantly improved by inhibiting prostaglandin synthesis and that in some instances these mitogenic responses could be completely corrected to normal values. To verify that the cell response for this suppression was, indeed, a monocyte as has been shown in other studies,[15] these studies were repeated with the PBL after the removal of the monocyte fraction. In these experiments, monocytes from the PBL preparation were first depleted by twice adherence for one hour on plastic tissue culture petri dishes. After this adherence procedure, the percent monocyte averaged about 1%. When indomethacin was added to either Con A or PHA stimulated, monocyte depleted, lymphocyte preparation no augmentation was seen. However, when autologous monocytes were added back to these same mitogen stimulated lymphocyte preparation, the PHA response was found to be augmented by indomethacin to the same degree as that of the original, unfractionated PBL preparation. To illustrate with a typical experiment, the cpm/well of PHA stimulated, monocyte depleted, lymphocyte preparation, averaged 56,600 cpm and 59,500 cpm for the ethanol control and indomethacin cultures, respectively. When approximately 10^4 autologous monocytes were added to the stimulated lymphocytes, the cpm per well for the ethanol controls averaged 50,200 cpm which was increased to 80,200 cpm/well with addition of indomethacin, a 53.6% enhancement. The addition of PHA to the monocyte preparation resulted in 1200 cpm/well, indicating that only a small number of lymphocytes were contaminating these monocyte preparations. The percent augmentation by indomethacin for the original PBL preparation before monocyte depletion averaged 47.8%, which was very similar to the percent value of 53.6% for the add-back experiments. Thus these studies rule out the possibilities that the indomethacin acts directly on lymphocytes to enhance their response to mitogen and that the monocytes are involved in this indomethacin effect.

TABLE 2. Indomethacin Induced Enhancement of PHA Reactivity of Various Concentration of Normal Lymphocytes

No. of lymphocytes added/well	No. of expts.	Cpm/well × 10^3 ± S.E. of control cultures	% Augmentation ± S.E. by indomethacin
1×10^4	8	16.6 ± 2.1	27.5 ± 9.6
2×10^4	8	36.2 ± 4.0	21.1 ± 6.6
10×10^4	16	95.4 ± 4.6	12.8 ± 2.0

Effects of Irradiation Therapy on the Mitogenic Responses and Degree of Augmentation by Indomethacin

Effects of either wide field irradiation therapy or medium field irradiation therapy on mitogen responsiveness and percent augmentation by indomethacin can be seen in Table 1. Both PHA and Con A reactivity is significantly lowered by irradiation therapy. However,

TABLE 3. Correlation Between Enhancement of PHA Reactivity by Indomethacin and Inhibition of PHA Reactivity by PGE_2

Study group	Addition of PGE_2 (1 ng/ml)		Addition of indomethacin (2 μg/ml)
	Cpm × 10^3 ± S.E. (control cultures)	% Inhibition ± S.E.	% Augmentation ± S.E.
Normal controls	92.5 ± 5.7	15.4 ± 2.7	12.8 ± 2.0
ENT Controls	80.0 ± 8.3	18.6 ± 4.4	6.8 ± 2.3
Cancer patients with normal individual augmentation values	70.9 ± 4.2	16.6 ± 3.8	5.4 ± 2.1
Cancer patients with elevated individual augmentation values	66.0 ± 10.9	50.8 ± 5.8	52.8 ± 5.3
Cancer patients finishing *extensive field irradiation*	45.7 ± 3.2	75.2 ± 5.1	80.5 ± 10.3

the percent augmentation by indomethacin was significantly increased, averaging 88.0% and 76.4% for the PHA and Con A response, respectively. These differences are highly significant ($P < 0.01$). In five patients with medium field irradiation the PHA reactivity was only mildly depressed and the percent augmentation was only modestly increased averaging 36.6% and 43.8% for PHA and Con A response respectively. To determine whether these large percent augmentations by indomethacin seen in the extensive field group was merely due to low mitogen reactivity, the experiments were repeated with decreased numbers of lymphocytes (1×10^4 and 2×10^4/well) instead of the usual 10^5 PBL/well. Monocytes were added to the cultures in numbers approximately equal to that found in 10^5 PBL, as described in the methods and materials portion. By this procedure, the PHA reactivity per well was decreased to values comparable to those of the irradiated patients. One can see in Table 2 that as the number of lymphocytes per well and the corresponding PHA response was decreased, the average percent augmentation by indomethacin was increased, but not to levels seen in the irradiated patients. For example, when 10^4 lymphocytes/well were added, which resulted in a baseline reactivity of 16,600 cpm/well, the percent augmentation averaged 27.5% as compared to an average of 88.0% for the irradiated patient group whose baseline PHA reactivity was similar, averaging 20,600 cpm/well (Table 1). The difference in percent augmentation was significantly different ($P < 0.01$). These results indicate that the mere low PHA reactivity is not solely responsible for the increased PHA reactivity by the addition of indomethacin for the irradiated patient group.

Correlation of PGE_2 Sensitivity of Stimulate Lymphocytes with the Degree of Augmentation by Indomethacin

Table 3 shows the percent inhibition caused by 1 ng/ml PGE_2 of PHA stimulated lymphocytes for both the control and patient groups. Although a linear dose response was seen for both controls and patient groups, a single concentration of 1 ng/ml was found to best illustrate the difference in sensitivity of normal and patient lymphocytes to PGE_2. As seen in this table, the most dramatic difference was seen in the untreated group of patients with elevated percent augmentation values by indomethacin and in the irradiation group where 1 ng/ml PGE_2 inhibited the PHA response by 75.2% as compared to 15–18% for the control group. In addition, the concentration of PGE_2 which inhibited control lymphocytes by 50% was approximately one μg/ml as compared to less than one ng/ml for the irradiation patients. Some patients were extremely sensitive to the prostaglandin with 1 picagram/ml still inducing significant inhibitions of blastogenesis induced by the maximum stimulatory concentrations of PHA. Often this amount of PHA was twice the amount necessary for the control cultures. The other patient group showing increased sensitivity to PGE_2 was the patients whose percent augmentation values by indomethacin was significantly higher than for the control values. In this group 1 ng/ml PGE_2 resulted in about 50% inhibition of maximal PHA response which is approximately 1000 times less than that necessary for 50% inhibition of control PHA reactivity. When the percent augmentation by indomethacin and the percent inhibition by PGE_2 or the maximal PHA response was done simultaneously with separate aliquots of the same PBL preparation and then the values compared (last two columns of Table 3), a striking correlation was seen. When the percent augmentation is low, so is the percent inhibition by PGE_2, and when the percent augmentation is increased, then the percent inhibition is likewise increased. In addition, when each individual percent augmentation value for all groups was correlated with each individual percent inhibition value, a significant correlation was obtained. Thus, these studies show that PHA stimulated lymphocytes from some untreated cancer patients and from all cancer patients receiving irradiation therapy were significantly more sensitive to the inhibitory effects of PGE_2 and that this effect cor-

TABLE 4. PGE_2 Induced Suppression of PHA Reactivity of Various Concentration of Normal Lymphocytes

No. of lymphocytes added/well	No. of expts.	Cpm/well ± S.E. of control cultures	% Inhibition ± S.E. by PGE_2 (1 ng/ml)
1×10^4	7	35.4 ± 3.4	40.7 ± 5.2
2×10^4	7	57.3 ± 3.0	5.0 ± 2.3
10×10^4	8	95.8 ± 4.7	16.3 ± 2.7

relates with the degree of augmentation by indomethacin.

The observation that there was a significant difference in baseline PHA reactivity between the controls and irradiation patient group (column 2, Table 3) raised the possibility that the percent inhibition by PGE_2 was merely a function of the degree of PHA reactivity in that the less reactivity, the greater the degree of inhibition even for normal lymphocytes. To study this possibility, the sensitivity of PGE_2 of various dilutions of normal, maximal stimulated PBL by PHA was determined. The results which are shown in Table 4 illustrates that as the number of reactive cells per well was decreased, the percent inhibition by one ng/ml PGE_2 was increased, however, not to the same degree as for the irradiated patient. For example, 2×10^4 normal lymphocytes/well, which gave an average cpm/well of 57,300 were inhibited by 5.0% as compared to 75.2% for the irradiated patient cells that were stimulated to approximately the same degree, averaging 45,700 cpm/well. Even with 10^4 cells/well the control PBL were stimulated maximally to 35,400 cpm and were inhibited by PGE_2 by 40.7%, which is still less than the 75.2% for the irradiated patient group. Thus, these studies indicate that other factors besides the mere low PHA response was responsible for this enhanced PGE_2 sensitivity of lymphocytes from this patient group.

Discussion

Impairment of cellular immunity, such as depressed PHA reactivity, can be demonstrated in many patients with head and neck carcinoma.[4,7,9] One possible explanation for the hyporeactive state is the existence of a suppressor cell. Such cells have already been described in various malignancies,[15,17,18] including squamous cell carcinoma of the lung[16,17] and head and neck region.[4,6] In the studies involving head and neck cancer, Berlinger *et al.*[6] and Zighelboim *et al.*[4] have described a population of cells with characteristics of monocyte that were capable of decreasing lymphocyte responsiveness to allogeneic cells and PHA respectively. In this communication, we have also presented evidence for the existence of a suppressor cell in about 1/3 of untreated patients with head and neck carcinoma and from all these patients finishing irradiation therapy. In these studies, we found that the reactivity of PHA or Con A stimulated lymphocytes from these patients were significantly enhanced by blocking prostaglandin synthesis with indomethacin, a phenomena that has already been observed by Goodwin *et al.*[15] in patients with Hodgkin's disease. In both studies, the cell responsible for this effect appeared to be an adherent cell, presumably a monocyte, and its effect most likely mediated by PGE_2 since the suppressor effect could be blocked by indomethacin and then recovered by the addition of PGE_2. The enhanced suppressor activity by the ISSC could result from either enhanced production of PGE_2 by these suppressor cells or increased sensitivity of the target cell, namely the PHA stimulated lymphocytes, to PGE_2. Our observation of a direct correlation between the degree of augmentation by indomethacin and degree of sensitivity to PGE_2 suggests that the latter alternative is responsible for this suppressor effect. Recently Stobo *et al.*[20] reported that PHA stimulated, low density lymphocytes were enhanced by PGE while intermediate or high density PHA stimulated lymphocytes were inhibited by this prostaglandin. Perhaps in our patients with enhanced ISSC suppressor activity, either the tumor burden or irradiation therapy had altered the lymphocyte subpopulation by enriching for the immediate and high density lymphocytes or by eliminating the low density T-cells in the peripheral blood, resulting in a more suppressible lymphocyte population by PGE_2. Thus either a shift in the lymphocyte population to the more sensitive fraction, as described by Stobo, or an enhancement of lymphocyte sensitivity to PGE could provide an explanation for the suppressed reactivity of PHA blastogenesis induced by the ISSC.

PGE_2 appears to play an adverse role in many immune reactions. Besides inhibiting blastogenesis induced by mitogens (5, and this study), PGE_2 has already been shown to inhibit NK activity,[21,22] lymphokine production,[23,24] lymphocyte cytotoxicity,[25] and macrophage cytotoxicity.[26] Thus it seems reasonable to assume that excess PGE_2 may have adverse effects on tumor control *in vivo* and that inhibiting the synthesis of prostaglandins E may be of importance in the therapy of head and neck carcinoma, especially in those that are receiving irradiation therapy. This beneficial effect assumes, of course, that giving oral indomethacin would have a similar enhancing effect on the immune system *in vivo* as it does *in vitro* and that enhancing the immune system would be of clinical benefit to these patients. Although still speculative, studies done with animal model systems and limited data from such studies done in man lend support to these assumptions.[22,27]

In humans, Goodwin *et al.*, found that giving oral indomethacin to patients with common variable im-

munodeficiencies enhance cellular immune response, such as the PHA response.[28] Thus these observations indicate that oral indomethacin may have a similar effect on the immune response as it does when added *in vitro* to the culture medium.

In other studies involving animals, adding indomethacin or other prostaglandin synthesis inhibitors to the drinking water has been shown to reduce the growth of various tumors and lengthen the survival of these mice.[29-32] In some cases, complete regression associated with cures has actually been reported. We have observed, in a pilot study, that daily indomethacin induced significant tumor regression in patients with head and neck carcinomas.[33] Although preliminary, this study suggests that oral indomethacin in some patients may have a similar effect in humans as it does in tumor-bearing animals. Whether *in vitro* assays such as those described in this study will be of value in helping to predict those patients that may potentially benefit from prostaglandin synthesis inhibitors is only speculative at this point. However, it seems reasonable and important to test this approach by initiating the appropriately designed clinical trials which would involve giving prostaglandin synthesis inhibitors such as indomethacin to these cancer patients.

REFERENCES

1. Lundy J, Wanebo HJ, Pinsky CM *et al.* Delayed hypersensitivity reactions in patients with squamous cell cancer of the head and neck. *Am J Surg* 1974; 128:530-533.
2. Pinsky CM, Domeini AE, Caron A. Delayed hypersensitivity reaction in patients with cancer: *Recent results. Cancer Res* 1976; 47:37-41.
3. Catalona WJ, Chretien PB. Abnormalities of quantitative dinitrochlorobenzene sensitization in cancer patients: Correlation with tumor and histology. *Cancer* 1973; 31:353-356.
4. Zighelboim J, Dorey F, Parker NH *et al.* Immunologic evaluation of patients with advanced head and neck carcinoma receiving weekly chemoimmunotherapy. *Cancer* 1979; 44:117-123.
5. Papenhausen PR, Kukwa A, Croft CB *et al.* Cellular immunity in patients with epidermoid cancer of the head and neck. *Laryngoscope* 1979; 89:538-549.
6. Berlinger NT, Hilal EY, Oettgen HF, Good RA. Deficient cell-mediated immunity in head and neck cancer patients secondary to autologous suppressive immune cells. *Laryngoscope* 1978; 88:470-480.
7. Wanebo HJ, Jun MY, Strong EW, Oettgen H. T-cell deficiency in patients with squamous cell cancer of the head and neck. *Am J Surg* 1975; 130:445-451.
8. Browder JP, Chretien PB. Immune reactivity in head and neck squamous carcinoma and relevance to the design of immunotherapy trials. *Semin Oncol* 1977; 4:431-439.
9. Sucin-Foca N, Buda J, McManus J. Impaired responsiveness of lymphocytes and serum inhibitory factors in patients with cancer. *Cancer Res* 1973; 33:2373-2377.
10. Ryan RE, Neel HB III, Ritts RE. Correlation of preoperative immunologic test results with recurrence in patients with head and neck cancer. *Otolaryngol Head Neck Surg* 1980; 88:58-63.
11. Olkowski ZL, Wilkins SA. T-lymphocyte levels in the peripheral blood of patients with cancer of the head and neck. *Am J Surg* 1975; 130:440-444.
12. Wara WM, Wara DW, Ammann AJ *et al.* Immunosuppression and reconstitution with thymosin after radiation therapy. *Int J Rad Oncol Biol Phys* 1979; 5:997-1001.
13. Rafla S, Yang SJ, Meleka F. Changes in cell-mediated immunity in patients undergoing radiotherapy. *Cancer* 1978; 41:1076-1086.
14. Tarpley JL, Potvin C, Chretien PB. Prolonged depression of cellular immunity in cured laryngopharyngeal cancer patients treated with radiation therapy. *Cancer* 1975; 35:638-644.
15. Goodwin JS, Messner RP, Bankhurst AD *et al.* Prostaglandin-producing suppressor cells in Hodgkin's disease. NEJM 1977; 297:963-968.
16. Han T, Takita H. Depression of T-lymphocyte response by non-T suppressor cells in lung cancer patients. *Cancer* 1979; 44:2090-2098.
17. Jerrells TR, Dean JH, Richardson GL *et al.* Role of suppressor cells in depression of *in vitro* lymphoproliferative responses of lung cancer and breast cancer patients. *J Natl Cancer Inst* 1978; 61:1001-1009.
18. Vosixa G, Thies J. Effect of indomethacin on blastogenesis of lymphocytes from cancer patients: differentiation of patient types. *Clin Immunol Immunopathol* 1979; 13:30-38.
19. Boyum A. Isolation of mononuclear cells and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Invest* (Suppl 21) 1968; 97:77-89.
20. Stobo JD, Kennedy MS, Goldyne ME. Prostaglandin E modulation of the mitogenic response of human T-cells. *J Clin Invest* 1979; 64:1188-1195.
21. Droller MJ, Schneider MU, Perlmann P. A possible role of prostaglandins in the inhibition of natural and antibody dependent allmediated cytotoxicity against tumor cells. *Cell Immunol* 1978; 39:165-177.
22. Brunda MJ, Herberman RB, Holden HT. Inhibition of murine natural killer cell activity by prostaglandins. *J Immunol* 1980; 124:2682-2687.
23. Gordon D, Bray MA, Morley J. Control of lymphokine secretion by prostaglandins. *Nature* 1976; 262:401-402.
24. Bray MA, Gordon D, Morley J. Regulation of lymphokine secretion by prostaglandins. *Agents and Actions* 1976; 6:171-175.
25. Strom TB, Carpenter CB, Garovoy MR *et al.* The modulating influence of cyclic nucleotides upon lymphocyte-mediated cytotoxicity. *J Expt Med* 1973; 138:181-187.
26. Schultz RM, Pavlidis NA, Stylos WA, Chirigos MA. Regulation of macrophage tumoricidal function: A role for prostaglandins of the E-series. *Science* 1978; 202:320-321.
27. Pelus LM, Strausser HR. Indomethacin enhancement of spleen-cell responsiveness to mitogen stimulation in tumorous mice. *Int J Cancer* 1976; 18:653-660.
28. Goodwin JS, Bankhurst AD, Murphy SA *et al.* Partial reversal of the cellular immune defect in common variable immunodeficiency with indomethacin. *J Clin Lab Immunol* 1978; 1:197-199.
29. Bennett A, Houghton J, Leaper DJ, Stamford IF. Cancer growth, response to treatment and survival time in mice: Beneficial effect of the prostaglandin synthesis inhibitor flurbiprofen. *Prostaglandins* 1979; 17:179-189.
30. Hial V, Horakova Z, Shaff RE, Beaven MA. Alteration of tumor growth by aspirin and indomethacin: Studies with two transplantable tumors in mouse. *Euro J Pharmacol* 1976; 37:367-376.
31. Lynch NR, Salomon J. Tumor growth inhibition and potentiation of immunotherapy by indomethacin in mice. *J Natl Cancer Inst* 1979; 62:117-121.
32. Trevisani A, Ferretti E, Capuzzo A, Tomasi V. Elevated levels of prostaglandin E_2 in Yoshida hepatoma and the inhibition of tumour growth by nonsteroidal anti-inflammatory drugs. *Br J Cancer* 1980; 41:341-347.
33. Panje WR. A prostaglandin synthesis inhibitor produced marked regression of head and neck epidermoid carcinoma. Proc Soc Head Neck Surgeons and Am Soc Head Neck Surgery (combined) Phoenix, 1981.