Excessive Prostaglandin E_2 Production by Suppressor Monocytes in Head and Neck Cancer Patients

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The proliferative response of peripheral blood mononuclear cells (PBMC) to the mitogens PHA and Con A was significantly depressed in 86% of 45 head and neck cancer patients compared with 44 normal controls. This depression of immune competence was greatest in older patients and in those with more advanced disease stages. The abnormal mitogen responses could be restored toward normal (especially with Con A stimulation) by incubating the cells with either of two prostaglandin synthetase inhibitors (indomethacin or RO-205720). This augmentation of immune response was independent of other factors, including the primary tumor site, disease stage, treatment (surgery, radiation therapy, or chemotherapy) or the patient's age or race. The most likely explanation for this depressed level of immunocompetence was an excessive production of PGE_2 by suppressor cells. This was confirmed by the finding that PBMC from patients produced more PGE_2 than PBMC from normal individuals (8.4 ng/ml *vs.* 5.2 ng/ml, $p = 0.002$). This difference was greatest among patients less than 60 years of age whose cultured PBMC produced 91% more PGE_2 than controls ($p < 0.0007$). Virtually all of the PGE_2 was produced by a population of monocytes defined by a monoclonal antibody and purified with a fluorescence-activated cell sorter. Patients with epidermoid cancer of the head and neck thus have an abnormality of immunoregulatory monocytes that can contribute significantly to their depression of cellular immunity by elaborating prostaglandin E_2. This abnormality could be partially corrected *in vitro* by incubating their PBMC with a prostaglandin synthetase inhibitor.

ABNORMALITIES in immune regulation by helper and suppressor cells represent an important new concept of altered immune competence in cancer patients.[1] It has been assumed that the decreased immunocompetence observed among cancer patients is caused by a deficiency of one or more types of effector cells. These include antibody-forming B lymphocytes, cytotoxic T lymphocytes, cytotoxic monocytes, natural killer cells, and antibody-dependent killer cells. However, recent information has demonstrated that the tempo, intensity, and even the choice of effector cells may be regulated in part by suppressor cells and by helper cells (also called amplifying cells, accessory cells or inducer cells). Since head and neck cancer patients often have profound abnormalities of immunologic function,[2] their immunologic abnormalities should be examined in the context of helper and suppressor lymphocytes and/or macrophages, for the immunosuppressed state in some head and neck cancer patients might be caused by too little "help" or too much "suppression."

Immune regulation by helper or suppressor cells can take place by cell-cell contact with effector cells, as well as by release of soluble mediators into the surrounding microenvironment. One important soluble mediator is prostaglandin E_2.[3] This hormone is produced by a glass-adherent suppressor cell that regulates various cellular immune responses.[4–6] Immunopharmacologic assays analyzing the prostaglandin system often utilize the drug indomethacin, a known inhibitor of prostaglandin synthesis. The authors have previously reported that indomethacin enhances the mitogen response in a series of 33 melanoma patients compared with 29 normal control subjects.[7] Others have reported indomethacin enhancement of immune competence in patients with Hodgkin's lymphoma[5] and lung carcinoma.[8]

In the experiments reported here, PBMC from 45 head and neck cancer patients were examined to determine whether T lymphocyte proliferation in response to either PHA or Con A was decreased, whether any depressed mitogen responses were related to prostaglandin-mediated suppression, and if the suppressor cells involved were lymphocytes or monocytes. These experiments were based upon a hypothesis that PGE_2-mediated suppression was abnormally increased in these patients. This postulate was tested indirectly by blocking PGE_2 effects with indomethacin *in vitro* and directly by

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TABLE 1. *Characteristics of Head and Neck Cancer Patients*

Total Number	45
Stage	
I & II	33%
III & IV	67%
Site	
Oral cavity	42%
Pharynx	9%
Larynx	42%
Unknown primary	7%
Age	
Median: 61 years	
Range: 35–75	
≤60 years	44%
>60 years	56%
Sex	
Male	100%
Female	0%
Treatment*	
Surgery	73%
Radiation	57%
Chemotherapy	24%

* Some patients received more than one treatment modality.

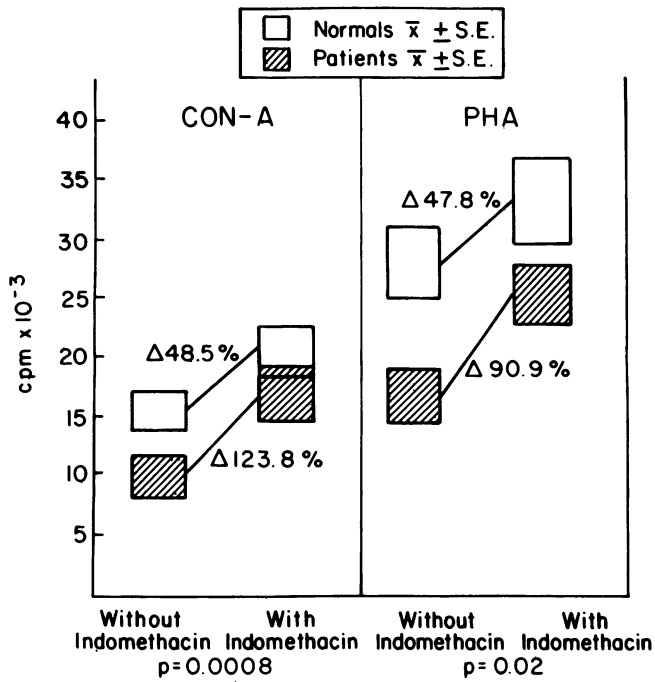

FIG. 1. Proliferative responses of blood mononuclear cells incubated *in vitro* with indomethacin (1 μg/ml). The 44 head and neck cancer patients had a depressed response to both mitogens that could be restored toward that of 45 normal individuals in the presence of a prostaglandin synthetase inhibitor. The Δ% shown in the figure represents the mean % change of the individuals in each group. The p values represent the comparison of mean Δ% for the patient *vs.* control values. The comparison of the mean CPM for the patients and control was also significant both for Con A ($p = 0.002$) and for PHA ($p = 0.02$).

measuring PGE_2 production by whole blood mononuclear cells or purified populations of lymphocytes and monocytes. The results were compared with those obtained from 44 normal individuals studied in parallel.

Materials and Methods

Subjects

A total of 45 patients with epidermoid carcinoma of the head and neck region were studied. The characteristics of these patients and their tumors are listed in Table 1. Their ages ranged from 35 to 75 years. Patients with parotid tumors, thyroid carcinomas, and skin carcinomas were excluded.

As controls, 44 healthy normal individuals were also studied in an identical fashion. Their ages ranged from 19 to 88 years.

Cells

Total blood mononuclear cells were separated from heparinized blood by Ficoll-Hypaque density centrifugation as described.[9] Peripheral blood mononuclear cells (PBMC) were washed and resuspended in RPMI 1640 with 20% fetal calf serum (FCS) and gentamycin. The proportion of lymphocytes and monocytes was determined morphologically by their typical appearance on slides after Wright staining.

In some experiments, a purified population of monocytes was identified by immunofluorescence using a monoclonal antimonocyte antibody, anti-CRP, (a generous gift from Dr. John Kearney), and then sorted with a fluorescence-activated cell sorter (FACS IV, Becton-Dickinson Company) as described.[10] A characterization of the anti-monocyte monoclonal antibody has been published.[11]

Mitogen Assay

One × 10^5 PBMC were cultured in microtiter plates with or without indomethacin (Sigma Chemical Company) at a concentration of 1 μg/ml (approximately 10^{-6} molar). It has been shown previously that this is the optimal dose in this laboratory for immune modulation studies since lower doses (to 10^{-8} molar) have a diminished capacity to inhibit PGE_2 production.[7] Additionally, cells were incubated with a suboptimal dose of either phytohemagglutin (PHA-M 25 μg/ml) or Concanavalin A (Con A at 5 μg/ml), since PGE_2-induced immunosuppression is obscured at higher mitogen doses.[7,12] The cells were incubated at 37 C in 5% CO_2 for a total of 72 hours, and then pulsed with ^3H labelled thymidine (0.5 μCi/well) eight hours before harvesting.

The net counts per minute (cpm) of triplicate cultures were calculated as cpm of cells with mitogen, minus cpm of cells without mitogen. Per cent change in the presence of indomethacin was calculated by the following formula:

$$\text{Per cent change} = \frac{(X - Y)100}{Y}$$

in which X is the net cpm with indomethacin and Y is the net cpm without indomethacin.

Prostaglandin Radioimmunoassay

PBMC were adjusted to a concentration of 5×10^5 lymphocytes/ml based on the Wright stain analysis. Duplicate aliquots (0.5 ml) of each cell suspension were added to sterile culture tubes and incubated upright for 48 hours at 37 C in 5% CO_2. Tubes were briefly vortexed, centrifuged, and the supernatants collected. The amount of PGE_2 produced in each sample was determined by a competitive inhibition radioimmunoassay using the method described by Taffet and Russell.[13]

Statistics

Univariate statistics were determined on all the descriptive variables for both patients and normal controls. Analysis of variance was utilized to analyze the significant factors relating to the mitogen effects on lymphocyte proliferative response. Both analyses of variance and covariance, with the initial lymphocyte response as a covariate, were used to determine which factors independently affected prostaglandin synthetase inhibition.[14]

Results

Mitogen Response

The mean lymphocyte proliferative response was significantly depressed in the 45 head and neck cancer patients compared with controls (Fig. 1) for both Con A (15,300 cpm *vs.* 9,600 cpm; p = 0.015) and for PHA (27,400 cpm *vs.* 16,100 cpm; p = 0.003). In 86% of the patients, the responses to one or both mitogens were below the normal range (±2 standard deviations). Compared with normals, the depressed mitogen response for the head and neck cancer patients was most marked in older individuals (p = 0.05).

Different trends in the mitogen responses were noted among certain patient subgroups (Table 2). Age had an important influence on the PHA response for the younger patients. Those patients less than 60 years old had a lower PHA response than age-matched normal subjects (18,781 *vs.* 31,462 cpm, p = 0.01). Similar trends were observed for the Con A response, but they were not statistically significant. Patients with metastatic or advanced local stages (Stages III and IV) had a lower mitogen response than those with a clinically localized cancer (Stages I and II). This observation applied to both Con A (8,440 *vs.* 12,450 cpm) and PHA (13,993 *vs.* 21,075 cpm) responses. The results were not statistically significant, primarily because of the inherent variability of the assay results. Patients undergoing radiation treatments had a lower mitogen response to both Con A (p < 0.007) and PHA (p = 0.02) compared with those not being so treated (Table 2). Chemotherapy did not influence the mitogen response of these patients. Sex differences could not be examined because all the patients were men.

TABLE 2. *Effect of Indomethacin (IM) on PGE$_2$-Mediated Suppression in Head and Neck Cancer Patients*

Patient Characteristics	Without IM		With IM	
	Con A	PHA	Con A	PHA
Stage				
I & II	12,450*	21,075	22,880	34,176
III & IV	8,440	13,993	13,736	21,392
Site				
Oral cavity	7,237	12,985	13,530	20,362
Pharynx	5,014	14,227	10,282	18,963
Larynx	12,236	19,906	20,188	3,114
Unknown primary	12,440	13,535	18,420	25,606
Age				
≤60 years	11,440	18,781	19,169	29,380
>60 years	8,363	14,159	14,654	22,169
Race				
Caucasion	9,620	15,598	14,600	24,612
Negro	12,722	17,705	22,343	27,130
Chemotherapy				
Yes	8,467	10,027	13,007	15,985
No	9,982	17,951	17,544	28,007
Radiation				
Yes	5,782	11,309	10,735	17,917
No	14,571	22,676	22,791	33,933

* Counts per minute of ^{3}H thymidine.

TABLE 3. *Enhancement of Mitogen Response in Head and Neck Cancer Patients with an Experimental Prostaglandin Synthetase Inhibitor (R0-205720)*

	Without drug	With drug	Net Change	p Value
PHA	16,522*	23,963	+31.1%	0.0001
Con A	9,651	13,232	+27.1%	0.0017

* Counts per minute of ^{3}H thymidine. Counts represent the mean value for 24 patients.

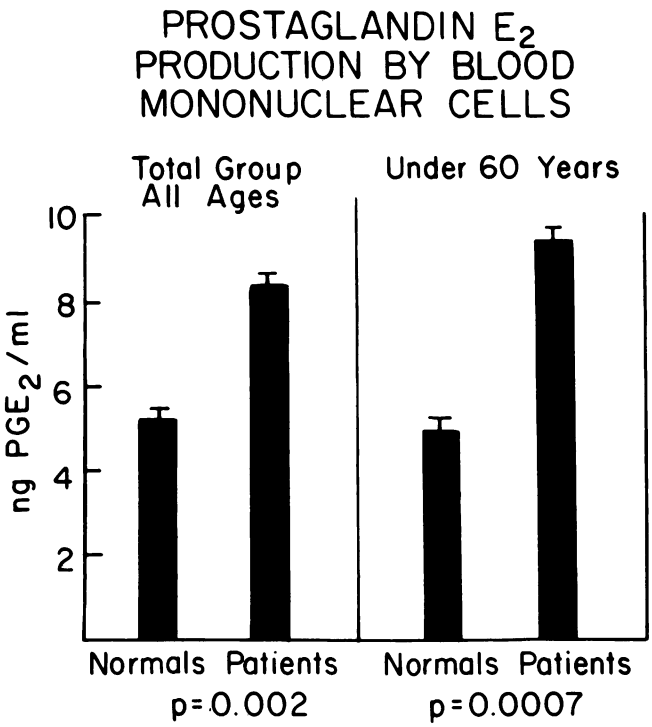

FIG. 2. *In vitro* production of PGE_2 by blood mononuclear cells during a 48-hour culture. Cells from the head and neck cancer patients produced significantly more PGE_2 than normal individuals. This difference was greatest in individuals under 60 years of age.

Effect of Prostaglandin Synthetase Inhibitors (Indomethacin and RO-205720)

Aliquots of PBMCs were tested in the presence of indomethacin at physiologic doses (1 μg/ml or about 10^{-6} molar) to determine whether this drug would enhance the mitogen response, presumably by blocking PGE_2-mediated suppression. Indomethacin significantly increased the mean proliferative response of PBMC for both the normal control group and the patient group (Fig. 1). However, the magnitude of increase for the patients was significantly greater than controls for both Con A (123.8% *vs.* 48.5%; p = 0.002) and PHA (90.9% *vs.* 47.8%; p = 0.0008). Responses to Con A increased almost to that of normal individuals, while the PHA response was still slightly depressed (Fig. 1). These differences between normal and patient groups were significant even after adjusting statistically for influences of age and race. Furthermore, there were no significant differences in the observed indomethacin enhancement of mitogen response (*i.e.*, rate of change) when the patients were subgrouped by primary tumor site, disease stage, age, race, or type of treatment (Table 2).

Indomethacin is known to alter lymphocyte function by mechanisms other than blocking PGE_2 effects.[3,7,15] Therefore, it was also tested whether mitogen response could be enhanced by another drug (RO-205720, Hoffman-LaRoche Pharmaceutical Company, Nutley, NJ), whose only known effect is to block prostaglandin synthetase activity.[16] In these experiments, it was observed that RO-205720 (at 20 μg/ml) also enhanced the magnitude of PBMC proliferation to both mitogens by almost one third (Table 3).

TABLE 4. *PGE_2 Production by Purified Monocytes and Lymphocytes*
ng PGE_2 Produced/ml Culture Supernatant

	Normal Subject	H & N Cancer Patient #1	H & N Cancer Patient #2
Unfractionated cells*	4.3	5.6	2.4
Sorted monocytes†	5.5	5.8	3.8
Sorted lymphocytes	<0.1	0.2	<0.1

* Stained but not sorted.

† Cells were stained with anti-CRP monoclonal antibody plus fluorescein-conjugated rabbit antimouse antibody. Anti-CRP selectively binds to monocytes.

PGE_2 Production

PGE_2 production by cultured PBMC from patients and controls was then compared in order to directly determine whether the indomethacin enhancement of mitogen response was related to elevated levels of PGE_2. After a 48-hour incubation, PBMC from patients produced significantly more PGE_2 compared with controls (Fig. 2). For the total group of head and neck cancer patients, a mean of 8.4 ± 0.8 ng/ml of PGE_2 was produced, compared with 5.2 ± 0.6 ng/ml for controls (p = 0.002). The differences were even greater among patients less than 60 years of age (Fig. 2). PGE_2 production did not correlate statistically with the primary tumor site, disease stage, or type of treatment.

Proportion of Blood Monocytes

It was observed that the proportion of monocytes in the blood of patients was significantly higher compared with that in normal individuals (14.3% *vs.* 9.5%; p = 0.01). However, the authors were not able to correlate the increased proportion of monocytes with an increased level of PGE_2 production in these patients either by statistical comparison or by adjusting the lymphocyte:monocyte ratio in the cultures and comparing PGE_2 production with the whole PBMC population (data not shown).

PGE_2 Production by Purified Monocytes

The cell producing PGE_2 has been shown to be a glass adherent blood mononuclear cell.[4] Although this type of cell is presumed to be a monocyte, it is known that some lymphocytes and granulocytes can adhere to plas-

tic or nylon wool as well.[17] To confirm the category of lymphoid cells that produces PGE_2, populations of blood monocytes were purified using an anti-monocyte monoclonal antibody and a fluorescence-activated cell sorter. Separated populations of monocytes and lymphocytes were then cultured for 48 hours in complete tissue culture medium and the supernatants assayed for PGE_2 using the radioimmunoassay. In one normal individual and two head and neck cancer patients, virtually 100% of the PGE_2 was produced by the monocyte fraction (Table 4).

Discussion

Prostaglandins have been demonstrated to inhibit the following immune responses: 1) proliferative responses to mitogens and alloantigens,[4] 2) lymphokine production,[18,19] 3) natural and antibody-dependent cellular cytotoxicity,[20,21] 4) cell-mediated cytolysis,[22,23] and 5) antibody production.[24–27] The authors have previously reviewed the role of PGE_2-mediated suppression on these immune functions in tumor-bearing mice.[3]

In this study, PGE_2-mediated suppression by monocytes was found by four criteria to be an important pathogenic mechanism contributing to depressed immunity in head and neck cancer patients: 1) the lymphocyte proliferative response to mitogens was significantly depressed in 86% of patients compared with controls; 2) this parameter of cellular immune function could be restored almost to that of normal individuals by incubating the cells with either of two prostaglandin synthetase inhibitors; 3) production of PGE_2 by blood mononuclear cells from head and neck cancer patients was significantly increased compared to controls; and 4) purified blood monocytes, but not lymphocytes, produced virtually all of the PGE_2 in a short-term culture.

Although it is generally assumed that indomethacin modulates cellular immune function by inhibiting prostaglandin synthetase, this drug has other direct effects on cellular function unrelated to PGE_2 metabolism.[3,15] For example, although indomethacin significantly enhances the mitogen response in melanoma patients compared with controls, this effect was not related to increased PGE_2 production.[7,28] It appears, in fact, that indomethacin may have a direct effect upon proliferating T lymphocytes in these melanoma patients.[28]

Increased prostaglandin production in older normal individuals is accompanied by an increased sensitivity of their lymphocytes to the suppressive effects of PGE_2 compared with young adults.[6] In addition, the monocyte production of PGE_2 is greater in older individuals (unpublished observation). These two observations explain in part the decreased immune responses to mitogens observed in elderly individuals. The patient's age is therefore an important variable to be controlled. Compared with age-matched controls, it was found that blood mononuclear cells from head and neck cancer patients produced more PGE_2 and responded better to mitogens in the presence of indomethacin. This effect was greatest in the cancer patients less than 60 years of age.

The experiments reported here concerning head and neck cancer patients provide an interesting and potentially important contrast in the function of their immune regulatory cells with those from patients having other types of cancer. Abnormal function of prostaglandin-producing suppressor cells was clearly operative in the head and neck cancer patients reported here and in Hodgkin's lymphoma patients previously reported by Goodwin.[5] This abnormality could be partially corrected *in vitro* by adding a prostaglandin synthetase inhibitor. In contrast, indomethacin can enhance the mitogen responses in melanoma patients, but its immune modulatory properties in these patients are independent of PGE_2-producing suppressor cells.[28] Whether such a dichotomy exists in other types of cancer is currently under investigation.

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