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Research Article

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Sensitivity of Lymphocytes to Prostaglandin E₂ Increases in Subjects over Age 70

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ABSTRACT We examined the sensitivity of lymphocytes from different age groups to inhibition by prostaglandin E₂. Phytohemagglutinin-stimulated cultures of peripheral blood mononuclear cells from 12 healthy subjects over the age of 70 were much more sensitive to inhibition by exogenously added prostaglandin E₂ than were cells from 17 young controls (ID₅₀ = 10 nM for the subjects over 70 vs. >3 μM for the young controls). The more sensitive lymphocytes from a subject over 70 were to prostaglandin E₂, the lower was his or her response to phytohemagglutinin ($r = 0.75$, $P < 0.01$). The mean responses to phytohemagglutinin of the peripheral blood mononuclear cells from the subjects over 70 were significantly depressed compared to the young controls. Addition of indomethacin, a prostaglandin synthetase inhibitor, to the cultures resulted in an increase in [³H]thymidine incorporation of 140 ± 16% in the cells of the subjects over 70 vs. a 36 ± 3% increase in the young controls (mean ± SEM, $P < 0.001$). The mean phytohemagglutinin response of the subjects over 70 was 40% of the control response without indomethacin. With addition of indomethacin the response of subjects over 70 rose to 72% of control. Thus, increased sensitivity to prostaglandin E₂ appears to be responsible in part for the depressed mitogen response of peripheral blood mononuclear cells from healthy subjects over 70.

INTRODUCTION

We have recently described the inhibitory effects of endogenously produced prostaglandin E₂ (PGE₂)¹ on the response of human lymphocytes to mitogens

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¹Abbreviations used in this paper: PBMC, peripheral blood mononuclear cells; PGE₂, prostaglandin E₂; PHA, phytohemagglutinin.

(1, 2) and have related this to the presence of a prostaglandin-producing suppressor cell, the increased activity of which appears to be responsible for the hyporesponsiveness to phytohemagglutinin (PHA) seen in some patients with Hodgkin's disease (3). The action of the prostaglandin-producing suppressor cell can be blocked in vitro (1-3) and in vivo (4-6) by administration of indomethacin, a prostaglandin synthetase inhibitor, with a resultant enhancement of humoral (4, 5) and cellular (6) immune responses.

In the present communication we report that sensitivity of lymphocytes to inhibition by PGE₂ increases with the age of the donor. Increased sensitivity to prostaglandin appears to be a major cause of depressed lymphocyte response to PHA in healthy subjects over 70.

METHODS

Subjects. The following subjects were studied: 12 newborns (cord blood samples), 10 children aged 2-10, 17 men and women aged 20-34 yr, 10 men and women aged 46-60 yr, and 12 men and women over the age of 70. The children had respiratory illnesses at the time of the study. All other subjects were healthy, ambulatory, and on no medications other than occasional laxatives. The 17 subjects in the 20-34-yr-old group were studied many times. Subjects from the other age groups were always tested in parallel with sex-matched subjects from the 20-34-yr-old group.

Preparation of lymphocytes. Peripheral venous blood was drawn in syringes containing preservative-free heparin. Mononuclear cells were isolated by centrifugation over Ficoll-Hypaque (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N J.) and were washed three times with phosphate-buffered saline.

Drugs. PGE₂ was a gift of Dr. John Pike (The Upjohn Company, Kalamazoo, Mich.); indomethacin was a gift of Dr. Clement Stone (Merck Sharp & Dohme, Div. of Merck & Co., Inc., Westpoint, Pa.). The drugs were dissolved in 95% ethyl alcohol at 10 mg/ml and diluted with phosphate-buffered saline. This resulted in final concentrations of 0.01% ethyl alcohol in the cultures. Ethyl alcohol concentrations of 0.0001-0.2% had no effect on control cultures.

Cell cultures. The various lymphocyte preparations were cultured in minimal essential media (Microbiological Associates, Walkersville, Md.) supplemented with L-glutamine,

penicillin-streptomycin, and 20% fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). Cells were cultured in microtiter plates, 1×10^5 cells in 200 μ l. PHA (lot 65C-5022, Sigma Chemical Co., St. Louis, Mo.) and drugs were added directly to the wells. The final volume in all the cultures was adjusted to 240 μ l. Cells were incubated at 37°C in 5% CO₂ for 72 h. The cultures were pulsed with [³H]thymidine (New England Nuclear, Boston, Mass., 0.5 μ Ci/well) at 48 h and harvested on glass wool filters at 72 h with a Mash II Harvester (Valcor Engineering Corp., Kenilworth, N. J.). The filters were counted in a liquid scintillation counter. All cultures were performed in quadruplicate. Dose-response curves to PHA were obtained for the different age groups, and all further experiments were performed at one sub-optimal concentration of the mitogen. We used a suboptimal level of mitogen because other investigators have shown that only at suboptimal mitogen levels is the [³H]thymidine incorporation proportional to the number of responding cells (7, 8). Net counts per minute were calculated as counts per minute of cells plus mitogen plus drug (prostaglandin, indomethacin, or nothing) minus counts per minute of cells plus drug. Percent stimulation of [³H]thymidine incorporation was calculated by dividing the net counts per minute of the mitogen cultures with indomethacin by the net counts per minute of the mitogen cultures without indomethacin. This number was expressed as a percent and 100% was subtracted from it to obtain percent stimulation. Percent inhibition of [³H]thymidine incorporation caused by PGE was calculated by dividing the net counts per minute of the mitogen cultures with PGE by the net counts per minute of the mitogen cultures without PGE. The fraction was expressed as a percent and subtracted from 100% to obtain percent inhibition. Whenever we measured the inhibiting effect of exogenously added PGE₂, we also added indomethacin (1 μ g/ml) to those cultures to remove the confounding variable of endogenously produced prostaglandins.

In some experiments we assayed lymphocyte proliferation directly by counting viable cells in the wells at 140 h after initiation of the cultures. Percent inhibition of PHA-stimulated proliferation caused by PGE₂ was calculated by dividing the net increase in cell number of the cultures with PGE by the net increase in cell number of the cultures without PGE. This fraction was expressed as a percent and subtracted from 100% to obtain percent inhibition. Net increase in cell number was the number of viable cells (those excluding trypan blue) in the PHA-stimulated cultures minus the number of viable

cells in the cultures without PHA. These experiments were done in triplicate.

RESULTS

PHA response of mononuclear cells from subjects of different ages. Table I shows the responses of the mononuclear cells from subjects in the different age groups to PHA. At each of the four PHA concentrations, the responses of the peripheral blood mononuclear cells (PBMC) from cord blood are increased, and the responses of the PBMC from the subjects over 70 are decreased compared to other groups. There are no significant differences among the responses of the children, the young adults, and the middle-aged subjects.

Sensitivity to inhibition by PGE₂ of mononuclear cells from subjects of different ages. Table II shows the percent inhibition caused by four concentrations of PGE₂ in PHA cultures from the five groups of subjects. At each concentration of PGE₂, [³H]thymidine incorporation is significantly more inhibited in the cultures from the subjects over 70 than in the cultures from the other age groups. The concentration of PGE₂ required to cause 50% inhibition in PHA cultures of cells from subjects over 70 is ~10 nM, which is two orders of magnitude less than the concentration required for the other age groups, ~3 μ M. In these experiments, indomethacin (1 μ g/ml) was added to block endogenous prostaglandin production. Similar results were obtained when indomethacin was not added.

We also measured the effect of added PGE₂ on inhibition of PHA-induced lymphocyte proliferation by counting the cells after a 5-d culture. PGE₂ at concentrations of 3 nM–3 μ M did not affect cell viability in the cultures of cells with or without PHA; that is the percentage of viable cells in cultures with PGE₂ was the same as the percentage of viable cells in cultures

TABLE I
Response in Counts Per Minute of PBM Cells from Subjects of Different Ages to Four Concentrations of PHA

PHA	Cord blood (15)*	2–10-yr old (10)	20–34-yr old (17)	46–60-yr old (10)	70–82-yr old (12)†
μ g/ml	cpm (mean \pm SD)				
0	1,994 \pm 1,350	478 \pm 104	495 \pm 104	483 \pm 177	374 \pm 120
2	18,864 \pm 10,914	8,437 \pm 4,413	7,873 \pm 5,166	7,578 \pm 4,786	3,347 \pm 2,116
4	41,298 \pm 17,724	17,621 \pm 6,125	16,394 \pm 7,604	16,581 \pm 6,904	6,663 \pm 4,807
10	55,678 \pm 20,199	27,984 \pm 10,517	31,363 \pm 13,679	29,368 \pm 12,081	15,417 \pm 6,986
20	58,023 \pm 18,030	38,836 \pm 14,721	37,414 \pm 15,528	31,478 \pm 15,699	18,730 \pm 7,764

* The mean responses of the mononuclear cells from the cord blood samples are significantly greater than the mean responses of the other age groups ($P < 0.01$ at each PHA concentration, comparing the cord blood to each of the other age groups).

† The mean responses of the mononuclear cells from the subjects over 70 are significantly less than the mean responses of the other age groups ($P < 0.01$ at each PHA concentration, comparing the PBMC from subjects over 70 to each of the other age groups).

TABLE II
Percent Inhibition of PHA (4.0 $\mu\text{g/ml}$)-Stimulated [^3H]Thymidine Incorporation Caused by PGE_2 in Different Age Groups

PGE ₂ concentration	Percent inhibition, mean \pm SEM				
	Cord blood (15)	2-10-yr old (10)	20-34-yr old (17)	45-60-yr old (10)	70-82-yr old (12)*
3 nM	11 \pm 3	NT†	10 \pm 3	14 \pm 3	34 \pm 3
30 nM	27 \pm 4	23 \pm 3	27 \pm 3	30 \pm 4	59 \pm 4
0.3 μM	36 \pm 6	37 \pm 4	37 \pm 4	39 \pm 4	75 \pm 4
3 μM	46 \pm 6	45 \pm 3	46 \pm 4	49 \pm 6	82 \pm 2

* At each concentration of PGE_2 , the mitogen responses of the subjects over 70 were significantly more inhibited than were the responses of the other age groups ($P < 0.001$ at each PGE_2 concentration, comparing the subjects over 70 to each of the other age groups). There are no significant differences between any of the other age groups.

† NT = not tested.

without PGE_2 . In cultures without PHA > 90% of the cells excluded trypan blue after 72 h, and in the cultures with 2 or 4 $\mu\text{g/ml}$ PHA > 85% of the cells excluded trypan blue at 72 h. At higher PHA concentrations there was increased cell death as previously reported (8).

PGE_2 inhibited the net growth in cell numbers in PHA (4 $\mu\text{g/ml}$)-stimulated cultures of PBMC from subjects over 70 and young controls. 3 nM PGE_2 caused a 37 \pm 11% decrease in cell proliferation in cultures of PBMC from six subjects over 70 vs. an 8 \pm 6 decrease in PBMC from six young controls (mean \pm SD, $P < 0.01$). Thus, PGE_2 inhibited lymphocyte proliferation as measured by [^3H]thymidine incorporation or by increase in cell numbers. By either assay

the lymphocytes of subjects over 70 were more sensitive to inhibition.

Thus, lymphocytes from subjects over 70 are more sensitive to inhibition by PGE_2 and this could play a role in their depressed PHA response. The more sensitive lymphocytes from a subject over 70 were to PGE_2 , the lower was the response to PHA. This is graphed in Fig. 1, showing a correlation between the percent inhibition caused by PGE_2 (30 nM) and the PHA response in counts per minute ($r = 0.75$, $P < 0.01$). There was no such correlation for the other age groups ($r \leq 0.13$, $P \geq 0.4$).

Effect of indomethacin on the mitogen response of mononuclear cells from subjects over 70 and young controls. Addition of indomethacin or other prosta-

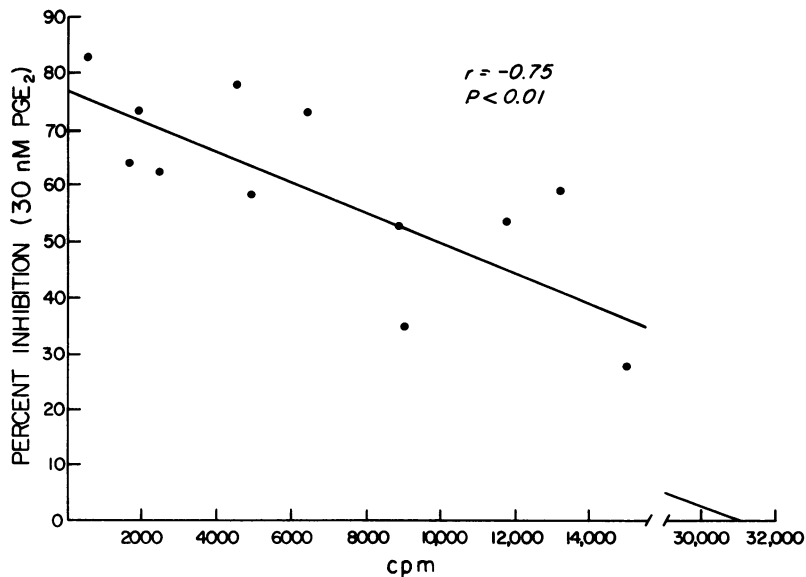


FIGURE 1 Percent inhibition of the PHA (4.0 $\mu\text{g/ml}$) response with PGE_2 (30 nM) vs. PHA response in subjects over 70. The more sensitive a subject's cells were to PGE_2 , the lower was the initial mitogen response ($r = 0.75$, $P < 0.01$).

TABLE III
 PHA (4.0 $\mu\text{g/ml}$) Response of PBMC from Different Age Groups:
 Effect of Addition of Indomethacin

Subjects	PHA	PHA + indomethacin	Percent increase
	<i>cpm (mean \pm SEM)</i>		
Cord blood (15)	41,298 \pm 4,579	55,339 \pm 4,912	34 \pm 7
2–10-yr old (10)	17,621 \pm 1,938	24,674 \pm 2,221	40 \pm 4
20–34-yr old (17)	16,394 \pm 1,845	22,295 \pm 1,840	36 \pm 3
46–60-yr old (10)	16,581 \pm 2,184	22,981 \pm 2,641	39 \pm 4
70–82-yr old (12)	6,663 \pm 1,389	16,003 \pm 2,406	140 \pm 16*

* The percent increase caused by indomethacin in the PHA cultures of the PBMC from subjects over 70 is significantly greater than the increase of the other groups ($P < 0.001$, compared to each of the other groups).

glandin synthetase inhibitors to PHA-stimulated cultures of PBMC will enhance [^3H]thymidine incorporation in the cultures, presumably by blocking endogenous prostaglandin production (1, 3). Because the PBMC from subjects over 70 were more sensitive to inhibition by PGE_2 , we reasoned that blockade of endogenous prostaglandin production in the PHA cultures might cause a greater enhancement of [^3H]thymidine incorporation in the PBMC from subjects over 70 than in the other age groups. This was indeed the case, as shown in Table III. The mean response of the cells from subjects over 70 to indomethacin was almost fourfold greater than the response of the other age groups. The response of the cells from subjects over 70 to indomethacin was greater not only in terms of "percent stimulation," but also in net increase in counts per minute caused by indomethacin (Table III). Before indomethacin, the mean response to PHA (4.0 $\mu\text{g/ml}$) of the subjects over 70 was 40% of the control response (20–34-yr-old age group). This increased to 72% of control with addition of indomethacin to the cultures.

The individual responses of the mononuclear cells from the subjects over 70 to PHA with and without indomethacin are graphed in Fig. 2. The subjects over 70 who respond in the normal range without indomethacin increase about the same as the young controls with addition of indomethacin, whereas the subjects over 70 who have depressed PHA responses have a much greater response to indomethacin, sometimes with as great as a 1,000% increase in counts per minute.

Also, as might be expected, the more sensitive were a subject's PBMC to inhibition by PGE_2 , the larger was the response to addition of indomethacin. These data are plotted in Fig. 3 for the subjects over 70 and young controls. Within each group there is a significant correlation between a subject's response to PGE_2 and his response to indomethacin. Most of the young controls form a tight group at the bottom left side of

the figure, at low inhibition by PGE_2 and low enhancement by indomethacin. The points representing the subjects over 70 are grouped in the upper right of the graph, showing $>50\%$ inhibition of the PHA response at this concentration of PGE_2 (30 nM) and a $>100\%$ increase in counts per minute with addition of indomethacin. The PHA response of some of these subjects was almost completely inhibited by 30 nM PGE_2 . In three of the subjects, addition of as little as

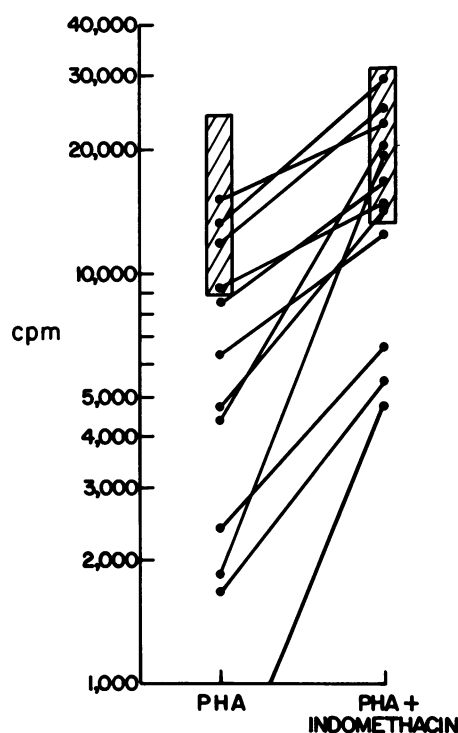


FIGURE 2 Response of PBMC from subjects over 70 to PHA (4.0 $\mu\text{g/ml}$) and to PHA + indomethacin (1 $\mu\text{g/ml}$). The hatched areas represent the mean \pm 1 SD of the responses of the young controls.

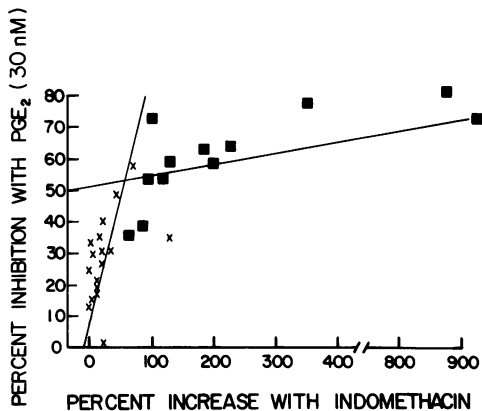


FIGURE 3 Percent inhibition of the PHA (4.0 $\mu\text{g/ml}$) response with PGE_2 (30 nM) vs. percent increase in the PHA response with addition of indomethacin. The results for the subjects over 70 (■) and young controls (×) are graphed. Within each group there is a significant correlation—the more sensitive to inhibition by PGE_2 , the larger the percent increase in counts per minute after addition of indomethacin ($r \geq 0.70$, $P < 0.01$ for each group).

3 nM PGE_2 caused $>70\%$ inhibition of the PHA response. These same subjects had very depressed responses to PHA and their cells responded to indomethacin with a $>300\%$ increase in [^3H]thymidine incorporation. The data for the other age groups overlap with the data for the young controls, and are not shown.

DISCUSSION

We can summarize the findings of this investigation as follows. First, the PHA response of PBMC from subjects over 70 is depressed as compared with young controls. This confirms the findings of several other laboratories (9–12). Second, PHA-stimulated cultures of PBMC from subjects over 70 are much more sensitive to inhibition by PGE_2 than are control cultures. Sensitivity to PGE_2 did not increase gradually with age; it was only apparent in subjects over 70 yr of age. The more sensitive cells from a subject over 70 were to inhibition by PGE_2 , the more depressed was the PHA response (Fig. 1). Third, the depressed PHA response of PBMC from subjects over 70 can be partly reversed by blocking the endogenous production of prostaglandin with the addition of indomethacin, a prostaglandin synthetase inhibitor.

Aging is associated with depressed humoral and cellular immunity in man (9–13) and experimental animals (14–16), although in humans T-cell function (cellular immunity) seems more depressed than B-cell function (humoral immunity) (9). The cause of this T-cell dysfunction has not been determined. Normal aging mice have both increased suppressor cells (14–16) and decreased helper cells (14), whereas in

man, the development of autoantibodies and monoclonal gammopathies with age would argue for loss of suppressor cell activity, at least in the humoral immune system (12, 17). Recent work by Inkeles et al. has demonstrated that the hyporesponsiveness of lymphocytes from aging humans to PHA is actually a sum of two deficiencies (10). First, the number of mitogen-responsive cells is reduced in lymphocyte preparations from old people. Second, the mitogen-responsive cells from old people do not proliferate as vigorously after exposure to PHA as do the lymphocytes from young persons. A decreased proportion of circulating T cells has also been noted in aging humans (18).

It is not clear what the significance of depressed cellular immunity in healthy old people is. The depressed mitogen responses are accompanied by depressed *in vivo* measurements of cellular immunity, such as delayed hypersensitivity skin testing (9). One could postulate that individuals with depressed cellular immunity would be more susceptible to certain infections, and this would appear to be the case in diseases where such a correlation has been looked for (19). Roberts-Thomson and co-workers studied the response of elderly subjects to a battery of five common skin test antigens (9), and reported that those subjects over the age of 80 who responded to only one or none of the antigens had a significantly greater mortality at 2 yr than those subjects who responded to two or more skin tests (80 vs. 35% dead at 2 yr, $P < 0.005$). These subjects were all ambulatory and well-nourished, and they would appear to resemble the subjects over 70 in our study. It would appear, then, that healthy old people with depressed cellular immunity are at greater risk of dying, though whether this is a direct or indirect effect is not known. Walford has theorized that the gradual loss of cellular immunity and immunological surveillance is the fundamental mechanism of aging (20).

Our data would suggest that increased sensitivity to PGE_2 is a major determinant of the depressed PHA response in healthy subjects over 70. Addition of indomethacin to PHA-stimulated cultures of mononuclear cells from healthy old people resulted in a percent increase in counts per minute that was almost four-fold greater than the percent increase in counts per minute caused by indomethacin in cultures of cells from the young controls. The mean PHA response of the subjects over 70 was 40% of control without indomethacin and this increased to 72% of control with addition of indomethacin. This indomethacin effect probably underrepresented the contribution of increased sensitivity to PGE_2 in the depressed PHA response of the subjects over 70. We have previously shown that addition of indomethacin (1 $\mu\text{g/ml}$) to PBMC cultures inhibits PGE_2 production by $\sim 80\%$, but this still results in ~ 1 nM PGE_2 production in the cultures

(1). PHA cultures from three subjects over 70 were >70% inhibited by as little as 3 nM PGE₂; so we can infer that the amount of PGE₂ produced endogenously even with indomethacin in the cultures was sufficient to cause a substantial inhibition of [³H]thymidine incorporation in the mononuclear cells from the subjects over 70. Another way to measure the contribution of sensitivity to prostaglandins to the depressed PHA response in old people is shown in Fig. 1, which illustrates the inverse relation between sensitivity to PGE₂ and PHA response in the subjects over 70. The linear regression line intercepts the abscissa at ~32,000 cpm, suggesting that if all inhibition by endogenous prostaglandins were eliminated, the resultant PHA response would be substantially higher than that obtained when indomethacin is added to partially block prostaglandin production.

Prostaglandins are produced in and have effects on almost every tissue in the body (21). A concept has been developed that these compounds work as universal local regulators of hormone action; that is, they are produced in a given tissue in response to a stimulus and they modulate the tissue's action by inhibiting or stimulating further response to the stimulus (21). It is interesting to speculate whether the increased sensitivity to PGE₂ found in lymphocytes from subjects over 70 would also be found in other tissues, perhaps explaining other physiologic changes seen with aging.

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