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Suppression of Lymphocyte Transformation by 16, (16) Dimethyl Prostaglandin E_2 and Unsaturated Fatty Acids[1] (38949)

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Current experimental evidence suggests that prostaglandins (PG) participate in the development of inflammatory and allergic reactions; however, there is conflicting evidence about the precise nature of their involvement. Several studies support the hypothesis that prostaglandins are mediators of the inflammatory response (1–3). In contrast, some investigators have presented evidence that certain prostaglandins can inhibit inflammation and hypersensitivity in both *in vitro* and *in vivo* models of these phenomena. Prostaglandin E_1 (PGE_1) prevents phytohemagglutinin (PHA)-induced transformation of lymphocytes (4) and inhibits lymphocyte mediated destruction of allogenic cells *in vitro* (5), suggesting an effect on T-lymphocytes. Recently evidence has been put forward that unsaturated fatty acids (UFA) are capable of suppression of the lymphocyte immune response in patients with multiple sclerosis (6) and in normal subjects as well (7). In this paper we present evidence that a synthetic prostaglandin E_2 (16,16-dimethyl PGE_2) and unsaturated fatty acids are efficient suppressors of one immune response, the lymphocyte transformation *in vitro*.

Materials and Methods. Lymphocyte responses of 12 normal subjects (aged 20–30 yr) were studied using the whole blood culture technique as described by Pauly *et al.* (8). Cell cultures were prepared by adding heparinized whole blood to 20 vol of prewarmed (37°) culture medium (RPMI 1640 with penicillin and streptomycin, Grand Island Biological Co., Grand Island, NY) containing 20% fetal calf serum. After mixing, 3 ml aliquots of the diluted blood were distributed to 16 × 125 mm culture tubes (Falcon) and PHA (Difco Laboratories, Detroit, Michigan) 10 μg in 0.4 ml of culture medium was added to each culture. 16,16-Dimethyl prostaglandin E_2 (PGE_2, Upjohn Company, Kalamazoo, Michigan) and unsaturated fatty acids (linoleic, oleic, and arachidonic acid, Sigma Chemical Co., St. Louis, Missouri) were dissolved in absolute ethanol and stored at −20° until use; 240 μg of each fatty acid and 20 μg of PGE_2 were added to each lymphocyte culture and the tubes were capped tightly and incubated at 37° in an upright position. Lymphocyte stimulation was measured by a [^{3}H]thymidine incorporation method; 1.0 μCi of [^{3}H]thymidine (sp act 2.0 Ci/mmole) was added to culture tubes after 5 days of incubation. The cultures were precipitated after additional incubation overnight. For harvesting, 2–3 vol of cold 3% acetic acid were added to lyse erythrocytes; the resulting leukocyte suspension was centrifuged, and the cell button was washed with 12 ml of 3% acetic acid. The final cell button was bleached with hydrogen peroxide 30%, dissolved in NCS solubilizer (Amersham/Searle Corp., Arlington Heights, Illinois) and prepared for counting in a liquid scintillation counter (Beckman Instruments, Palo Alto, California). Viability of the lymphocytes was determined by the trypan blue dye exclusion test. All experiments were done in triplicate cultures.

Results. The inhibitory effect of 16,16-PGE_2 and unsaturated fatty acids on lymphocyte transformation is shown in Fig. 1. PGE_2 suppressed the PHA-induced lymphocyte activation by 59% ± 1.97% (mean ± SE). Suppression values for linoleic acid, arachidonic acid, and oleic acid were 89 ± 0.71%, 89 ± 0.4%, and 91 ± 1.79%, respectively. In most instances the suppressive effect of each of the fatty acids was comparable. The possibility that PGE_2 and unsaturated fatty acids were

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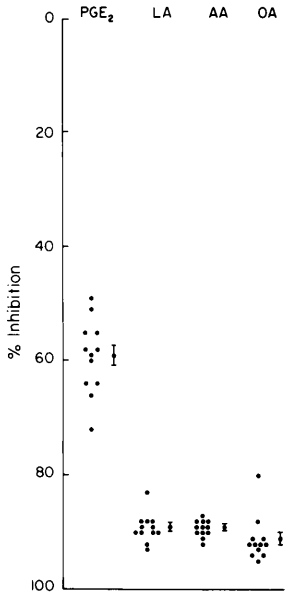

FIG. 1. Inhibitory effect of 16, (16) prostaglandin E_2 (PGE_2), Linoleic acid (LA), Arachidonic acid (AA), and Oleic acid (OA) on PHA-induced lymphocyte transformation from 12 normal subjects.

toxic to lymphocytes was excluded by the observation that the small lymphocytes exposed to PHA and these substances remained morphologically intact and excluded 0.4% trypan blue. Also, the concentration of ethanol (0.4%) used in this study had no inhibitory effect on lymphocyte transformation.

Concentrations of PGE_2 in relation to its suppressive effect were titrated on lymphocytes for four different individuals. PGE_2 solution containing 5, 10, 15, 20, 25, and 30 μg/0.01 ml were added to each culture tube. The suppressive effect was remarkably uniform for the four different cells with increasing concentrations of PGE_2 (Fig. 2). The degree of inhibition of lymphocyte transformation 56% obtained when 20 μg of PGE_2 was added to the cultures was comparable with that described in Fig. 1 (59%).

Discussion. The presented data demonstrate that PGE_2 and unsaturated fatty acids are potent inhibitors of PHA-induced lymphocyte transformation. The inhibition does not appear to be the result of toxicity to the lymphocytes as judged by their

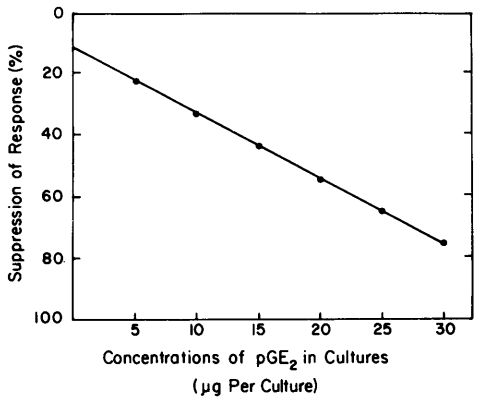

FIG. 2. Relation of PGE_2 concentration to its suppressive activity on PHA-induced lymphocyte transformation.

morphologic appearance and ability to exclude trypan blue as well. The degree of inhibition by PGE_2 observed in our experiments is comparable with that reported by other investigators (6, 7). The dose, however, was considerably smaller than theirs, indicating the great potency of the synthetic compound used in this study. The exact mechanism by which prostaglandins act is still a subject of speculation. However, many studies (9, 10) indicate that a close relationship frequently exists between prostaglandins and cyclic AMP formation or action. It has been postulated that prostaglandins stimulate the activity of adenyl cyclase and increase cyclic AMP in leukocytes and in several other tissues as well (11, 12). Recently a number of investigators reported that there seems to be a close relationship between cyclic AMP (and possibly GMP) system and prostaglandins on cell proliferation; cyclic AMP and its derivatives restore the morphology of transformed fibroblasts toward normal and decrease their growth rate (13). This suggests that cyclic AMP has a pathophysiological role in regulating the growth and morphology of fibroblasts and that a defect in cyclic AMP metabolism may explain some of the abnormal properties of transformed cells.

With regard to the suppressive effect of fatty acids on lymphocyte transformation, our results support earlier observations that unsaturated fatty acids cause significant

inhibition of PHA-induced lymphocyte activation (7). The degree of inhibition observed in our experiments, however, was significantly higher than that reported previously in normal subjects and comparable with that seen in patients with multiple sclerosis (6). However, our results may not be directly comparable with theirs, mainly due to significant differences in design between their experiments and ours. Although there is general agreement that fatty acids may exert some regulatory control of lymphocyte reactivity to various stimulants (14), no definite mechanism of action has been demonstrated. It has been postulated that inhibition of lymphocyte transformation by UFA (and possibly prostaglandins) may be related to direct blocking of the membrane associated receptor or to changes in the permeability of the lymphocyte membrane (7).

Summary. A new synthetic prostaglandin (16,16-PGE_2) and unsaturated fatty acids have been shown to inhibit the incorporation of [^{3}H]thymidine in the lymphocyte transformation reaction in response to phytohemagglutinin (PHA). This inhibition was not due to toxicity of these substances, for the lymphocytes remained intact and capable of excluding trypan blue.

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