CHILD syndrome. Phenotypic dichotomy in eicosanoid metabolism and proliferative rates among cultured dermal fibroblasts.

M E Goldyne, M L Williams

J Clin Invest. 1989;84(1):357-360. https://doi.org/10.1172/JCI114163.

Research Article

Dermal fibroblasts from a patient with CHILD syndrome (an acronym for congenital hemidysplasia with ichthyosiform erythroderma and limb defects) were obtained and successfully maintained in culture. Fibroblasts from an area of chronically hyperkeratotic skin were compared with fibroblasts from the corresponding contralateral area of normal skin in regard to proliferative activity and to both unstimulated and stimulated generation of PGE2, an eicosanoid with documented effects on both epidermal cell and fibroblast function. Compared with the uninvolved skin fibroblasts, those from involved skin showed (a) a slower rate of proliferation, (b) a cyclical pattern of PGE2 synthesis, and (c) an approximately 20-fold greater synthesis of PGE2 in response to human purified IL-1, a cytokine known to be secreted by epidermal keratinocytes. Furthermore, we were able to demonstrate that the cyclical generation of PGE2 by the involved skin fibroblasts is responsible for their slower rate of growth when compared with the uninvolved skin fibroblasts. These data document a phenotypic dichotomy between the uninvolved and involved skin fibroblasts in CHILD syndrome that may be exploited to increase our understanding of the nature of dermal influences that may affect epidermal growth and differentiation.

CHILD Syndrome

Phenotypic Dichotomy in Eicosanoid Metabolism and Proliferative Rates among Cultured Dermal Fibroblasts

Marc E. Goldyne and Mary L. Williams
Departments of Dermatology, Medicine, and Pediatrics, Veterans Administration Medical Center and University of California, San Francisco, California 94121

Abstract

Dermal fibroblasts from a patient with CHILD syndrome (an acronym for congenital hemidysplasia with ichthyosiform erythroderma and limb defects) were obtained and successfully maintained in culture. Fibroblasts from an area of chronically hyperkeratotic skin were compared with fibroblasts from the corresponding contralateral area of normal skin in regard to proliferative activity and to both unstimulated and stimulated generation of PGE_2, an eicosanoid with documented effects on both epidermal cell and fibroblast function. Compared with the uninvolved skin fibroblasts, those from involved skin showed (*a*) a slower rate of proliferation, (*b*) a cyclical pattern of PGE_2 synthesis, and (*c*) an approximately 20-fold greater synthesis of PGE_2 in response to human purified IL-1, a cytokine known to be secreted by epidermal keratinocytes. Furthermore, we were able to demonstrate that the cyclical generation of PGE_2 by the involved skin fibroblasts is responsible for their slower rate of growth when compared with the uninvolved skin fibroblasts. These data document a phenotypic dichotomy between the uninvolved and involved skin fibroblasts in CHILD syndrome that may be exploited to increase our understanding of the nature of dermal influences that may affect epidermal growth and differentiation.

Introduction

Studies by Billingham and Silvers (1) and by Melbye and Karasek (2) have demonstrated that the dermal component of skin can influence the growth and differentiation of the epidermal component. While the factors that constitute this influence are not well defined, some may be of a soluble and diffusable nature (2). For example, PGE_2, one of the soluble factors generated by dermal fibroblasts (3, 4), has been shown to affect the proliferation of epidermal keratinocytes (5).

During efforts to identify the possible dermal influences that could affect epidermal function, we had the opportunity to obtain dermal fibroblasts from a patient with CHILD syndrome. The name, coined by Happle et al. (6), is an acronym for congenital hemidysplasia with icthyosiform erythroderma and limb defects. As shown in Fig. 1, patients with this syndrome have a striking unilateral, hyperkeratotic erythroderma that abruptly terminates at the midline; the contralateral skin is clinically and histologically normal (7). Dermal fibroblasts were obtained from both involved hip skin and contralateral normal-appearing hip skin and evaluated for several metabolic parameters. Our studies demonstrate a phenotypic dichotomy between the involved and uninvolved skin fibroblasts with regard to proliferative rate and to unstimulated as well as stimulated generation of PGE_2. The data to be presented document that the difference in proliferative rates between the two fibroblast populations is due to their different patterns of PGE_2 synthesis.

Methods

Cell preparation. Fibroblasts were obtained from diagnostic biopsies of both involved (right lateral hip) and contralateral uninvolved skin (left lateral hip). Fibroblasts from the cultured explants were passaged in DME 21 medium containing 5% newborn calf serum, 4.5 mg/ml glucose, 2.5 μg/ml fungizone, 1 μg/ml of an equal mixture of penicillin and streptomycin, and 0.1% glutamine. Fibroblasts were harvested for passage using 0.1% trypsin in calcium- and magnesium-free PBS. The fibroblasts were subjected to at least two passages before carrying out the initial studies. In the studies to be described, fibroblasts were initially plated at a density of 1.5×10^5 cells/dish in 60-mm culture dishes. Replicate dishes (2–4) were used for each time point studied.

Measurement of PGE_2. Because previous studies (8) using radiolabeled AA demonstrated that PGE_2 was the major eicosanoid synthesized by both normal and CHILD syndrome dermal fibroblasts, we used an established RIA for PGE_2 to monitor the levels of this eicosanoid generated by the different fibroblast populations (9, 10). Background levels of PGE_2 possibly in the cell-free culture medium (due to the 5% serum) were always checked and subtracted from values obtained in the various cell culture supernatants.

Measurement of DNA. Cell proliferation was assessed by directly measuring total cellular DNA using the method described by LaBarca and Paigen (11). This technique involves treating sonicated cells with the fluorochrome bisbenzamidazole, which binds to DNA, and measuring the resulting fluorescence with reference to known quantities of DNA.

Stimulation and inhibition of PGE_2 synthesis. Human purified IL-1, an established stimulus for PGE_2 synthesis in fibroblasts (12), was obtained from Collaborative Research Inc. (Lexington, MA) and used at a concentration of 3 U/ml for stimulation studies. To inhibit PGE_2 synthesis, indomethacin (Sigma Chemical Co., St. Louis, MO) was added to cultures at a concentration of 10^{-6} M. In replacement studies, PGE_2 (Advanced Magnetics, Cambridge, MA) was added to indomethacin-treated cultures at a concentration of 3×10^{-7} M.

Address reprint requests to Dr. Marc E. Goldyne, Departments of Dermatology and Medicine, Veterans Administration Medical Center, 4150 Clement Street, MS190, San Francisco, CA 94121.

Received for publication 2 March 1989.

The Journal of Clinical Investigation, Inc.
Volume 84, July 1989, 357–360

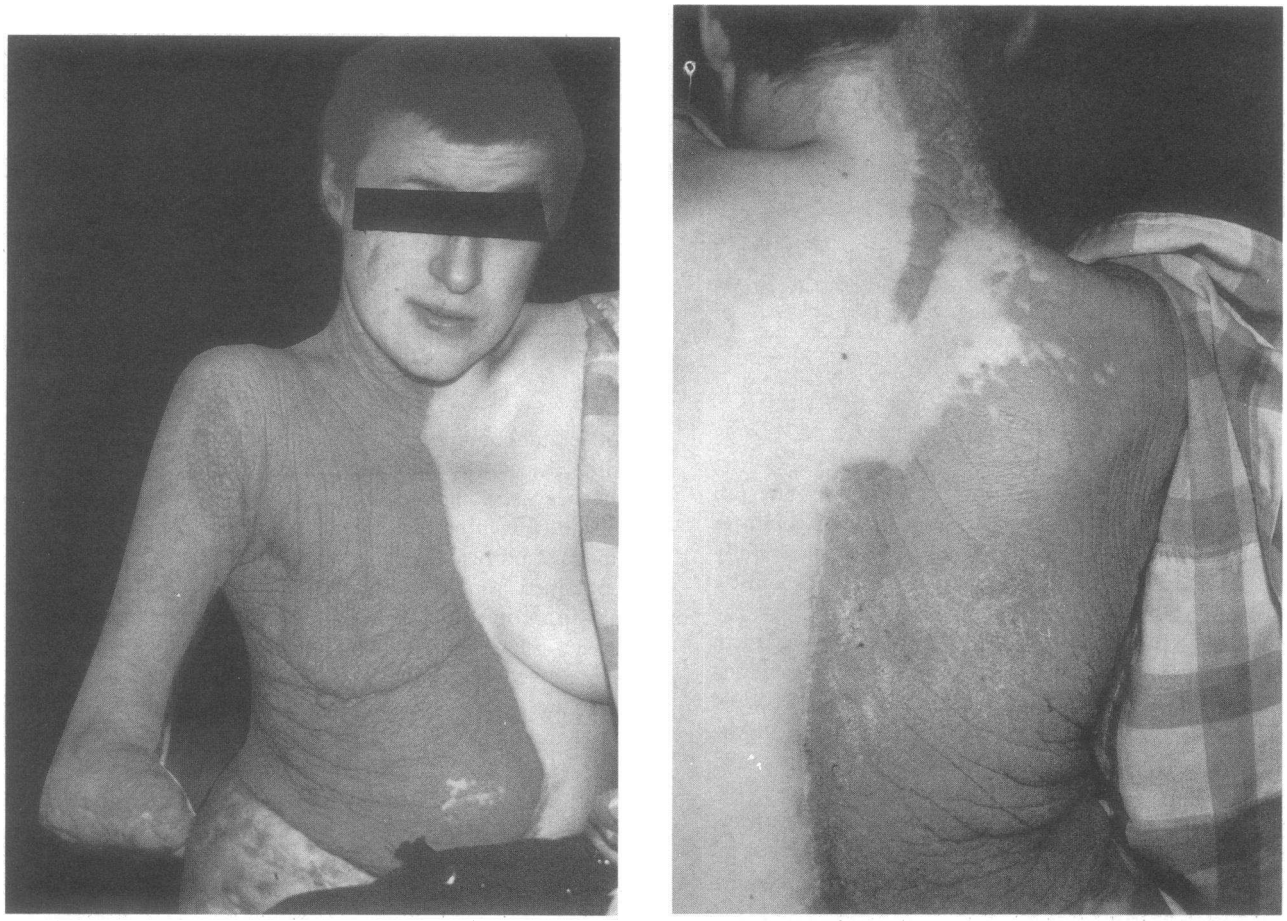

Figure 1. CHILD syndrome. The patient studied presented with the characteristic striking unilateral distribution of the hyperkeratotic erythroderma.

Results

Phenotypic dichotomy in proliferative rate among CHILD syndrome fibroblasts. Fig. 2 illustrates the DNA accumulation curves for the two fibroblast populations. Starting at 96 h, the growth slopes begin to diverge, and by 10 d the mean DNA level of the uninvolved skin fibroblasts (*solid circles,* 4.9±0.35 μg/dish) was significantly higher than that of the involved skin fibroblasts (*open circles,* 3.5±0.04 μg/dish; $P < 0.01$, t test for independent means; $n = 4$).

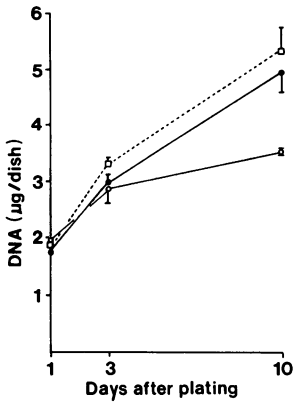

Figure 2. CHILD syndrome comparison of the DNA accumulation curves for the uninvolved (●) and involved (○) skin fibroblasts. Involved skin fibroblasts cultured with 10^{-6} M indomethacin (□) enhanced their proliferative rate to a level equivalent to that of the uninvolved skin fibroblasts. Indomethacin had no effect on the proliferative rate of the uninvolved skin fibroblasts (data not graphed).

Phenotypic dichotomy in PGE_2 synthesis among CHILD syndrome fibroblasts. Fig. 3 shows the levels of PGE_2 accumulating during successive 24-h intervals (media changed every 24 h) in the supernatants from the two fibroblast populations over 10 d. The uninvolved skin fibroblasts (*bottom*) failed to generate detectable levels of PGE_2 until almost achieving cul-

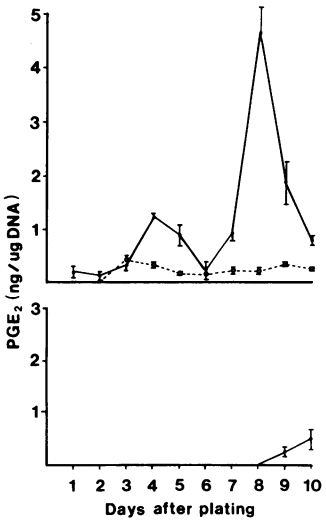

Figure 3. CHILD syndrome: profile of PGE_2 generation by the involved and uninvolved skin fibroblasts. (*Top*) Involved skin fibroblasts. *Dotted line,* PGE_2 levels generated in the presence of indomethacin. (*Bottom*) Uninvolved skin fibroblasts.

ture confluency on day 9. In complete contrast, the involved skin fibroblasts, during the same period, generated two significant peaks of PGE_2 activity (*top*). Incubating the fibroblasts with 10^{-6} M indomethacin virtually abolished the PGE_2 activity, ruling out nonspecific crossreactivity as an explanation for the peaks that were observed.

Indomethacin corrects the depressed proliferative rate of the involved CHILD syndrome fibroblasts. Since endogenous PGE_2 production by unstimulated human lung fibroblasts has been shown to exert a suppressive effect on proliferation (13), studies were performed to determine if treatment of the involved skin fibroblasts with indomethacin, an inhibitor of PG synthesis, would alter their proliferative rate. Fig. 2 also includes the growth curve for indomethacin-treated involved skin fibroblasts cultured in parallel with the other experimental groups illustrated. Indomethacin was able to enhance the proliferative rate of the involved skin fibroblasts to a level equivalent to that of the uninvolved skin fibroblasts. Although not shown for the sake of clarity, culturing the uninvolved skin fibroblasts in the presence of indomethacin had no effect on their proliferative rate.

The cyclic generation of PGE_2 by the involved skin fibroblasts is responsible for their slower rate of proliferation. The ability of indomethacin to significantly enhance the rate of proliferation of the involved skin fibroblasts suggested that their cyclic generation of PGE_2 may serve as a suppressive stimulus. To test this possibility, exogenous PGE_2 was added to indomethacin-treated involved skin fibroblasts on days 3, 4, 5, 7, 8, and 9 of a 10-d incubation period to mimic the pattern of endogenous PGE_2 generation shown in Fig. 3. On days 3, 5, 8, and 10, cells were harvested and the total DNA levels per dish calculated. As shown in Table I, whereas indomethacin significantly enhanced the amount of DNA present on days 5, 8, and 10 when compared with untreated involved skin fibroblasts, the addition of PGE_2 to the indomethacin-treated cells resulted in reestablishment of the slower rate of proliferation which was not statistically different from that of the untreated involved fibroblasts.

Table I. CHILD Syndrome: Effect of Exogenous PGE_2 on the Proliferation of Indomethacin-treated, Involved Skin Fibroblasts

	DNA			
Additions	Day 3	Day 5	Day 8	Day 10
	μg/dish, mean±SEM			
None	1.50±0.25	1.93±0.08	4.63±0.13	7.53±0.08
Indomethacin	1.38±0.13	2.33±0.08	5.50±0.00	9.19±0.06
Indomethacin + PGE_2	1.49±0.11	1.90±0.00	4.35±0.25	7.65±0.10

Fibroblasts were cultured for 10 d either alone, with indomethacin (10^{-6} M), or with indomethacin plus PGE_2 (3×10^{-7} M added on days 3, 4, 5, 7, 8, and 9). Media were changed every 24 h. For each time point, no significant difference in mean DNA values between untreated cells and those treated with indomethacin plus PGE_2 were found. However, on days 5, 8, and 10 the mean DNA values for indomethacin-treated cells were significantly greater than for the corresponding populations of either untreated cells or cells treated with indomethacin plus PGE_2 ($P < 0.05$, one-tailed t test for independent sample means).

Phenotypic dichotomy in IL-1-induced PGE_2 synthesis among CHILD syndrome fibroblasts. In response to 3 U/ml of IL-1, the involved skin fibroblasts generated a mean of 1,054±32 pg $PGE_2/\mu g$ DNA ($n = 2$) vs. 48±41 pg $PGE_2/\mu g$ DNA ($n = 2$) for the uninvolved skin fibroblasts. In these experiments both populations of fibroblasts failed, in the absence of IL-1 stimulation, to generate detectable levels of PGE_2 over background during the 72-h interval evaluated.

Discussion

These studies document, for the first time, the existence of a phenotypic dichotomy in several metabolic parameters between the dermal fibroblasts from the involved and uninvolved skin of a patient with CHILD syndrome. Compared with the uninvolved skin fibroblasts, those from involved skin demonstrated a significantly lower proliferative rate, a cyclical pattern of PGE_2 generation, and an approximately 20-fold greater synthesis of PGE_2 in response to a given concentration of IL-1.

The depressed proliferative rate of the involved skin fibroblasts appears intimately related to their synthesis of PGE_2. First, the divergence of the growth curves for the two fibroblast populations (Fig. 2) begins in association with the first peak of PGE_2 generation by the involved skin fibroblasts. Second, indomethacin treatment of the involved skin fibroblasts elevates their proliferative rate to that of the uninvolved skin fibroblasts, whereas indomethacin has no effect on the proliferative rate of the uninvolved skin fibroblasts that are not producing PGE_2 during the active growing phase. Third, adding PGE_2 back to indomethacin-treated involved skin fibroblasts in the cyclic manner in which it is endogenously released reestablished the slower proliferative rate of the untreated cells. These findings are in agreement with those of other investigators (13, 14) who have demonstrated the suppressive effect of PGE_2 on normal human lung fibroblast proliferation. The mechanism responsible for the cyclical generation of PGE_2 by the involved skin fibroblasts remains to be elucidated.

The difference in PGE_2 generation between the two fibroblast populations after exposure to IL-1 is of particular interest. It has recently been shown that IL-1 stimulates transcription of mRNA for the PG-generating cyclooxygenase (15). It is therefore not totally unexpected to find the involved skin fibroblasts, which generate significantly more PGE_2 under unstimulated conditions, synthesizing significantly more PGE_2 after stimulation with IL-1.

In light of the data presented, possible relationships between the phenotypic dichotomy in fibroblast eicosanoid metabolism and the cutaneous changes associated with CHILD syndrome are worth considering. Our results, while not excluding the possibility of a primary genetic alteration in the involved skin epidermal cells themselves, provide evidence of definite dermal fibroblast alterations in the involved skin. Since it has been shown that PGE_2 can induce proliferation in human keratinocytes (5), the continuous cyclical generation of PGE_2 by the involved skin fibroblasts, if occurring in vivo, might serve as a persistent stimulus for inducing keratinocyte hyperproliferation. The question of metabolically altered dermal fibroblasts contributing to epidermal hyperkeratosis has already been addressed by other investigators in the case of psoriasis (16), wherein fibroblasts from involved skin caused a hyperproliferative response in cocultured normal keratino-

cytes. Unlike psoriasis, however, the hyperkeratotic state of the involved skin in CHILD syndrome is not subject to remissions and exacerbations; it remains constant. Therefore, strong justification is provided for further studies using available coculture technology for comparing the effects that these two fibroblast populations may exert on normal human epidermal keratinocytes. Such studies are currently in progress.

Acknowledgments

The authors thank Ms. Laidler Rea and Ms. Mara Hincenbergs for their technical assistance.

This work was supported by grants from the Medical Research Service, the Veterans Administration (to Dr. Goldyne) and by grant AM-29908 from the National Institutes of Health (to Dr. Williams). Dr. Goldyne is the recipient of a Clinical Investigator Award from the Veterans Administration.

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