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USE OF *IN VITRO* EPIDERMAL CELL CULTURES TO STUDY GROWTH MECHANISMS IN HYPERPLASTIC SKIN DISORDERS

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Abstract. Both atopic dermatitis and psoriasis are proliferative skin disorders that are self-limited, show hereditary tendencies and are postulated to result from changes in the cyclic AMP-beta adrenergic system of the epidermis. Cyclic nucleotides, polyamines, arachidonic acid and its metabolites, and several drugs are associated with changes in the hyperproliferative epidermis. These biologically active compounds have been shown to affect the *in vitro* function of both neonatal and adult human primary culture systems. These *in vitro* model systems can be used to study the growth mechanisms of hyperplastic skin disorders.

Key words: Atopic dermatitis; Psoriasis; Cell model systems; Cyclic nucleotides; Prostaglandins; Polyamines

Two benign proliferative skin disorders used for biological studies are atopic dermatitis and psoriasis. As reviewed by Voorhees & Duell (16), a defect in beta receptor of atopic dermatitis has been postulated as an important aberration in this disease, i.e., the Szentivanyi beta-adrenergic theory of atopy, although investigations to date using biopsy epidermal tissues are still inconclusive (16). In psoriasis, a similar cyclic AMP-adrenergic receptor abnormality (5, 11, 17), along with changes in polyamine (13) and arachidonic acid metabolism (3, 4) have been demonstrated.

However, analyses of biopsy material can only suggest hypotheses that postulate the individual role, and the interplay of these factors and therapeutic drugs in the disease state. The investigation of these working hypotheses require isolated growing epidermal cells. Both a neonatal mouse and adult human epidermal keratinocyte system have been described and are presently being used in our laboratories to study epidermal cell function. We feel that these and similar technologies are reliable tools to study changes that may exist in the function of the psoriatic and atopic epidermis. In this report we will present some of the studies that have been done and are in progress using these *in vitro* systems.

METHODOLOGY

To prepare neonatal mouse primary cultures, the basal cells are trypsinized from 50 to 60 neonatal mouse skins. The basal cells are isolated from debris and any dermal fibroblasts using a discontinuous Ficoll gradient. Two to four $\times 10^6$ cells per cm^2 are plated in medium 199 + 13% fetal calf serum + antibiotics and grown at 31 to 32°C. The cultures proliferate, form multilayers (up to 10–11) and differentiate over a 4–5 week growth period. The exact details are presented in Marcelo et al. (12).

To establish adult human keratinocyte cultures, two 1 × 3 cm epidermal strips are removed from normal volunteers by use of a keratome. After trypsinization, approximately 1×10^6 cells in MEM + 10% calf serum + antibiotics are plated per 16 mm collagen coated well. After 24 h, the medium is changed to McCoy's 5A + 10% fetal calf serum + 4×10^{-4} M L-serine. The adult human cells grow, stratify and differentiate (at least, partially) at 37°C for up to 3 to 4 months. The details of this system are reported by Lui & Karasek (7).

DISCUSSION

The effect of elevated intracellular cyclic AMP levels on the neonatal mouse cultures has been extensively investigated (8). Briefly, we have shown that 1) both high, and moderate to low levels of cyclic AMP are associated with enhanced cell proliferation, 2) cyclic GMP (the other major biologically active cyclic nucleotide) is apparently not involved in the cyclic AMP stimulated growth, and 3) cyclic AMP enhanced keratinocyte growth is associated with increased specialization of the cultures.

Similar studies using the adult human epidermal cell cultures indicate that adult cells are also stimulated by increases in intracellular cyclic AMP levels although there is a marked difference in the dose and time response of the cultures (10). The most important aspects of these two investigations is the demonstration of an effect for cyclic AMP in both the neonatal and adult epidermis, thus suggesting a role for cyclic AMP in the pathology of the psoriatic and atopic epidermis.

As discussed, in psoriasis other mediators have been implicated in the disease process. Both the neonatal mouse and adult human systems are being used to study the association between arachidonic acid metabolites and epidermal cell growth. Hammarström et al. (4) report that the neonatal mouse keratinocyte in culture produces HETE (hydroxyeicosatetraenoic acid), prostaglandin (PG) E_2 and $PGF_{2\alpha}$, and that the epidermal hyperplastic agent TPA (tetradecanoyl phorbol acetate) stimulates cell production of PGE_2. This response, i.e., increased PGE_2 production, was prevented by the drugs triamcinolone acetonide and indomethacin. Sadowski (14), employing the same cultures, has similar findings and, in addition, reports that calcium ionophore A 23187 and retinoic acid also stimulated PGE_2 production. Sadowski & Marcelo (15) have reported PGE_2, $PGF_{2\alpha}$, PGD_2 and PGA_2 production by the adult human keratinocytes in culture which was inhibited by indomethacin. Both these cell systems possess arachidonic acid metabolic systems that may be suitable models for studies of keratinocyte growth regulation by these biologically active compounds.

The diamine and polyamine system, i.e., the levels of ornithine, putrescine, spermine and spermidine, and the enzymes involved in their interconversion: ornithine decarboxylase (ODC) and S-adenosyl-L-methionine decarboxylase (SAMD) are augmented in the hyperproliferative psoriatic epidermis (13). By use of the neonatal mouse model system, the role of the polyamines in epidermal cell proliferation (6) and potential use of ODC inhibitors on epidermal cell proliferation is being studied.

In addition, drugs that can be therapeutic in hyperproliferative skin disorders (glucocorticoids; retinoids (2)) are being studied using the *in vitro* systems. Glucocorticoids have been demonstrated inhibit the proliferation of both normal and hyperplastic primary neonatal mouse epidermal cell cultures (9). Retinoid drugs may have both stimulatory and inhibitory responses (mouse and human) concomitant with changes in epidermal cell differentiation (1, 18).

CONCLUSION

Use of the primary neonatal mouse and adult human keratinocyte culture systems is contributing to our understanding of epidermal cell function, and of the changes in these functions induced by normal cell modulators and several drugs. The results of these studies can be applied to understanding the pathologies of the epidermal components of various cutaneous disorders, i.e., psoriasis and atopic dermatitis. However, the use of in vitro technology to grow the diseased epidermis itself will be necessary for a clear demonstration of the difference between the normal and diseased epidermis.

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DISCUSSION

Saurat (Paris). Q: Is atopic dermatitis an epidermal defect?

A: I don't know.