Epidermal Cell Cultures as Models for Living Epidermis

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The epidermis is composed of closely packed cells with very little intercellular matrix. Most of epidermal cells are keratinocytes which synthesize keratin and are eventually lost by desquamation. This loss is compensated by permanent proliferation of basal cells so that the biology of living epidermis is dominated by keratinization and regulation of growth. Mainly 2 mechanisms have been proposed for this regulation. The first is based on the fact that the epidermis is always in contact with the underlying dermis. The idea is that growth as well as keratinization are controlled by connective tissue factors passing through the basement membrane. According to the second, signals are emitted by maturing, keratinizing cells, which control the activity of basal cells by blocking them at various stages along the cell cycle. One major point in using epidermal cell cultures as models for living epidermis is to isolate epidermal cells to eventually study the various factors influencing their growth at the molecular level.

Besides keratinocytes there are other epidermal cells such as melanocytes and Langerhans cells. But in contrast to keratinocytes which accomplish their biological function (keratinization) independently of other cell types, the functions of melanocytes and Langerhans cells are more or less dependent upon their interactions with adjacent keratinocytes with which they form some sort of epidermal “symbionts.” For example skin pigmentation is largely influenced by interactions between melanocytes and keratinocytes. Also, contacts between these latter cells and Langerhans cells are important in epidermal immunology.

Thus, the epidermis is a complex tissue, the biology of which rests not only on that of the keratinocyte taken as a separate element, but also on the interactions that it (the keratinocyte) has with other epidermal cells.

Consequently, when epidermal cell cultures are considered as models for living epidermis at least 2 types of culture systems must be envisaged: one for the study of the biology of keratinocytes, proper, and one for that of keratinocytic-nonkeratinocytic cell interactions.

In addition, living epidermis is morphologically organized. The proliferative compartment is composed of basal cells which appear like a palisade on vertical histology sections whereas the maturing compartment is made of flattened cells which pile up into adjacent columns. This raises the question as to whether epidermal morphogenesis can be approached through tissue culture methods. Finally, living epidermis can regenerate after wounding and, here again the question is to see if regeneration can be studied in culture.

EPIDERMAL CELL CULTURE MODELS FOR THE BIOLOGY OF KERATINOCYTES

An important point was made in 1960 [1] when it was shown that adult guinea pig epidermal cells would grow in culture, fraction of the elimination of dermal connective tissue. It was shown thereafter, that not only growth, as evidenced by mitotic activity, but also differentiation, as demonstrated by multilayering, tonofilament formation, desmosome reconstruction and keratohyaline granules, occurred in pure cultures of adult[2,3] as well as neonate[4,5] epidermal cells.

The method used to culture pure epidermal cells was to separate epidermis from dermis with trypsin thus, eliminating connective tissue elements prior to cultivation. With this method, cell suspensions used to initiate a culture can be very pure: less than one connective tissue cell per 10,000 epidermal cells.

In cultures of keratinocytes dissociated with trypsin, subcultures are usually difficult. The reason for this is that the speed at which epidermal cells slough off into the culture medium (and in so doing loose their ability to divide), is so great that it is barely compensated by the mitotic activity of the culture [6]. Recent experiments [7] have been made to compare the growth of adult guinea pig epidermal keratinocytes with that of dermal fibroblasts in weighing daily (for 8 days) the amount of cellular matter present in the culture flasks. Each day, the culture medium was harvested and filtered; the cells were detached with trypsin + EDTA, suspended and filtered. After 8 days, all the filters were weighed and their weights were plotted according to time. It was found that the total amount of cellular matter (resulting from the addition of cell + medium filters) increased in both cultures as a direct function of time. However, in the case of dermal fibroblasts, this increase was due exclusively to the amount of cells attached to the culture support whereas in that of epidermal keratinocytes it was the result of progressive augmentation of the amount of cells (and cell debris) floating in the medium. The situation in culture is then comparable to living epidermis in which the basal cell population (proliferative - corresponding to the cells attached to the culture substrate) is never compensated by the loss of cornified elements by desquamation (corresponding to the sloughed cells floating in the culture medium).

The life expectancy of the culture evidently depends upon 2 fundamental factors (i) the number of cells able to proliferate that are present in the cell seed at the start of the culture and (ii) the speed of desquamation (i.e., maturation). When adult guinea pig epidermal cells are cultured, the activity of the proliferative pool (as expressed in terms of DNA synthesis) tends to decrease with time and eventually the cultures die [6]. The number of cells of the proliferative pool can be increased by the use of collagen gels as substrates, because the
attachment of epidermal cells is better on collagen than on plastic or glass [8]. In fact, pure adult human epidermal cells can be grown on collagen gels, and subcultured 2 or 3 times, whereas on glass or plastic subcultures are usually impossible. It has been shown recently that human neonate cells can be grown through serial subcultures (up to 50 or more) in planting them on a plastic substrate on which X-irradiated mouse 3T3 cells are already present [9]. It is not certain that this dramatic effect of 3T3 cells is due to their connective tissue (rather to mouse) origin since nonmouse fibroblasts do not significantly increase the growth of epidermal cells. One may wonder if the enhancing effect of mouse 3T3 cells is due to the release by these cells of a factor with epidermal growth factor (EGF) like properties. It has been shown [10] that EGF has no effect on the growth of small epidermal cell colonies (in which there is little or no differentiation), but significantly increases that of large colonies (in which differentiation has already started). One explanation can be that EGF (and EGF-like factors) slows down the speed of maturation thus maintaining intact the proliferative pool for an extended time. According to this view, the action of connective tissue cells on epidermal cell growth would be through the release of molecules which modulate the differentiation of keratinocytes. Mouse connective tissue cells may then be remarkable only because of the amount of modulating molecules they can release.

An interesting point about the cultivation of keratinocytes on 3T3 feeder cells is that pure cultures of epidermal cells can be obtained by differential trypsin (or EDTA) treatment of the cultures. This treatment can be adjusted to remove all connective tissue cells (including 3T3 and possible adventitious fibroblasts) without detaching epidermal cells. In such a system, the purification of the epidermal culture follows instead of preceding the culture itself.

Evidence that cultured keratinocytes keratinize has been given practically all cell culture systems including embryonic [4,5] and postembryonic skin cells [2,3,11].

Morphologically, cultured cells produce intracellular prekeratin tonofilaments and form fiberdesmosome complexes. They pile up, flatten out, lose their nuclei [6,11] and desquamate; depending upon culture conditions, keratohyaline-like granules have been found.

Biochemically, the cornified envelope is synthesized in culture [12]. Keratins also are synthesized [13,14], which bear some similarities with in vivo keratins. However, there are differences in molecular weight, number of components and charge heterogeneity [14]. In addition, incorporation of labeled histidine which occurs preferentially in high level cells in vitro has been found evenly distributed in culture [3]. Thus it is clear that keratin genes express themselves in culture but their expression is significantly modulated by the artificial milieu in which the cells are maintained.

In vitro, inhibitory signals emitted by maturing (keratinizing) cells block basal cells at various stages along the cell cycle [15]. Some block these cells in G2 (G2 chalone) immediately before mitosis and others arrest the cells in G1, before the entry in S phase (G1 chalone). In vitro, it has been shown that adult guinea pig epidermal basal cells can be isolated as pure populations before culture. These basal cells remain susceptible to G1 and G2 inhibitions in a manner which is not unlike the living system [16,17]. Basal cell cultures produce a G2 inhibitor which blocks part of the basal cell population in the tetraploid stage. DNA synthesis is greatly reduced in basal cell cultures by adding either high level maturing keratinocytes or semipurified skin extracts to the culture medium.

However, although cultures of adult guinea pig epidermal basal cells are susceptible to the G1 inhibitory action of such skin extracts, those derived from neonate mouse skin are not [15]. The hypothesis can be made that adult epidermal cells possess "receptors" for G1 inhibitory messages that neonate cells have not. Along this view, growth enhancing effects like that of EGF could be due to an interaction (competition) at the cell surface level, between the "Growth enhancing factor" and inhibitory signals.

Thus the proliferative pool of neonate cells (which do not respond to inhibitory signals) would be maintained in this nonresponsive state by EGF and/or EGF-like molecules. This would explain why neonate human keratinocytes can be grown in large quantities on 3T3 cells (and even larger on 3T3 plus EGF) whereas adult epidermal cells cultured in the same conditions can not.

In conclusion, the growth of basal cells seem to be under the combined influences of inhibitory messages from high level keratinizing keratinocytes and anti-inhibitory factors originating from dermal connective tissue. The culture of isolated adult basal cells appears then as the model of choice to study the molecular aspect of epidermal growth regulation.

EPIDERMAL CELL CULTURE MODELS FOR KERATINOCYTIC-NON KERATINOCYTIC CELL INTERACTIONS

When epidermal cell suspensions are prepared by enzymatic disruption of the skin, they ordinarily contain melanocytes. These latter cells can in turn be extracted from the epidermal cell suspension by taking advantage of differences in cell adhesiveness or resistance to microenvironmental factors [18].

When isolated, melanocytes attach to the culture support and exhibit typical dendritic morphology. Electron microscopy reveals that the synthesis of melanosomes persists at least during the first week in vitro. Mitotic activity has been reported, it varies from very low to moderate. Serial subcultures have not been possible.

Heavily pigmented melanocytes extracted from black earring of adult guinea pig have been co-cultured with keratinocytes isolated from nonpigmented albino skin. It was seen that the transfer of melanin from pigmented melanocytes to albino keratinocytes did not occur if there was no contact between the 2 cell types. However, contacts between these 2 cell types did not always result in pigment transfer. This indicates that for pigment transfer to occur, there must be "something" happening in addition to cell contact.

Interactions between keratinocytes and Langerhans cells have been the subject of much speculation. The hypothesis that Langerhans cells are carriers of immunologic information was made back in 1969. This implied that the Langerhans cells were a kind of antigen processing macrophage [19], a supposition which has received considerable support recently. Along such a view, the Langerhans cell would keep the lymphoid system informed of changes occurring in the immunogenicity of keratinocytes, and, since the location of the cell is always close to where keratohyaline containing keratinocytes are situated, it may be thought that the role of Langerhans cells is to capture information regarding the self versus nonself nature of the proteins which are synthesized during the terminal phases of keratinocyte maturation.

There are some indications that the role of Langerhans cells can be studied in culture. First, epidermal cell suspensions enriched in Langerhans cells have been made and maintained in vitro for short periods of time. Second, when epidermal cells are co-cultured with auto or isologous lymphocytes, there is definite (but constant) stimulation of DNA synthesis in lymphoid cells (unpublished observations). It might be that Langerhans cells are responsible for this stimulation, either directly or by processing potentially immunogenic materials which would be rendered accessible by the cultivating process (including treatment of the cells with trypsin).

EPIDERMAL CELL CULTURE MODELS FOR EPIDERMAL MORPHOGENESIS

The morphogenesis of epidermis has been the object of a large number of studies using recombinants of isolated epider-
mis on different kinds of connective tissue beds at various stages of development. Through these experiments the notion has been gained that epidermal morphogenesis is markedly influenced by the underlying connective tissue. In cultures of dissociated keratinocytes, however, the cells are plated on plastic and morphogenesis is only grossly comparable to what it is in vivo. As the living epidermis, cultures exhibit 2 compartments: the proliferating one, which is made of dividing keratinocytes attached to the culture support and the maturing one, which consists of keratinizing and desquamating flat, cornified elements. However, vertical sections through piled up cultures reveal that neither the dividing layer nor the piled up cornifying cells are organized like they are in vivo. There is neither polarization nor palisading of the cells of the dividing layer. There is no orderly arrangement of the upward ascending cells. Electron microscopy reveals the presence of desmosomes at the cell interfaces but keratohyaline granules are rare and keratinosomes are absent.

Some progress toward better organization of cultured keratinocytes have been made recently. When small explants of adult human skin are cultured on pig dermis [20], epidermal multilayered outgrowths extend rapidly. In these outgrowths, the low level dividing layer exhibits some palisading and with the electron microscope, typical keratohyaline granules as well as intracellular keratinosomes have been seen in high level cultures [21]. It was noted that such improved organization was found in cultures maintained at the air-liquid interphase but not in sister cultures completely immersed in the culture medium. This suggests that the underlying connective tissue may not control all aspects of epidermal morphogenesis. More efforts along this line may prove to be rewarding in the future.

EPIDERMAL CELL CULTURE MODEL FOR REGENERATION

Early after wounding, epidermal cells start moving on the fibrin clot which has appeared in between the 2 edges of the wound. In culture also, epidermal keratinocytes are capable of migration as shown by total encapsulation of the dermal aspect of skin fragments floating in tissue culture medium (epiboly). In vivo, when split-thickness skin grafts have been taken with a dermatome, the excised surface rapidly heals through the regeneration of appendage remnants (sweat ducts and hair bulbs). A somewhat similar reconstitution of the epidermis has been observed in organ cultures of large pieces of skin (22). It was noted that such improved organization was found in cultures maintained at the air-liquid interphase but not in sister cultures completely immersed in the culture medium. This suggests that the underlying connective tissue may not control all aspects of epidermal morphogenesis. More efforts along this line may prove to be rewarding in the future.

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