Experimental Models for Psoriasis

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Evidence suggests that inherent in skin of psoriatic subjects are cells, architectural structures, and/or mediators, which are, at a minimum, responsible for its hyperproliferative epidermis. An objective of our laboratory has been to establish an in vitro definition of this inherent aberration. Fibroblasts are important to epidermal proliferation/differentiation. This, and an unconfirmed report that fibroblasts from psoriatic subjects might drive the abnormal epidermal proliferation in psoriasis, have caused further focus on the fibroblast. Data show that fibroblasts from patients with psoriasis, both involved and uninvolved, in the presence of human serum, either normal or psoriatic, have an increased rate of proliferation. Fibroblasts from uninvolved psoriatic sites are most responsive. To determine if fibroblasts from psoriatics could induce the psoriasiform phenotype on normal keratinocytes, an interactive skin equivalent system has been developed. With this system, fibroblasts from uninvolved and involved sites cause normal keratinocytes to have an enhanced outgrowth. Uninvolved fibroblasts cause the greatest changes. The nature of the skin equivalent system calls for this to occur via message over distance. We conclude that fibroblasts from psoriatic subjects can induce a psoriasiform phenotype via a soluble message. *J Invest Dermatol 95:56S–58S, 1990*

The objective of this paper is to delineate the reasons for studying the pathogenesis of psoriasis using the defined conditions of experimental models and to present our experience with selected models.

Psoriasis is a dynamic disease that is genetically driven, is expressed almost exclusively in the skin, and has an expressed and a non-expressed phenotype. An observation by our group supports this concept. Relative to normal skin, the epidermis of both involved and uninvolved skin of psoriatic subjects becomes equally hyperproliferative upon transplantation to nude mice; however, the scaling and erythema that typify a lesion of psoriasis do not persist [1]. It follows that inherent in skin of psoriatic subjects are the cells, their architectural structures and/or mediators, which are, at a minimum, permissive for the unexpressed phenotype and, at a maximum, substituents that drive the expressed phenotype once humoral aspects inherent in psoriatic subjects are also present.

It is our thesis that the expression of disease in a psoriatic subject at any given time is a composite of the basic defect that gives rise to the excess epidermal proliferation seen in all skin of psoriatics, and the subsequent mediator-regulated events that induce the lesions or the lesions induce. This thesis suggests that measurement of putative mediators in lesional skin is likely to be, as it has proved to be, highly variable [2]. These aspects suggest that the further definition of the pathogenesis of psoriasis will require an in vitro system that employs psoriatic tissue where at least one parameter of the psoriatic phenotype emerges as a function of time.

We hypothesize that the cellular constituents from the epidermis and dermis of skin of psoriatic and normal subjects can be brought together in vitro and that under appropriate culture conditions a "skin equivalent" (see below) can be generated that simulates the in vivo state sufficiently to permit emergence of a psoriasiform phenotype, a hyperproliferative epidermis. This hypothesis is supported by the as yet unconfirmed experiments reported by Saiag et al [3]. In a system that uses punch biopsies of skin inserted into a fibroblast-impregnated collagen gel in which epidermal proliferation is measured as outgrowth of keratinocytes from the biopsies, these investigators report that fibroblasts from either involved or uninvolved sites of psoriatic subjects promote the outgrowth of keratinocytes from either normal or psoriatic biopsies. Furthermore, we predict that those cellular constituents from uninvolved skin of psoriatic subjects that drive a hyperproliferative phenotype house at least a component of the defect common to psoriatic subjects.

Because fibroblasts regulate keratinocyte proliferation/differentiation [4] in simple in vitro systems, the initial hypothesis was that fibroblasts from psoriatic subjects might modulate keratinocytes to an enhanced level of proliferation (a psoriasiform phenotype) in a defined interactive system that avoids the complexities inherent to using the skin biopsies for a read-out, as reported by Saiag et al [3].

GENERATION OF A SIMPLE INTERACTIVE SKIN EQUIVALENT SYSTEM

Inherent to exploration of the foregoing hypothesis is the notion that cellular constituents of skin are interactively regulatory in a paracrine fashion, i.e., "message acting over distance." To facilitate the characterization of these modulatory interactions, the system housing the cellular constituents has to be permissive of message interaction but yet not be in direct contact. An additional requirement is that the system permit each of the cell types present to proliferate and differentiate. The system chosen for exploration is illustrated in Fig 1. In this system, outgrowth of cells (keratinocytes) in the upper chamber can be measured noninvasively as a function of time, and this outgrowth can be correlated with vertical growth by histologic assessment of the keratinocytes on the membrane at the end of the experiment.

To validate this system, a series of experiments was undertaken that used normal fibroblasts and epithelial cell lines derived from squamous cell carcinomas of the skin. These were chosen because both the carcinoma lines and the fibroblasts grow equally well in the same medium (DMEM-HEPES + 10% FBS). Data demonstrate that normal fibroblasts in the under chamber cause overlying carci-

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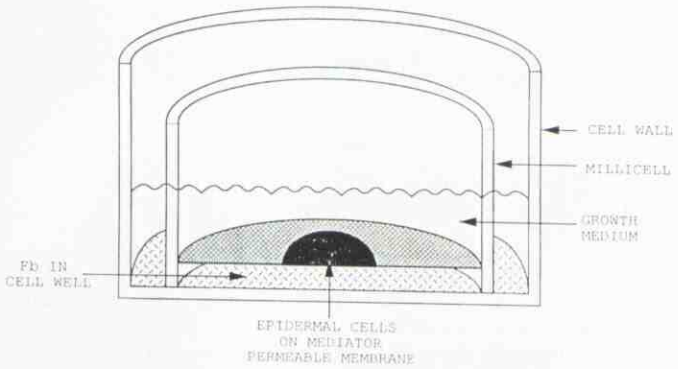

Figure 1. Cross-section of the interactive cell culture system, Millicell-HA, 30 mm diameter, inserted into a 36-mm-diameter well of a 6-well Corningware plate. Epidermal cells are seeded, via gravity, in a 1-cm diameter area with the aid of a Teflon sleeve. The sleeve is removed 12–16 h later and the Millicell is placed over the lower chamber already seeded with fibroblasts. At various times, usually 9 d, the Millicell is raised 3 mm to expose the keratinocytes to air. Area is measured on the overlying cover with the assistance of a computerized planimeter at various intervals (see text). Fibroblast growth can be monitored. The keratinocyte growth and differentiation into the epidermis can be monitored by histologic assessment of the membrane.

noma cells to have an enhanced outgrowth and a much more organized phenotype. This, as well as the fact that these carcinoma cells suppressed fibroblast proliferation, served to validate the system as being quantitatively interactive [5].

Various protocols were used to develop culture conditions that promoted the simultaneous growth of both keratinocytes and fibroblasts. The fibroblast medium (DMEM-HEPES + 10% FBS) supported the growth of rapidly differentiating keratinocytes but only if fibroblasts were present in the lower chamber. Keratinocyte growth medium (KGM, Clonetics Corp., San Diego, CA) supported keratinocyte growth but not fibroblasts. Conveniently, it was found that keratinocytes and fibroblasts could be grown together under at least two protocols. In one, they were cocultured in a 60:40 mixture of KGM:DMEM-HEPES + 10% FBS for 9 d and then air-exposed for terminal differentiation. In the other, they were cultured in this combination for varying time periods, e.g., 1 d, 2 d, 3 d, 5 d, etc., and then were switched to DMEM-HEPES + 10% FBS and managed as above (media being changed every 3–4 d). In the first, an impressive epidermal equivalent emerged; however, the influence of fibroblasts on growth was not easily recognized, i.e., changes in outgrowth were not evident, fibroblasts vs no fibroblasts. In the second, the epidermal equivalent was not as impressive, i.e., thinner and prematurely differentiated. Here, however, the influence of fibroblasts on keratinocyte outgrowth could be quantitatively established (manuscript in preparation).

CHARACTERIZATION OF FIBROBLAST GROWTH, PSORIASIS VS NORMAL

Prior to addressing the hypothesis of whether fibroblasts from psoriatics could induce a phenotype of psoriasis on normal keratinocytes, we deemed it necessary to characterize the growth of fibroblasts in vitro. Unconfirmed reports showed that some fibroblasts derived from involved and uninvolved lesions of psoriatic subjects were hyperproliferative [6]. Using these reported culture conditions, we were unable to show that any of 16 fibroblast lines derived from involved ($n = 8$) or uninvolved ($n = 8$) sites of psoriatic subjects displayed a hyperproliferative mode [7].

Experiments carried out by Saiag et al (L. Dubertret, personal communication, 1987) revealed that human serum was requisite for fibroblasts from psoriatic subjects to have an effect on keratinocyte outgrowth. This, coupled with the observation that psoriasis frequently develops in a background of tissue injury and the fact that serum contains growth factors common to inflammatory wound-repair processes, prompted experiments where normal or psoriatic serum was added to the fibroblast cultures. To analyze growth, fibroblasts derived from 3-mm biopsies of lesional and non-lesional skin were seeded, in early passage, 3–5, at 2.2×10^4 per well of a 24-well Costar plate containing DMEM-HEPES and varying concentrations of sera. Cell counts were made at days 6 and 9. Highlights of these observations (manuscript in preparation) are presented.

1. Fibroblast number, derived from biopsies of normal subjects and grown in 10% FBS, was similar, $84 \pm 24\%$ (not a significant difference), to that seen in 10% normal human serum.

2. Comparative testing of the effects of sera of 14 psoriatic and 11 normal subjects on the growth of six normal fibroblast lines and 12 psoriasis fibroblast lines (six uninvolved, six involved) showed that uninvolved fibroblasts were more proliferative in either normal or psoriatic sera than fibroblasts from involved sites ($p < 0.02$). Furthermore, both involved and uninvolved fibroblasts were more responsive than normal fibroblasts to these sera. Finally, sera from psoriatic subjects were more stimulatory than normal sera for all types of fibroblasts ($p < 0.05$).

3. Sera from five psoriatic subjects were pooled and tested for the effect on fibroblast growth. These data showed (a) An initial 6-h pulse with the pooled psoriasis sera (10%) resulted in 26% more fibroblasts by the end of the experiment (day 6), (b) There was a dose response that was significant and linear from 2.5% through 20%, such that the mean ± SD number of fibroblasts from uninvolved sites of psoriatic subjects in psoriasis sera was $470{,}000 \pm 3{,}000$ vs normal fibroblasts in normal sera, where the comparative value was $180{,}000 \pm 90{,}000$ (values being at the 20% sera concentration at day 9), (c) 65% of the enhancement of growth of psoriasis fibroblasts was secondary to the serum source (psoriatic) and 35% was secondary to the fibroblast phenotype, and (d) The mean values for growth of fibroblasts in individual sera were nearly identical to those for the pooled sera.

We concluded that psoriasis fibroblasts contain an inherent aberration (enhanced proliferation) that can be detected in the presence of normal human serum and is enhanced in the presence of serum from psoriatic subjects, and that fibroblast lines from uninvolved sites of psoriatic subjects are most responsive. Whether this proliferative aberration is linked to other "more classic" manifestations of psoriasis that might not require human serum has been analyzed (see below).

INDUCTION OF PSORIATIC PHENOTYPE ON NORMAL KERATINOCYTES

Validation of the interactive skin-equivalent system and the observation that psoriasis fibroblasts displayed an inherent aberration led to testing the hypothesis that psoriasis fibroblasts can induce a psoriasiform phenotype ("enhanced growth") on normal keratinocytes. We conjectured that the induction of enhanced outgrowth on normal keratinocytes would likely be linked to the processes that result in uninvolved and involved skin of psoriatic subjects both having inherently elevated levels of epidermal proliferation [1,2]. Keratinocyte growth was measured using a computerized planimeter in a non-invasive manner by tracing the outline of the periphery of the keratinocytes on the cover of the system as a function of time, 0, 5, 9, 14, and 17–21 d after the deposition of 1×10^5 normal keratinocytes in 0.79 cm^2 on the Millicell HA membrane.

Initial experiments showed that KGM:DMEM-HEPES with 10% FBS at a 60:40 ratio provided good vertical and horizontal growth. However, this protocol did not distinguish enhanced outgrowth secondary to psoriasis fibroblasts ($n = 6$). This prompted the further modification of the feeding protocol noted above. Data demonstrated that the influence of normal fibroblasts on normal keratinocytes was minimal if the system was held in KGM:DMEM-HEPES with 10% FBS, 60:40, for one day and then switched to DMEM-HEPES with 10% FBS. However, the influence was maximal for normal fibroblasts when the system was held for 3 days in KGM:DMEM mix before being switched to DMEM-HEPES with 10% FBS. Accordingly, the effect of psoriasis fibroblasts on normal keratinocyte growth was monitored follow-

ing a day-1 switch. Using seven different fibroblasts from involved and uninvolved sites of seven patients and seven normal fibroblasts and keratinocytes from a 33-year-old female, keratinocyte outgrowth measurements were made. These experiments showed (manuscript in preparation):

1. Involved (5/7) and uninvolved (6/7) fibroblast lines caused these normal keratinocytes to have an outgrowth that exceeded the range delineated by the SEM for the normal fibroblasts.

2. Some psoriasis fibroblast lines (3/14) consistently failed to induce this change.

3. When the 11/14 inducers of the enhanced outgrowth, the psoriasiform phenotype, were considered as a group, the group means were substantially and significantly above the range of normal, $p < 0.01$. These changes represent a $40 \pm 8\%$ greater surface area covered by keratinocytes on any given day in the presence of uninvolved fibroblasts than normal fibroblasts, and $26 \pm 18\%$ enhancement of surface coverage for fibroblasts from involved sites when compared with outgrowth in the presence of normal fibroblasts.

Histologic analysis revealed a correlation that coincided with the outgrowth, i.e., psoriasis fibroblasts induced a greater vertical growth as well as a greater horizontal growth.

The significant reproducible ability of both uninvolved and involved fibroblasts of psoriatic subjects to induce a psoriasiform phenotype on normal keratinocytes is seen as a major breakthrough, i.e., cells from the dermis of both involved and uninvolved sites of psoriatic subjects induce an effect on normal keratinocytes via a "message" over distance. We conclude that for this to occur, the "message" must, at times, be in the media, and hence is amenable to experimental isolation and characterization.

Inherited diseases that wax and wane suggest at least one of two possibilities. First, the up- or down-regulatory factors are generated in cells adjacent to the tissue of expression and modulate the basic defect(s), or, second, that the basic defect is autoregulatory. If the former is true, removal of fibroblasts of psoriatic subjects from their in situ setting should permit aberrations to emerge. This does occur, both involved and uninvolved fibroblasts having an inherent propensity to hyperproliferate and the capacity to induce a psoriasiform phenotype on normal keratinocytes. The fact that a few fibroblasts from psoriatic subjects do not induce the phenotype suggests that this potential defect, which may be central to psoriasis, can be down-regulated, an observation that fits the well-recognized waxing and waning of this disease [2].

THE RAT-HUMAN SKIN SANDWICH FLAP

The need for an accurate assessment of the dynamic processes surrounding the penetration of solutes through the stratum corneum and epidermis of human skin led to the development of a rat-human skin sandwich flap system [8,9]. This system contains a flap of viable human skin that is transplanted to the back of an athymic rat and is generated in a manner that causes it to be supplied by a single artery and drained by a single vein, both of which are accessible. The recent development of skin equivalents using an artificial dermal matrix impregnated with fibroblasts that is then coated with cultured keratinocytes, and the observation that these skin equivalents can mature into a functional skin in vivo suggest that psoriatic components can be adapted to this system [10]. Specifically, it is predicted that flaps with skin equivalents containing normal keratinocytes with psoriasis fibroblasts, or psoriasis keratinocytes with psoriasis fibroblasts, can be generated and compared and contrasted with flaps generated from normal keratinocytes and normal fibroblasts. Having these cell types on an experimentally accessible flap will permit the induction of psoriasiform features with injury, drugs (e.g., lithium), etc. Furthermore, humoral aspects from psoriatic subjects, e.g., cloned cells, serum, etc., can be applied to the flap. Finally, mediator message and production can be compared and contrasted with the proliferation response and other clinical features as a function of time. As this conference has abundantly demonstrated, skin has the capacity to generate and respond to a plethora of mediators and modulators of growth and differentiation. Investigators at this conference have suggested that only those that are present in significant quantities should be considered in the initial evaluation of those mediators/modulators that are important. It seems probable that the rat-human skin sandwich flap with skin equivalent diseased and non-diseased components can be used to target the most critical players.

Table I. IL-6 Levels Following 1,000 mJ/cm² of UVB

Time of Collection	Flap Blood (ng/ml)	Systemic Blood (ng/ml)
0	1.42	0.45
1	17.04	3.08
4	6.67	4.81
24	0.34	0.14
48	0.30	0.20

Recently, an experiment was conducted on the human side of a rat-human skin sandwich flap in conjunction with Dr. David Lynch (Immunex Corp., Seattle, WA) that illustrates the utility of this system for exploring these types of questions. In this experiment, the human side of the flap was exposed to 1,000 mJ/cm² of ultraviolet B (UVB), a dose equivalent to a moderate sunburn. Immediately prior to UVB, 100 μl of flap and contralateral systemic blood were drawn in a 1-min period. This was repeated at 1, 4, 24, and 48 h after UVB. Table I compares the appearance and disappearance of IL-6 in the flap and systemic blood. Note that the levels in the blood from the flap peaked earlier than the systemic blood. The increased amounts released by the flap result in measurable amounts in the systemic circulation. This experiment illustrates the probability that such a system can be used to further explore the pathogenesis of this facile disease.

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