REGULATION OF EPIDERMAL PROLIFERATION AND DIFFERENTIATION IN PSORIASIS

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Psoriasis is a genetic disease with an environmental component. The importance of the genetic versus the environmental component varies considerably from patient to patient. Nonetheless, a considerable amount of research indicates that psoriasis is apparently a multifactorial genetic disease (1). In psoriasis patients the frequency of HL-A 13 and HL-A 17 cell surface antigens is elevated fourfold (2). This information does little to suggest fruitful avenues of research which will lead to a molecular understanding of the essence of this disease.

It is necessary to therefore choose an area of research in psoriasis which is technically feasible and likely to have pathophysiological importance. We have chosen the epidermis. Stimulated epidermal cell proliferation is a major feature of psoriasis. The disease would probably not exist as we know it, if the epidermis were not a major component. The vascular compartment or dermal signals to the epidermis may be more "primary" than the epidermal disorder. However, the epidermis with difficulty can be sampled and the specimens obtained for biochemical analysis are reasonably free of dermal and vascular contamination. The vessels, dermis and other potential candidates can not be adequately

sampled. To obtain sufficient tissue of either type would require a surgical technique which would produce serious scarring and the procured tissue (vessels and dermis) would be unacceptably heterogenous. From heterogenous tissue comes uninterpretable data. It is for these reasons that our research has focused on epidermal regulation in psoriasis.

In this type of research sequential biopsies of psoriatic epidermis are generally not feasible. The amount of tissue needed for most biochemical techniques and the numbers of patients it is necessary to study due to biological variation make sequential biopsies almost impossible. Therefore, one can not usually decide what is primary or secondary. Until this type of sequential technology is available, research on psoriasis will not advance rapidly. However, until then one can circumvent this serious drawback by studying those aspects of the molecular pathophysiology of psoriasis which are hypothetically amenable to pharmacological modulation. Using this philosophical approach one is not concerned about what is primary or secondary, if the correction of a pathophysiological event normalizes the lesion. An important corollary to our research strategy is that in the initiation and/or maintenance of a lesion, certain, but not all molecular events, whether they are primary or secondary, are critical.

We have termed such events "critical molecular variables" in psoriatic epidermal pathophysiology. In this approach the key ingredient is to make the best educated guesses

as to which of several thousand events are critical. Today it is my great honor to be here in Yonago to address the Japanese Dermatological Association. My lecture will discuss and explain these "critical variables" which we have studied. There are four such areas which I will consider sequentially: 1) the epidermal cell surface; 2) cyclic nucleotides; 3) arachidonic acid-prostaglandin cascade; 4) polyamines.

THE CELL SURFACE

The cell surface acts as a switchboard to receive incoming signals (hormonal and environmental stimuli) and to coordinate the cyclic nucleotide and arachidonic acid systems. The cell surface is composed of the plasma membranes, specialized external macromolecules (glycoproteins and glycolipids), and structures on the inner face of the membrane to which the microtubule-microfilament (MT-MF) system is attached (3).

In psoriatic lesions the cell surface as visualized by electron microscopy is markedly reduced in thickness (4). The area of reduced thickness is external to the plasma unit membrane and is the putative binding region of cell surface hormones. Intuitively, it would seem that if cell surface defects in psoriasis are sufficiently gross as to be detected by microscopy, that serious defects in hormone binding and cell-cell communication may exist. DNA synthesis in cultured cells can be regulated by the addition of membranes (5). Also, cell surface membrane recognition factors regulate cyclic AMP and cyclic GMP levels in reaggregating sponge cells (6). Perhaps in psoriatic lesions the misregulated cyclic nucleotide systems are secondary to reduced cell surface hormone binding or reduced quantities of macromolecular cell surface recognition factors. However, in cultured cells cyclic AMP regulates the formation of the cell surface (7, 8). A delicate balance appears to

exist between the regulation of the cyclic AMP system by the cell surface and the regulation of the cell surface by cyclic AMP.

Defective cell surfaces are also associated with changes in membrane fluidity. The amount of cholesterol relative to phospholipid is one determinant of membrane fluidity. As the cholesterol/phospholipid ratio increases, membrane fluidity decreases (9). Since the free cholesterol content of lesional psoriasis is high, membrane fluidity should be decreased (10). Changes in membrane-lipid composition of this kind have been reported to alter the activity of membrane bound enzymes such as adenylate cyclase (9). Therefore, an alteration in cell surface glycolipids and glycoproteins and in plasma membrane fluidity could have major influences on the communication of the cell surface to the nucleus via cyclic nucleotides.

Psoriasis is said to be a multifactorial genetic disease (1). The frequency of the cell surface gene products HL-A 13 and HL-A 17 is increased three to four-fold (11). Whether these HL-A abnormalities are genetic markers or participants in the pathophysiology of the disease is unknown. However, the H-2 gene complex of the mouse is analogous to the HL-A gene complex in man. In mice the H-2 genotype is one regulator of the liver cyclic AMP level (12). The increased frequency of HL-A 13 and HL-A 17 cell surface components in psoriasis might frustrate normal cell surface regulation of the cyclic AMP system.

The MT-MF network is associated with the cell surface and is thought to relay growth regulatory information from the cell surface to the nucleus. Microtubules are composed of tubulin and microfilaments of actin (13). Wanger and Sundquist (14) have reported an accumulation of actin in psoriatic lesional epithelial cells suggesting the possiblity of an alteration of the MT-MF system as an important event in the deranged growth control

of psoriasis. Puck (13) has shown that for a normal organized array of MT-MF one must have normal or increased levels of cyclic AMP. In psoriasis the cyclic AMP function is reduced and it might be this reduction which alters the MT-MF array, thus rendering the array more prominent when viewed by immunofluorescent antibody techniques.

Clearly the cell surface of involved areas is abnormal. Whether this abnormality is primary or secondary, it is likely to be important in lesional pathophysiology for reasons discussed above. The unusual frequency of the HL-A cell surface phenotype in psoriasis suggests that the psoriatic cell surface membrane may be a substantial aspect of the predisposition to the disease. Cell surface biochemistry and biophysics are at an advanced state of technology. Unfortunately many of these methods require large quantities of relatively undisturbed membranes. The isolation of epidermal membranes of any quantity, and sufficiently intact to be useable, has not yet been achieved.

Perhaps this membrane technology could be used to compare psoriatic with normal membranes from blood lymphocytes. From lymphocytes adequate membrane preparation can be achieved. The use of lymphocytes may be successful if the psoriatic genotype is expressed in the lymphocyte and in the lymphocyte membrane specifically. If so, hormone receptor binding, glycoprotein/glycolipid profiles, and membrane fluidity could be studied in normal subjects in comparison with psoriasis patients. However, it is possible that psoriasis genes are expressed only or predominantly in the skin. If so, the psoriatic skin membrane studies will await a new technology. As mentioned previously, the cell surface and cyclic AMP are a functioning unit with the cell surface serving as a switchboard for incoming stimuli (injury or hormonal etc.).

We must therefore discuss the cyclic AMP system in psoriasis at this point.

THE CYCLIC AMP SYSTEM

The characteristic features of involved psoriatic epidermis are increased basal cell proliferation (15, 16), increased glycogen content in the center of the hyperplastic rete peg (17), and reduced morphological differentiation in the outer one third of lesional epidermis (18). In 1971 we proposed that these abnormalities might be due to a defective cyclic AMP system (19). Metabolic regulation mediated by cyclic AMP depends not on cyclic AMP per se, but on the differentiated state of the cell in which the regulation occurs. In various cells and cell layers, cyclic AMP, at least in many cases, is a promoter of the expression of differentiated properties (20). Thus a defective epidermal cyclic AMP system could be expected to produce one kind of abnormality in one epidermal layer (i.e., increased proliferation) and yet a different abnormality in a higher layer.

This theory was based on several earlier observations. Bullough and Laurence (21) showed that epidermal cell division was inhibited by epinephrine. Johnson and Pastan (22) reported that cyclic AMP elevating agents stimulated morphological differentiation in cultured fibroblasts. Sutherland and Rall (23) had previously demonstrated that cyclic AMP was the mediator of beta adrenergic epinephrine induced phosphorylase activation and glycogen breakdown. Thus, it seemed that decreased function of the cyclic AMP system might result in proliferation, decreased differentiation and glycogen storage within the lesion and that stimulation of the cAMP system might normalize these changes.

This concept was supported by studies showing a statistically significant 43 percent decrease in lesional cyclic AMP content in

comparison with uninvolved or normal epidermis (24). Subsequently, using a different assay we demonstrated a lesser but statistically significant 36% decrease in the cyclic AMP content of lesional epidermis (25). These lesional cyclic AMP reductions have not been confirmed by others. Härkönen et al. (26) reported increased lesional cyclic AMP levels and Yoshikawa et al. (27) detected no change. Since quite different psoriatic tissue preparations have been used by each research group, it is not surprising that different data have been generated. Considering the technical difficulties in procuring biopsies from psoriatic patients and the large number of patients already studied, it seems unlikely that further study of the cyclic AMP level will be productive.

Also the cyclic AMP level per se is but one possible defect in the cyclic AMP system. This system is composed of several sequential steps from the epidermal cell surface receptor to the as yet unstudied putative protein substrates in the epidermis which are phosphorylated by cyclic AMP dependent protein kinases (28). A defect in this system or interference with its function should reduce the extent of cyclic

AMP mediated epidermal protein phosphory-lation (Fig. 1). Thus a reduced "cyclic AMP effect" could be produced by interference with one of the several components of the cyclic AMP system. This is a tenable postulate since all evidence to date suggests that the epidermal cyclic AMP system is similar to that of other tissues (28).

As yet no study of cyclic AMP dependent protein phosphorylation in psoriatic epidermis has been reported. However, the functional state of the psoriatic epidermal cell surface receptor-adenylate cyclase complex has been studied. Yoshikawa et al. (29) have reported that involved psoriatic epidermis contains less cyclic AMP than uninvolved epidermis after both have been stimulated by beta catacholamine. Also involved areas produce less cyclic AMP than uninvolved when stimulated by prostaglandin of the E series (30). In confirmation of these date Mahrle and Orfanos (31) have found reduced cyclic AMP formation in response to isoproterenol stimulation using cytochemical techniques in psoriasis. Thus the capacity of involved psoriatic epidermis to produce cyclic AMP in response to hormonal signals appears to be
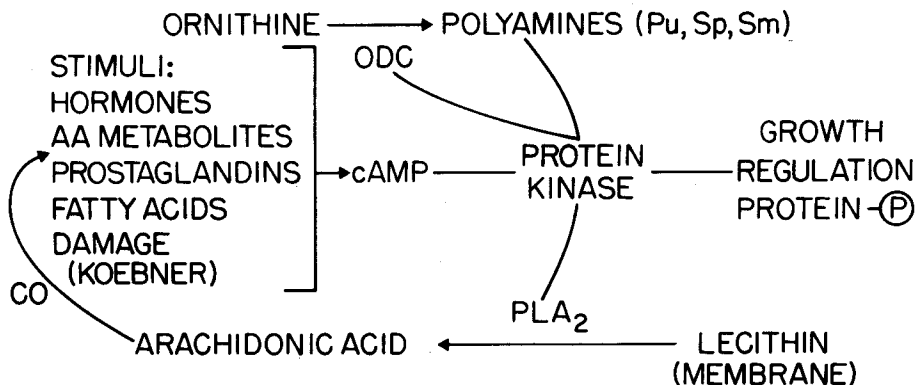

Fig. 1. Hypothetical Interrelationships of Cyclic AMP, Arachidonic Acid and Polyamines in the Regulation of Epidermal Proliferation and Differentiation in Psoriasis. Abbrev: ODC, ornithine decarboxylase; Pu, putrescine; Sp, spermidine; Sm, spermine; AA, arachidonic acid; cAMP, cyclic adenosine 3', 5' monophosphate; CO, cyclooxygenase; PLA$_2$, phospholipase A$_2$.

reduced. It is difficult to see how this reduced cyclic AMP synthetic activity could result in increased lesional cyclic AMP levels as reported by Härkönen et al. (26). However, it is compatible with either normal or reduced lesional cyclic AMP levels as shown by Yoshikawa et al. (27) and Voorhees et al. (24), respectively.

There are several more recent pieces of information which, although indirect, are compatible with a defective cyclic AMP system in psoriasis. Lithium is a known inhibitor of adenylate cyclase (32). Furthermore, if one incubates psoriatic epidermis in lithium at a concentration found in the blood stream of a lithium controlled manic depressive patient, cyclic AMP formation in response to isoproterenol is markedly inhibited by the lithium (33). Psoriatic patients who develop manic depressive illness have been reported in whom as the mania improves, the psoriatic lesions become resistant to methotrexate and topical steroids (34) and uninvolved areas become involved (35). The possible involvement of inhibited cyclic AMP synthesis in this response of psoriatic patients to lithium must be considered.

A beta adrenergic blocking agent, practolol, formerly available in Europe, and propranolol, another beta blocker, have been reported to precipitate several types of rashes, one of which is psoriasis-like (36). Intracutaneous testing failed to support the possibility that this reaction was the result of type I or type IV hypersensitivity (36). Possibly beta blockers prevent cutaneous beta catecholamines from maintaining a normal epidermal cyclic AMP content. As in the case of lithium, psoriatic patients taking oral practolol were resistant to antipsoriatic therapy and new psoriatic lesions developed.

Subsequently, Wiley and Weinstein (37) injected propranolol into clinically uninvolved psoriatic skin and into the skin of normal subjects. By autoradiography they demonstrated that propranolol produced a 6-fold increase in the number of proliferating cells in psoriatic epidermis. These data might be explained by a genetically impaired cyclic AMP generating system in psoriatic skin which is further compromised to a point of malfunction (i.e., increased cell proliferation) by propranolol. In summary, it is interesting that two structurally different agents (i.e., lithium and beta blockers) both inhibit cyclic AMP formation and both promote the formation of new lesions and render existing lesions refractory to both systemic and topical antipsoriasis drugs.

Other molecular observations have been made in lesional psoriatic epithelium which are compatible with a malfunctioning cyclic AMP system. Vassalli et al. (38) demonstrated that plasminogen activator production in cell culture is inhibited by compounds which raise the cellular cyclic AMP level and is inhibited by glucocorticoids. The plasminogen activator activity in lesional epithelium is increased (39). Glucocorticoids are known to markedly improve psoriasis and substances which can elevate intracellular cyclic AMP content also improve psoriasis when applied topically (40, 41).

The presence of increased glycogen levels in psoriatic lesions is perhaps of minimal metabolic significance. However, its presence is interesting because when normal or involved psoriatic epidermis is incubated with epinephrine, glycogen breakdown occurs (42). Thus, epidermal glycogen can be regulated by cyclic AMP as can glycogen in other cells. The location of glycogen in the more central areas of lesional epidermis may indicate that cells in that location are experiencing a reduced cyclic AMP signal.

As discussed above, our earliest theory proposed that cyclic AMP in epidermis might

serve as an antiproliferative compound and a morphogen (19). This theory has been studied by several researchers using epidermal cells in culture. Delescluse et al. (43) demonstrated that elevated cyclic AMP inhibited the proliferation of guinea pig epidermal cells in vitro. Flaxman and Harper (44) later demonstrated that the proliferation of human keratinocytes in culture were markedly inhibited by several compounds which resulted in cyclic AMP elevation. Delescluse et al. (45) reported that epidermal cell differentiation was stimulated in vitro by cyclic AMP additions to the cultures. This observation was extended by Chopra (46) who saw that keratohyalin granule production was stimulated by cyclic AMP added to human epidermal cells in culture.

If the net cyclic AMP effect is decreased in psoriatic lesional epithelium, lesions might improve if stimulated by cyclic AMP. Posternak et al. (47) injected dibutyryl cyclic AMP with and without theophylline directly into psoriatic lesions with modest results. In China (48) cyclic AMP or dibutyryl cyclic AMP was administered intramuscularly or intravenously to psoriatic patients. Of 69 patients so treated the authors concluded that this therapy was effective in 80 percent. Stawiski et al. (40) applied papaverine topically to psoriatic lesions in 45 patients and observed a statistically significant improvement. These investigators (41) then reported that another topical cyclic AMP elevating agent known as Ro 20-1724 produced statistically significant improvement of psoriasis.

Thus the several molecular and clinical lines of evidence presented here suggest that the cyclic AMP system has decreased function in psoriasis. However, the nature of this misregulation is not understood and requires further research. How this research could be approached experimentally can be visualized in Figure 1. Cell surface stimuli may not be properly received by the defective cell surface (4). Iizuka et al. (49) have provided some evidence that the cell surface can not properly receive an incoming beta catecholamine signal but can normally receive other hormonal signals which produce elevated cellular cyclic AMP. Whether this defect is due to generalized in vivo desensitization (50) or very selective desensitization (51) is unknown. Thus, the reduced capacity of lesional epidermis to form cyclic AMP in the presence of isoproterenol could be due to prior in vivo receptor desensitization with a genetically normal membrane beta receptor phenotype. If this is true, the reduced catecholamine sensitivity should be temporary and reversible under definable in vitro conditions. The elevation of lesional cyclic AMP content according to our theory (19) should improve the lesion. Therefore, a key question is whether beta receptor linked cyclic AMP generation specifically can be related to pathophysiology. That is, when the lesional beta receptor is again able to promote the synthesis of cyclic AMP in vivo, does the lesion begin to resolve.

Figure 1 also shows how a defect distal to cyclic AMP formation could produce a functional decrease in the cellular response to cyclic AMP. The binding of cyclic AMP to the receptor subunit of cyclic AMP dependent protein kinase may be impaired. The kinase could be primarily altered by a genetically produced amino acid substitution in an area of the kinase molecule which reduces receptor binding. Reduced binding, depending on its extent, could produce reduced function. Alternatively, the "milieu interieur" (52) of lesional epidermal cells may not and quite likely is not normal. That is to say, the molecular constituents of the intracellular cell fluid within the epidermal cell plasma membranes may not be optimal. Enzymic systems in regulatory biology must have a

normal microenvironment to properly perform their catalytic functions. Thus in Figure 1, cyclic AMP dependent protein kinase and its specific phosphoprotein substrate(s) may be normal but the intracellular enzymic microenvironment may prevent the kinase from providing normal phosphorylation or the protein substrate(s) from accepting phosphorus. Either functional defect distally could produce the same "cyclic AMP deficiency state" as could an actual reduction in the cyclic AMP level per se, proximally.

It is apparent from examining Figure 1 that there are several ways by which the necessary cellular cyclic AMP function can be reduced: 1) reduced ability of the cell surface receptors to stimulate adenylate cyclase activity thereby producing reduced cellular cyclic AMP levels; 2) reduced ability of the kinase to carry out the distal functions of the cyclic AMP which was formed in response to hormone binding to the epidermis; 3) reduced ability of the protein substrate for kinase to act as a phosphate acceptor. In all these situations, it makes no difference whether the interference in the cyclic AMP cascade is primary or secondary. The end result is the same — reduced epidermal lesional cyclic AMP function. What does make a difference is whether the reduced cyclic AMP signal is biologically important. Throughout this part of my lecture, I have discussed various data which suggests that cyclic AMP is important. However, all these data (eg., glycogen, lithium, practolol, in vitro cyclic AMP effects, topical application of papaverine and Ro 20-1724, etc.) are indirect and subject to several interpretations.

Evidence must be developed which directly proves that cyclic AMP can modulate epidermal function. If it does, then the potential participation of cyclic AMP in the mismodulated psoriatic epidermis is greater. To this end we have labored over the last three years on the cultivation of mouse epidermal cells in vitro. This endeavor has met with scuccess (53). We are very reproducibly cultivating primary epidermal cells from neonatal mice on plastic dishes without the need for a collagen substrate or 3T3 fibroblasts. Using this system we were quite surprised but delighted to observe that cyclic AMP when added to these cells, causes a several fold increase in their rate of proliferation. Other hormones which also raised intracellular cyclic AMP caused increased proliferation. Other studies showed that in these cells, it was the rise in cyclic AMP that caused the proliferation and not a rise in cyclic GMP (54).

We were surprised by these results in that stimulation rather than inhibition (the expected result) occurred. However, we were pleased because any time one speaks of inhibited proliferation, there is always the question of whether the inhibition is due to the known mechanism of action of the inhibitor or whether the dose response curves were generated by a fundamentally cytotoxic substance. It is most unlikely that any cell system can respond to a cytotoxic agent with stimulated cell proliferation. It is quite likely that cell proliferation induced by cyclic AMP in these cultures is a cyclic AMP specific, noncytotoxic response. Therefore, in these cultures it is likely that physiologically occurring cyclic AMP can modulate proliferation. If one examines the cyclic nucleotide literature, it is apparent that the tissue response to elevated cyclic AMP levels is dependent on the tissue type and the differentiated state of the tissue. It is therefore possible to advance the hypothesis that cyclic AMP may stimulate neonatal mouse epidermis and inhibit adult human epidermis either due to different ages, species or both. To test this hypothesis we are developing similar cultures of human adult

epidermis. However, the stimulated prolifera-
tion in neonatal tissue described above makes it
possible to state that cyclic AMP probably does
have an important role in epidermal physiology
and probably in pathology as well. This is an
excellent area for future research and we are
currently continuing along these lines. Since
cyclic GMP has been implicated in psoriatic
pathophysiology, we will discuss cyclic GMP at
this point.

THE CYCLIC GMP SYSTEM

Many investigators agree that cyclic AMP
may modulate in vitro cell proliferation. It ap-
pears that cyclic AMP may be a significant
regulator of proliferation in epidermis as well.
However the role of cyclic GMP in the regula-
tion of cellular growth either in vitro or in vivo
is unclear. Hadden et al. (55) were the first to
discover an association between stimulated
lymphocyte proliferation and elevated levels of
cellular cyclic GMP. Several proliferative cell
systems in which a similar association exists
have been recently reviewed by Goldberg and
Haddox (56). However, several cell prolifera-
tion systems have been described in which the
cyclic GMP content either does not rise or in
fact, falls when proliferation occurs. Perhaps
the best studied situation is that reported by
Nesbitt et al. (57). Rapidly dividing trans-
formed cells in culture were found to have a
low cyclic GMP content and markedly reduced
guanylate cyclase acitivity in comparison with
their nontransformed counterparts. The true
role of cyclic GMP in the regulation of cell
division is unknown.

In psoriasis Voorhees et al. (58) reported an
increased lesional cyclic GMP content. More
recently, Marcelo et al. (59) found elevated
cyclic GMP levels in lesions of a larger group of
psoriatic subjects confirming the earlier report.
Perhaps this elevation of cyclic GMP in
psoriatic lesions is significantly associated with

the increased lesional cell proliferation.
Presently, the elevated cyclic GMP in psoriasis
is an interesting observation, the importance of
which in psoriatic pathophysiology is yet to be
discovered.

From this discussion it seems that misregulat-
ed cyclic AMP action in psoriasis may well be a
fruitful area of future research. Furthermore,
the role of cyclic GMP in lesional patho-
physiology may be more important than can
currently be predicted.

THE ARACHIDONIC ACID-PROSTAGLANDIN SYSTEM

For this reason we have been interested in
studying the regulation of cyclic nucleotide
(cyclic AMP and cyclic GMP) synthesis in skin.
Agents which raise cyclic AMP in skin are
similar to agents which raise cyclic AMP in
other organs. One of these compounds is
prostaglandin. Prostaglandin is an excellent
candidate in the regulation of cyclic AMP
formation because a locally acting agent is
required. Lesions of psoriasis are in patches
with sharp borders and adjacent normal ap-
pearing skin. If a circulating hormone were
involved in the misregulated cyclic nucleotide
metabolism of psoriasis, the disease would be
generalized rather than located in patches.
Prostaglandins are formed and degraded
locally in tissues and cells and do not circulate
in a hormonally active state (60).

Prostaglandins are synthesized from
arachidonic acid. It is the release of free
arachidonic acid from arachidonic acid
esterified into cell membrane phospholipids
that is the rate limiting reaction in the cascade.
The release of arachidonic acid occurs when
tissues are injured or are hormonally
stimulated (Fig. 2). Released free arachidonic
acid is transformed to a substance known as 12-
L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid
(HETE), cyclic endoperoxides, thromboxane

PHARMACOLOGICAL
BLOCKERS:

☓ GLUCOCORTICOID

— INDOMETHACIN
LIKE DRUGS

● ETA (TYA)

PL

☓ PHOSPHOLIPASE A$_2$

AA

● CYCLOOXYGENASE

LIPOXYGENASE

PGG$_2$

HPETE   PGH$_2$
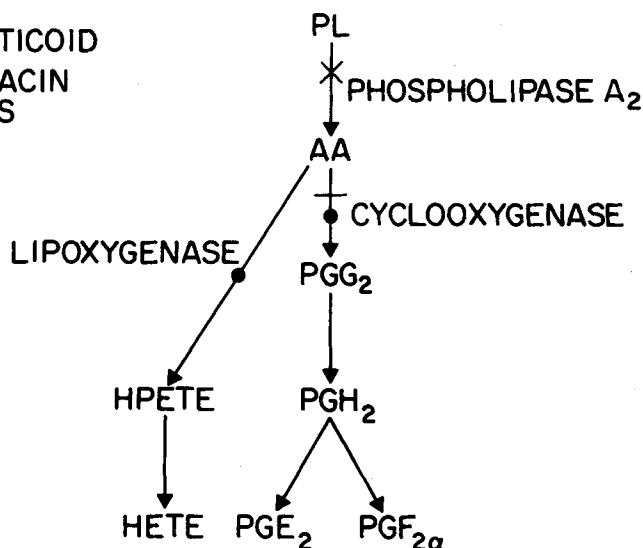
HETE   PGE$_2$ PGF$_{2\alpha}$

Fig. 2.   Arachidonic Acid-Prostaglandin Cascade in Psoriatic Epidermis. The 26-fold increase in lesional AA content is 95% inhibited by plastic film occluded glucocorticoid plus cream vehicle. Psoriatic cyclooxygenase is markedly inhibited by indomethacin whereas both lipoxygenase and cyclooxygenase are inhibited by ETA. Arachidonic acid in psoriatic epidermis is transformed into the metabolites seen in this figure. In other tissues different metabolites have been detected such as thromboxane and prostacycline. Such metabolites are not detectable in either psoriatic or normal epidermis. Abbrev: PL, phospholipid; AA, arachidonic acid; PGG$_2$ and PGH$_2$, the two prostaglandin cyclic endoperoxides; PGE$_2$, prostaglandin E$_2$; PGF$_{2\alpha}$ prostaglandin F$_{2\alpha}$; HPETE, hydroperoxy-HETE; HETE, 12L-hydroxy-5, 8, 10, 14-eicosatetraenoic acid; ETA (TYA), 5, 8, 11, 14-eicosatetraynoic acid.

and the classical prostaglandins, PGE$_2$, PGF$_{2\alpha}$ and PGD$_2$ (60). These various intermediates all can modulate the content of cyclic nucleotides in tissue as reviewed by Voorhees et al. (61).

In an attempt to determine how these metabolites might regulate cyclic nucleotides in psoriasis, it was first necessary to identify these metabolites and their levels in psoriatic tissue. This was done by Hammarström et al. (62) who reported a 26-fold increase in the content of free arachidonic acid and an 82-fold increase in HETE levels in involved versus uninvolved epidermis. This was a striking finding since the content of free arachidonic acid in normal tissues is barely detectable and that minimal detectable level is usually thought

to have been released by inadvertent tissue manipulation.

Hammarström et al. (62) also detected a 40 and 80% increase in the lesional levels of PGE$_2$ and PGF$_{2\alpha}$, respectively. This minimal increase was unexpected since high arachidonic acid levels in tissue are usually transformed into classical prostaglandins. Thus it appears that in lesional tissue of psoriasis, there is an endogenous inhibition of prostaglandin biosynthesis in vivo. In relation to the large quantity of released arachidonic acid, the lesion may be said to have a relative prostaglandin deficiency. This relative prostaglandin deficiency may be the result of an endogenous inhibitor of prostaglandin biosynthesis

described by Penneys et al. (63). This inhibitor is dialyzable but is not susceptible to ribonuclease, deoxyribonuclease or proteases.

As previously mentioned, the major disturbance in psoriasis is the large increase in free arachidonic acid (26-fold) which is transformed primarily into HETE. Turner et al. (64) first reported that HETE was chemotactic for polymorphonuclear leukocytes (PMNs). This was confirmed by Goetzl et al. (65) using human PMNs. Psoriatic lesions have considerable numbers of PMNs. It is possible that the high HETE content is an important factor in the chemoattraction of PMNs into the lesion. In fact, Penneys et al. (66) injected arachidonic acid metabolites, one of which was presumably HETE, intradermally into human volunteers. They found that this injection attracted large numbers of PMNs into the injection site suggesting that the endogenous HETE content in psoriasis could indeed be one important chemoattractant.

In psoriasis the release of arachidonic acid and its transformation into HETE may disturb cyclic nucleotide metabolism. Wallach and Pastan (67) showed that arachidonic acid per se stimulated guanylate cyclase activity and thus is a compound which might promote the accumulation of cyclic GMP in tissues. Subsequently, Hidaka and Asano (68) found that the hydroperoxy-form of HETE, which is always formed as arachidonic acid is transformed into HETE, stimulated guanylate cyclase activity.

Unsaturated fatty acids also inhibit adenylate cyclase activity thus preventing the accumulation of cyclic AMP in tissue. Anderson and Jaworski (69) showed that arachidonic acid per se inhibited adenylate cyclase activity. Also lysolecithin not only is a potent activator of guanylate cyclase, but it is a potent inhibitor of adenylate cyclase activity (69, 70). Since lysolecithin is formed as arachidonic acid is released in tissues, it is possible that the

lysolecithin content of psoriatic lesions is elevated. Lysolecithin is also known to be a potent inhibitor of prostaglandin biosynthesis at concentrations which are physiologically plausible (71). Thus the putative elevation in psoriatic lesional lysolecithin may disturb cyclic nucleotide synthesis and is also a candidate for the dialyzable inhibitor of lesional prostaglandin biosynthesis described by Penneys et al. (63).

Although the levels of lesional prostaglandin E_2 and $F_{2\alpha}$ reported by Hammarström et al. (62) may represent a relative deficiency, the absolute levels are minimally increased. Since prostaglandin $F_{2\alpha}$ has been reported to stimulate the formation of cyclic GMP and prostaglandin E_2 the formation of cyclic AMP, the elevated lesional levels of these two prostaglandins in relation to the lesional cyclic nucleotide content requires comment. In our hands, prostaglandin $F_{2\alpha}$ has not been capable of elevating lesional cyclic GMP and only at high (1×10^{-3} to 1×10^{-5} M) concentrations does prostaglandin E_2 elevate epidermal cyclic AMP. Also, lesional prostaglandin levels remain elevated for the life of a lesion. It seems quite possible that this would possibly lead to in vivo desensitization of prostaglandin receptors. When this desensitization phenomenon occurs cells could be refractory to prostaglandin induced increases in cyclic nucleotide content (50). In essence, the cells could perform as though the increased prostaglandin content did not exist. Thus the steady state lesional prostaglandin level may bear little relationship to the steady state cyclic nucleotide content. When considering the role of prostaglandins in the regulation of lesional cyclic nucleotide content, it is clearly preferable to consider the role of all the products of arachidonic acid. As discussed above, the various metabolites of arachidonic acid are capable of regulating cyclic nucleotide biosynthesis. In psoriasis

therefore, it is of interest to know the effect of arachidonic acid per se, HETE and hydroperoxy HETE on cyclic nucleotide synthesis.

Since arachidonic acid levels are markedly increased in psoriasis, we will briefly consider its regulation in other tissues for what value such information may contribute to psoriasis. The rate limiting enzyme in the synthesis (release) of free arachidonic acid from membrane phospholipids is known as phospholipase A_2 (60). Lapetina et al. (72) and Minkes et al. (73) have shown that in horse and human platelets respectively, cyclic AMP inhibits the release of arachidonic acid presumably by inhibiting the activity of phospholipase A_2. Thus in psoriasis by stimulating the sluggish cyclic AMP system, it may be possible to correct the abnormally high release of arachidonic acid. The cyclic nucleotide system and the arachidonic acid-prostaglandin cascade appear to be functionally interdependent. The arachidonic acid metabolites switch on cyclic AMP formation which in turn switches off arachidonic acid release. Whether cyclic AMP does in fact inhibit arachidonic acid release in psoriatic lesions is currently under investigation.

THE POLYAMINES

Putrescine, spermidine and spermine are three substances which are collectively called polyamines. The rate limiting step in the conversion of ornithine to putrescine, putrescine to spermidine, and spermidine to spermine is the decarboxylation of ornithine by ornithine decarboxylase (ODC). An elevated rate of polyamine formation by stimulating ODC activity appears to be the most constant correlate of increased cell proliferation (74). Russell et al. (75) reported that the level of polyamines is significantly higher in lesional psoriasis than in uninvolved epidermis and that
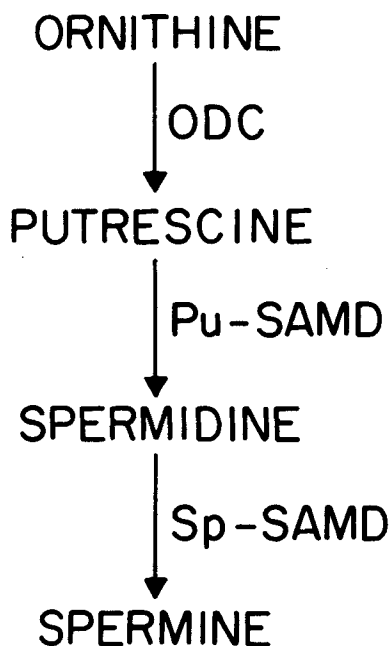

Fig. 3. Polyamine Biosynthesis in Normal and Psoriatic Human Epidermis. Abbrev: ODC, ornithine decarboxylase; Pu-SAMD, putrescine-stimulated S-adenosyl-L-methionine decarboxylase; Sp-SAMD, spermidine-stimulated S-adenosyl-L-methionine decarboxylase.

the uninvolved levels are significantly higher than epidermis from normal volunteers. This increased polyamine content of uninvolved epidermis is perhaps a molecular correlate of the two-fold increase in the labelling index of uninvolved epidermis versus normal epidermis (16). Certainly the increased labelling index and polyamine content of "uninvolved" areas indicates that there is no such thing as an uninvolved area at the molecular level.

Although elevated polyamine levels have been associated with rapid proliferation for several years (74), it has been only recently that a possible cause and effect relation between polyamines and proliferation has been suggested. Mamont et al. (76) recently showed that blocking the synthesis of polyamines by an inhibitor of ODC reduced cell proliferation.

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This inhibition could be overcome by combining polyamines with the ODC inhibitor, thus bypassing the ODC blokade. Thus the inhibition of polyamine synthesis by analogs of ornithine and polyamines per se may offer new approaches to psoriasis and other proliferative diseases.

In some cells where cyclic AMP induces hypertrophy and hyperplasia, cyclic AMP induces ODC activity and polyamine formation to accompany the proliferation (77). in lesional epidermis of psoriasis the studies of Stawiski et al. (40, 41) indicate that cyclic AMP elevation probably inhibits cell proliferation. Since polyamines are already elevated in psoriasis the cyclic AMP rise could inhibit ODC activity in lesions of psoriasis. Such an inhibition of ODC activity has been shown by Insel and Fenno (78) in lymphoma cells where cyclic AMP also inhibits cell growth. Whether cyclic AMP inhibits ODC activity in psoriasis and in this way participates in the regulation of cell proliferation in lesions is under current study.

CONCLUSION

Today I have discussed several aspects of the molecular basis of psoriasis which we consider to be of unusual importance. I have reviewed our pragmatic approach to psoriasis research. This approach has been used because in my opinion the research tools for studying the etiology of psoriasis are not available. Until then, hopefully a dissection of the molecular systems I have described today will provide potentially better therapies.

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