Elevated phosphorylase kinase activity in psoriatic epidermis: correlation with increased phosphorylation and psoriatic activity

M.C.Y.HENG, M.K.SONG AND M.K.HENG

Division of Dermatology and the Laboratory of Vascular Biology, Department of Medicine, UCLA San Fernando Valley Program, Veterans Administration Medical Center, Sepulveda, California, U.S.A.

Summary   To determine whether abnormal activity of a calmodulin-containing enzyme which catalyses phosphorylation reactions may play a pathogenetic role in psoriasis, the presence and activity of phosphorylase kinase (PK) in human epidermis were determined in patients with untreated/active psoriasis ($n=10$), treated/resolving psoriasis ($n=10$), and non-psoriatic controls ($n=10$). Biopsies were taken from involved and uninvolved skin for PK, organic phosphorus, and inorganic phosphate estimation, and light and electron microscopy. The enzyme was present in involved and uninvolved skin of every patient in the study. PK activity (units/mg protein) was significantly higher in active psoriasis than in resolving psoriasis and controls. PK activity correlated directly with organic phosphorus levels, and inversely with the extent of cellular glycogenolysis measured by the depletion of glycogen granules within the keratinocytes. The study demonstrates that PK is present in both psoriatic and normal epidermis, with significantly higher levels in active psoriasis. Furthermore, higher levels of PK activity, glycogenolysis and phosphorylation are associated with increased clinical psoriatic activity. We conclude that PK, a calmodulin-containing enzyme, is involved in regulating calcium-dependent phosphorylation events in human epidermis, and disturbance of its activity may play a key role in the clinical manifestations of psoriasis.

Psoriasis is a hyperproliferative skin disease in which the causal mechanisms are not completely understood, despite many studies into its pathogenesis.[1-20] Abnormal activity of some compounds involved in signal transduction events has been reported in both involved and uninvolved psoriatic epidermis. These include elevated levels of calmodulin[21] and phospholipase A2,[22] a calmodulin-dependent enzyme.[23] In this study, we report the discovery of the presence of the calmodulin-containing enzyme, phosphorylase kinase (PK), in human epidermis, and the finding of an abnormally increased activity of the enzyme in patients with active psoriasis.

Phosphorylase kinase, through activation of glycogen phosphorylase and inhibition of glycogen synthase, is the key enzyme in glycogenolysis. This enzyme is, therefore, involved in providing the essential energy for the production of high energy phosphate bonds used in many phosphorylation reactions involving proteins.[24-27] In addition, PK transfers high energy phosphate bonds from ATP to convert phosphorylase-b to phosphorylase-a, the active form of the enzyme. This reaction is a necessary step in the activation of phosphorylases which are regulated by phosphorylation and dephosphorylation. Although PK is known to be abundant in muscles,[24-26] it has never been reported to be present in human epidermis. However, a number of abnormalities which are known to occur in psoriatic epidermis, such as keratinocyte terminal differentiation,[28-31] and ligand-dependent epidermal growth factor receptor activation,[32-37] appear to involve calcium-dependent phosphorylation.[37] Accordingly, we postulate that disturbed activity of a calmodulin-containing enzyme which catalyses calcium-dependent phosphorylation reactions may be a key pathogenetic mechanism in psoriasis. This hypothesis is supported by preliminary observations in our laboratory that periodic acid Schiff-staining glycogen, which is decreased in active untreated psoriasis, is increased in the healing stage of the disease. The present study was designed to demonstrate the presence of PK in human epidermis, and to determine whether changes in activity of PK in the skin are related to clinical psoriatic activity.

Correspondence: Dr M.C.Y.Heng, UCLA School of Medicine, Chief, Division of Dermatology, Veterans Administration Medical Center, 16111, Plummer Street, Sepulveda, CA 91343, U.S.A.

Methods

Patients

Thirty subjects for the study were recruited from the dermatology clinics at the Veterans Administration Medical Center, Sepulveda, California. They consisted of 10 men with clinically active psoriasis who had not received any form of treatment for the disease (untreated/active group); 10 men with resolving lesions of psoriasis after therapy with diltiazem 60 mg t.i.d.,[12] with or without tar or methotrexate (treated/healing group); and 10 men with the following untreated non-psoriatic skin diseases—three eczema, one urticaria, one mycosis fungoides, one Darier's disease, one melanoma, one tinea corporis and two squamous cell carcinoma (control group). Six punch biopsies, each 4 mm in diameter and weighing about 50 mg, were obtained from each patient—three from involved skin, and three from uninvolved skin. In psoriatic patients, 'involved skin' refers to the active lesions from the active/ untreated group, or resolving lesions from the healing/ treated group. In the non-psoriatic control patients, 'involved skin' refers to the active untreated lesions of non-psoriatic skin disease. In each patient, biopsies from the uninvolved skin were taken at least 10 cm distant from those obtained from involved skin. Of the three biopsies obtained from each involved or uninvolved site, one was processed for microscopy, and the others for biochemical assays. The study was approved by the Human Studies Subcommittee of Sepulveda VA Medical Center, and informed consent for the biopsies was obtained from all patients.

Light and electron microscopy

Each biopsy obtained for microscopy was divided longitudinally. One half was fixed in 10% neutral-buffered formalin and processed for light microscopy. The paraffin sections were stained with haematoxylin and eosin. The other half, for electron microscopy, was fixed in 2·5% glutaraldehyde, buffered to pH 7·3 with 0·1% sodium cacodylate, post-fixed in osmium tetroxide, treated en bloc with tannic acid, dehydrated in ethanol and propylene oxide, and embedded in a mixture of Epon 812 and Araldite 502. Silver sections (70–80 nm thick) were cut on a Sorvall MT 2B ultramicrotome with a diamond knife (Dupont) and examined with a Philips EM201 electron microscope.

Cytosolic preparation of epidermal cells

Two skin biopsy samples, weighing approximately 100 mg, were used for biochemical analysis. The samples were stored at $-70°C$ until use. Each frozen sample, with the epidermal surface upwards, was placed in a glass tube to which 3 ml Tris-HCl buffer (10 mM Tris-HCl, pH 7·8, 1 mM dithiothreitol (DDT), 3 mM $MgSO_4$ and 1 mM EGTA) was added, and homogenized vigorously with a Teflon plunger in a Tris-R model K41 homogenizer for 1 s. Homogenization was repeated if necessary. The lysate of epidermal cells, separated from the dermis, was in the cytosolic solution. The fibroblasts which were inadvertently detached, together with the relatively intact piece of dermis, were removed by decanting the cytosolic solution into a 5-ml-capacity polypropylene test-tube. The lysates were centrifuged at 3000 g for 15 min. Membranes and other cytosolic organelles which formed a pellet at the bottom of the tube were removed. The supernatant, which contained the cytosolic component of epidermal cells, was then subjected to biochemical analysis.

A separate study, using biopsies from 12 subjects, was performed to validate the technique of separating the epidermis from the dermis in the skin samples. The samples, after thawing, were placed in a glass tube with the epidermis upwards, and suspended in Tris-HCl buffer solution containing 0·9% NaCl, for the separation of intact epidermal cells. The currents generated by stirring with the Teflon plunger caused the separation of the epidermis from the dermis at the dermo-epidermal junction, which was confirmed by histological examination after the tissue samples, separated from the dermis by this procedure, were fixed in formalin and processed through paraffin sections for light microscopy. They consisted of 94·1 ± 4·2% epidermal cells for samples from psoriatic patients ($n = 6$). These results show that the tissue from which our cytosolic preparation was obtained consisted mainly of epidermal cells.

Assay of phosphorylase kinase concentration

Phosphorylase kinase activity was assayed by measuring the conversion rate of phosphorylase-b to phosphorylase-a, according to a modification of the method of Cohen.[38] Briefly, PK was assayed by measuring radioactive phosphate transferred from [^{32}P]ATP (Du Pont Co., Wilmington, DE, U.S.A.) to the phosphorylase-b, suspended in 30 mM cysteine solution, pH 7·0, in the process of conversion to the phosphorylase-a form. Forty microlitres of 30 mM cysteine solution, 50 μl of 0·25 M β-glycerophosphate solution, 50 μl of phosphorylase-b

solution, and 20 μl of either PK standard solution or cytosolic samples were transferred to each 5·0 ml polypropylene test-tube. The tubes were incubated at 30°C for 3–5 min to equilibrate temperatures. At 0 min, 40 μl of [^{32}P]-ATP solution was added to each tube, mixed thoroughly by vortexing, and incubated at 30°C for 15 min. One millilitre of ice-cold 5% trichloracetic acid (TCA) solution was then added to each tube. The tubes were then placed in ice, and cooled for 10 min or more. Each reaction mixture was then filtered through a millipore filter paper (pore size 0·45 μm), and washed three times with 2 ml of cold 5% TCA solution. The filter paper containing phosphorylase-b was counted in a liquid scintillation counter. Enzyme activity was determined based on a standard curve prepared with PK of known activity supplied by Sigma Co (St Louis, MO, U.S.A.).

The reproducibility of our methodology was assessed by duplicate measurements of unknown concentrations of the enzyme from the randomly selected patients in the study population against known concentrations of PK. Firstly, 10 solutions of standard PK with known concentrations, ranging from 0 to 38 μg/mg protein, were assayed, and a linear standard graph was obtained. The average per cent variation of each duplicate measurement from the mean was 4·70±4·24% (range 0–11·5%), and the linear correlation between the paired measurements was $r = 0.98$. Secondly, enzyme activities from 10 patients in the study population were measured twice for each skin sample. The corresponding reproducibility measurements for this assessment were 3·35±2·27% (range 0·27–8·81), and $r = 0.98$.

Determination of total phosphorus in the epidermal cytosol

To each 5-ml-capacity glass test-tube containing the test sample (1–10 μmol of phosphorus or 10–100 μl cytosolic sample), 40 μl of perchloric acid solution (2·0 M) was added, and boiled on a hot-plate. Bumping was minimized by placing a capillary tube, sealed at the top, into the tube. One drop of potassium iodide solution (0·5 mg KI/ml) was added when the volume of each sample was reduced to about 30 μl. When the sample had evaporated almost to dryness, it was removed from the hot-plate and cooled. Twenty microlitres of dilute ammonia solution (5·6 M) was added to the sample, and the preparation heated again on a hot-plate until it was dry. The sample was dissolved in exactly 1·0 ml distilled water when the test-tube had cooled. Exactly 40 μl of ammonium molybdate reagent [2·0 mM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in 5·0 M sulphuric acid solution]

was added, followed by 10 μl of sodium sulphate solution (0·4 M). Precisely 10 min later, 20 μl of stannous chloride working solution (20 mM $SnCl_2 \cdot 2H_2O$ in 0·5 M HCl solution) was added. Thirty minutes later, the absorbance at 700 nm was read in a spectrophotometer. The reagent blank was subtracted from the absorbance reading of the sample. The concentration of inorganic ion in the sample was determined against standards of inorganic phosphate.

Inorganic phosphate concentration in each 1·0 ml of cytosolic sample was determined by adding molybdate reagent, followed by the addition of stannous chloride solution, and reading of the absorbance at 700 nm. The value of organic phosphorus was determined by subtracting the value of inorganic phosphate from the total phosphorus concentration.

Determination of the density of glycogen granules

For each biopsy sample, the glycogen granules within 10 adjacent keratinocytes situated mid-way between the stratum corneum and the basal keratinocyte layer were photographed at a magnification of ×20,000, and printed at a further ×2·5 magnification on 203×254 mm Kodak paper, to a final magnification of ×50,000. The density of the glycogen granules was counted and determined for each square micron. Areas occupied by mitochondria, tonofilaments and nuclei were eliminated from the count.

Statistical analysis

Comparison of the differences between mean measurements of the three patient groups was performed by multiple analysis of variance (MANOVA) and Tukey's *post-hoc* test. The results were considered statistically significant when $P < 0.05$. All results in this report are expressed as mean ± SD.

Results

Comparison of phosphorylase kinase activity in the cytosolic supernatant of epidermal cells

The results are shown in Figure 1.

The PK activity (U/mg protein) in the epidermis of the three groups of patients is given below: active/untreated psoriasis – involved epidermis 25·9±11·48, uninvolved epidermis 16·11±9·95; healing/treated psoriasis – involved epidermis 5·71±2·67, uninvolved epidermis 4·45±2·67; controls – involved epidermis 2·95±1·75, uninvolved epidermis 4·07±3·18. In untreated psoria-
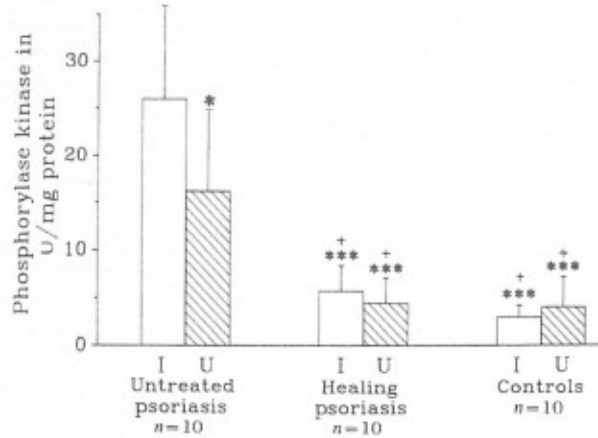
Figure 1. Phosphorylase kinase activity (U/mg protein) in epidermal cytosolic preparations in involved and uninvolved skin from active/untreated psoriatics, resolving/treated psoriatics, and non-psoriatic controls. *$P<0.05$ compared with involved untreated psoriasis; ***$P<0.001$ compared with involved untreated psoriasis; +$P<0.01$ compared with uninvolved treated psoriasis. I, involved; U, uninvolved.
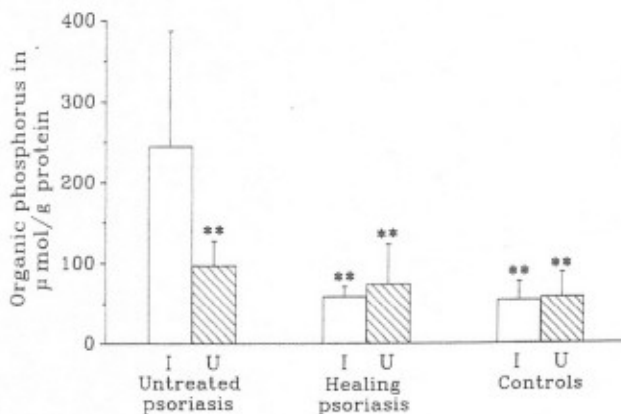

Figure 3. Inorganic phosphate concentrations (μmol/g protein) in epidermal cytosolic preparations in involved and uninvolved skin from active/untreated psoriasis, involved and uninvolved skin from treated/resolving psoriasis, and involved and uninvolved skin from non-psoriatic controls. There is no statistical difference between the involved and uninvolved skin of the three groups. I, involved; U, uninvolved.

Figure 2. Organic phosphorus concentrations (μmol/g protein) in epidermal cytosolic preparations in involved and uninvolved skin from active/untreated psoriatics, involved and uninvolved skin from resolving/treated psoriasis, and involved and uninvolved skin from non-psoriatic controls. **$P<0.005$ compared with involved untreated psoriasis. I, involved; U, uninvolved.

tic patients, the PK activity of the involved epidermis was significantly higher than in the uninvolved epidermis ($P<0.05$). The enzyme activity in both involved and uninvolved epidermis of untreated psoriatic patients was significantly higher than that of patients with treated psoriasis and non-psoriatic controls. The differences between PK levels of the involved epidermis in untreated psoriatics and both involved and uninvolved skin in the other two groups were all significant at the $P<0.001$
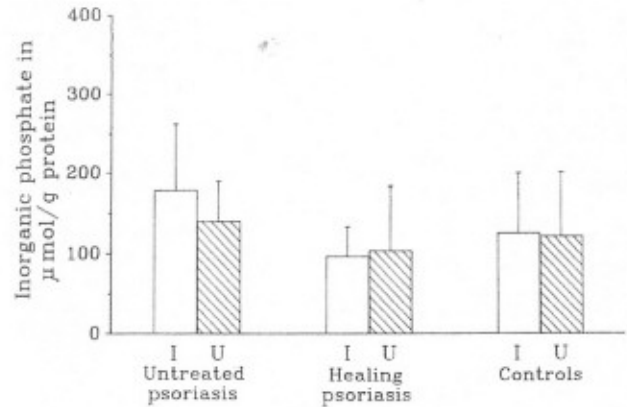
level; the corresponding differences for the uninvolved epidermis in untreated psoriatics were all significant at the $P<0.01$ level. The PK activities of the involved and uninvolved epidermis in the healing psoriatics and controls were all statistically similar.

Epidermal organic phosphorus concentrations in psoriatic patients and controls

The results are shown in Figure 2.

The organic phosphorus concentrations (μmol/g protein) in the epidermis of the three groups of patients were as follows: untreated psoriatic patients – involved epidermis 244.3 ± 142.2, uninvolved epidermis 96.9 ± 30.2; treated psoriatic patients – involved epidermis 58.9 ± 12, uninvolved epidermis 74.2 ± 49.1; controls – involved epidermis 54.8 ± 23.6, uninvolved epidermis 58.7 ± 30.4. The organic phosphorus concentration in the involved epidermis of the untreated psoriatics was significantly higher than the uninvolved skin of the same group, and the involved and uninvolved skin of the other two groups; the differences were all significant at the $P<0.005$ level. The values of the uninvolved skin in untreated psoriatics and the involved and uninvolved epidermis in the treated psoriasis group and controls were statistically similar.

The levels of organic phosphorus were directly related to the levels of PK activity; the correlation coefficient between the two parameters was $r=0.934$ ($P<0.001$).
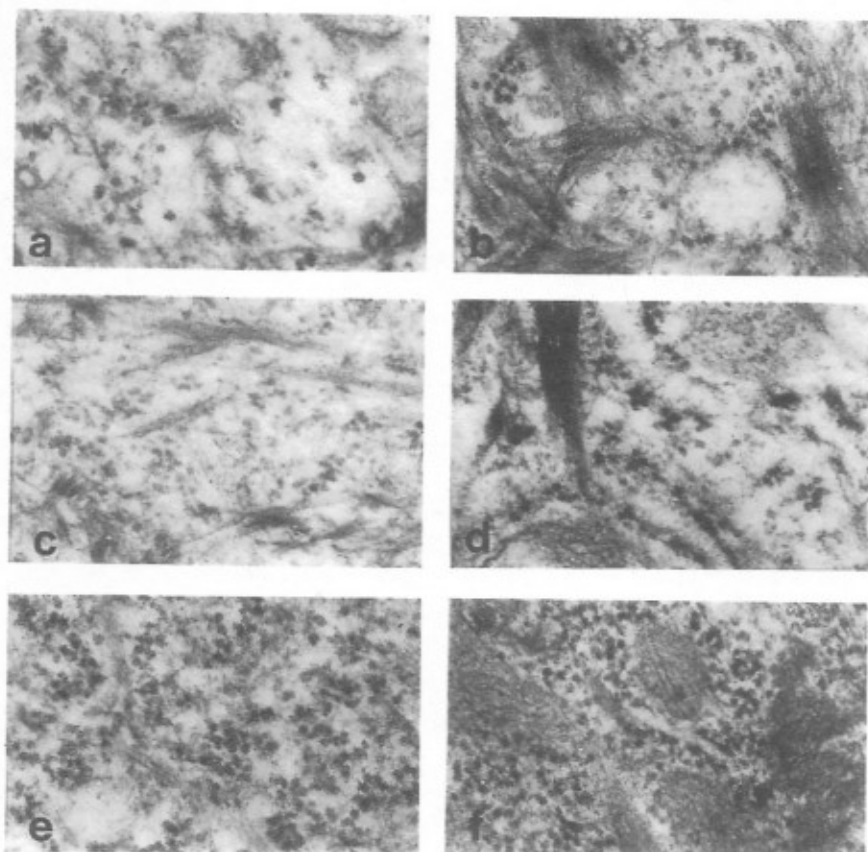
Figure 4. Electron micrographs showing glycogen granules in (a) involved and (b) uninvolved skin of active/untreated psoriasis, (c) involved and (d) uninvolved skin of resolving/treated psoriasis, and (e) involved and (f) uninvolved skin of non-psoriatic controls. Note the sparse granules in active psoriatic disease (a, b), with intracellular oedema caused by increased osmotically active glucose particles from glycogenolysis, as a result of increased phosphorylase kinase activity; numerous glycogen granules in non-psoriatic controls (e, f), and intermediate changes in resolving/treated psoriasis (c, d). Note also the presence of well-developed tonofilaments in uninvolved skin (b, d, f) compared with sparse tonofilaments in involved skin (a, c, e) (× 50,000).
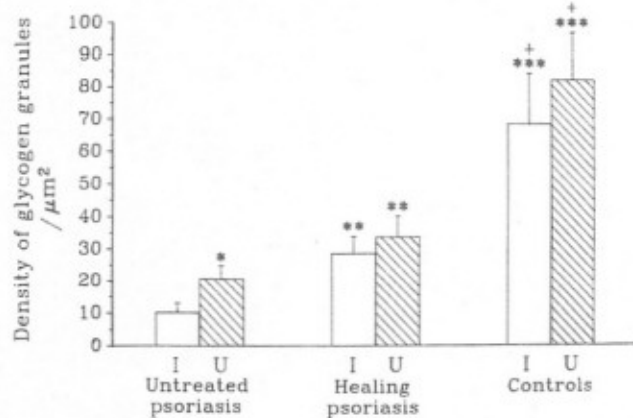

Figure 5. Density of glycogen granules in number/μm^2 in keratinocytes from involved/untreated and uninvolved/untreated psoriasis, involved/treated and uninvolved/treated psoriasis, and involved and uninvolved non-psoriatic controls. *$P<0.05$ compared with involved untreated psoriasis; **$P<0.01$ compared with involved untreated psoriasis; ***$P<0.001$ compared with involved untreated psoriasis; +$P<0.005$ compared with involved and uninvolved treated psoriasis. I, involved; U, uninvolved.

Inorganic phosphate concentrations in psoriatic patients and controls

The results are shown in Figure 3.

The levels of inorganic phosphate ($\mu mol/gm$ protein) in the epidermis of the three groups of patients were as follows: untreated psoriatic patients – involved epidermis $178\cdot3\pm84\cdot23$, uninvolved epidermis $139\cdot44\pm50\cdot98$; treated psoriatic patients – involved epidermis $96\cdot69\pm36\cdot24$, uninvolved epidermis $103\cdot63\pm79\cdot91$; controls – involved epidermis $125\cdot03\pm74\cdot95$, uninvolved epidermis $121\cdot43\pm79\cdot91$. The levels in the involved and uninvolved skin in all three groups were statistically similar.

Density of glycogen granules in involved and uninvolved keratinocytes of psoriatic patients and controls

Electron microscopic examination of the skin specimens showed a paucity of glycogen granules in both involved (Fig. 4a) and uninvolved (Fig. 4b) skin of untreated psoriasis, intermediate values in involved (Fig. 4c) and uninvolved (Fig. 4d) epidermis of treated psoriasis, and

abundant glycogen granules both in involved (Fig. 4e) and uninvolved (Fig. 4f) epidermis of non-psoriatic controls.

The density of glycogen granules, measured as the number of granules/μm^2, were as follows (Fig. 5): untreated psoriasis – involved skin $10 \cdot 1 \pm 2 \cdot 8$, uninvolved skin $20 \cdot 5 \pm 4 \cdot 3$; treated psoriasis – involved skin $28 \cdot 5 \pm 5 \cdot 2$, uninvolved skin $33 \cdot 6 \pm 6 \cdot 5$; controls – involved skin $60 \cdot 3 \pm 15 \cdot 7$, uninvolved skin $82 \cdot 0 \pm 14 \cdot 8$. The glycogen density in the involved skin of untreated psoriasis was significantly lower than the uninvolved skin of the same group of patients ($P < 0 \cdot 05$), and both involved and uninvolved skin of patients with treated psoriasis ($P < 0 \cdot 01$) and controls ($P < 0 \cdot 001$). In the treated and control groups, there was no significant difference between the density of glycogen granules in involved and uninvolved skin biopsies. However, the density in the treated psoriatics was significantly lower than the controls ($P < 0 \cdot 01$).

Discussion

Calcium binding by calmodulin, an intracellular calcium binding protein discovered by Cheung,[23] is the commonest translator of the intracellular message following the generation of the calcium signal.[39] Calmodulin-containing enzymes catalyse the formation or breaking of bonds between phosphorus and other molecules.[24–27,39,40] Calmodulin levels have been reported to be elevated in psoriatic epidermis.[21] PK is a calmodulin-containing enzyme which plays a crucial role in glycogenolysis. PK catalyses the conversion of phosphorylase-b to phosphorylase-a,[24–27,39,40] which in turn is the mechanism of activation of several phosphorylases, including glycogen phosphorylase, the enzyme responsible for the breakdown of glycogen into glucose during glycogenolysis. Because the end-product of glycogenolysis is ATP, which is required by many cellular protein kinases for phosphorylation of their target proteins, PK is critically important in many cellular functions. Its abundant presence in muscles, where ATP is the essential fuel for the high energy requirements of the organ, has long been known.

The presence of PK in the skin, however, has not been reported previously. In the skin, the breakdown of glycogen stores in the keratinocytes provides the energy for the calcium-dependent reactions involved in terminal differentiation of keratinocytes,[28,29] including reactions such as the conversion of profilaggrin to filaggrin.[30] The process of keratinocyte terminal differentiation, as indicated by the expression of L-fucose lectin binding

sites on keratinocytes, has been shown to be greatly enhanced in active psoriatic epidermis.[31] In addition, the expression of the L-fucose moiety has been shown not only to be associated with, but also to precede marked epidermal proliferation in psoriatic plaques.[31] Increased PK levels in active psoriatic epidermis increase the availability of ATP for phosphorylation reactions such as those involved in the enhancement of calcium-dependent processes in keratinocyte terminal differentiation.

Our data in patients with active psoriasis show that involved epidermis contains significantly elevated PK activity (Fig. 1). In addition, both involved and uninvolved epidermis from active psoriatic disease contain significantly higher PK activities than involved and uninvolved skin from treated/healing psoriasis and non-psoriatic controls (Fig. 1), suggesting that enhanced PK activity parallels clinical psoriatic activity. The elevated PK levels in uninvolved active psoriatic skin may explain the tendency for the uninvolved skin in active psoriatic disease to develop psoriasis. The much lower enzyme level in involved epidermis of the resolving psoriatic plaques in patients treated with diltiazem, a calcium-channel blocker, suggests not only that decreased PK activity may be a prerequisite for the induction of the healing phase of the disease, but that both PK activity and psoriatic activity appear to be calcium-dependent. It must be pointed out that although a combination of treatment modalities may have been responsible for induction of the healing phase, the only treatment common to all patients in the healing/treated group was diltiazem. In a previous study,[12] we found that diltiazem was effective in preventing the development of trauma-induced psoriasis.

Glycogen granules were decreased in density in active/untreated psoriatic keratinocytes, with involved skin containing fewer granules than uninvolved skin. In addition, there was a paucity of glycogen granules on electron microscopy in the keratinocytes of both involved (Fig. 4a) and uninvolved untreated (Fig. 4b) psoriatic patients, compared with similar biopsies of control patients (Fig. 4e and f). The density of glycogen granules is inversely related to glycogenolytic activity, so that increased glycogenolysis results in a lower density of glycogen granules. Accordingly, these findings are consistent with increased glycogenolysis in both involved and uninvolved active psoriatic skin, but not in involved and uninvolved non-psoriatic biopsies. Thus, elevated PK activity correlates with increased glycogenolysis in active psoriatic epidermis, with involved epidermis showing a greater degree of glycogenolytic activity than uninvolved psoriatic epidermis. This find-

ing is expected, because PK activates glycogen phosphorylase, which in turn breaks down glycogen to glucose-1-phosphate. In resolving psoriasis, the decrease in PK activity is associated with the cessation of enhanced glycogenolysis, which is reflected in the accumulation of glycogen granules within the keratinocytes.

Differences in cytosolic (epidermal cells) organic phosphorus concentrations reflect intracellular phosphorylation reactions. In this study, organic phosphorus in the involved skin of active psoriatics was significantly elevated, whereas levels of inorganic phosphates were similar in all groups (Fig. 3). Thus, the increased concentrations of organic phosphorus parallel the increased activity of PK in involved, untreated psoriatic epidermis, suggesting that increased PK activity is required for the enhanced phosphorylation reactions in involved epidermis of active, untreated psoriasis. Because many protein kinases responsible for increased protein phosphorylation utilize ATP from glycogenolysis, increased activity of PK in psoriasis may result in the enhancement of many of the epidermal processes which are dependent on protein phosphorylation. For example, protein phosphorylation is a necessary event following receptor binding. In particular, phosphorylation of epidermal growth factor-receptor (EGF-R) after binding by its ligand epidermal growth factor, or its natural ligand transforming growth factor-α (TGF-α), is associated with epidermal growth and proliferation. Phosphorylation of EGF-R has been shown to be calcium-dependent, and occurs at threonine and serine residues.[41] It is significant that PK has also been shown to phosphorylate serine-14 of glycogen phosphorylase.[42] In psoriasis, phosphorylation of the receptor proteins follows the binding of TGF-α, a cytokine secreted by activated macrophages, to its ligand EGF-R, the expression of which has also been found to be calcium-dependent.[37] It is significant that enhanced expression of both TGF-α[35] and EGF-R[36] has been observed in psoriatic epidermis. We propose that PK enhances calcium-dependent phosphorylation reactions. Support for this belief lies in the fact that we were able to reduce both PK levels and organic phosphorus levels in psoriatic epidermis after treatment with calcium channel blockers.

Phosphorylase kinase is a calmodulin-containing enzyme which is sensitive to the calcium signal.[24-27] This enzyme has the subunit structure of $(\alpha\beta\gamma\delta)$,[4] where α and β are the subunits which are phosphorylated. The δ subunit is calmodulin, which binds calcium ions, and then triggers the activity of PK. Another molecule of calmodulin also binds to the apoenzyme when the molecule is activated. In this way, the activity of PK is affected both by calcium ions and by calmodulin levels. The activity of PK is also regulated by two cAMP-dependent protein kinases—one which phosphorylates the β subunit at low intracellular cAMP concentrations, thus activating the enzyme, and the other which phosphorylates the α subunit at high intracellular cAMP concentrations, thus deactivating the enzyme. The dependence of PK activity on cAMP levels may explain why drugs which decrease cAMP, such as β-adrenoceptor blocking agents[43] and lithium,[44] have been reported to aggravate psoriasis.

The fact that uninvolved skin in untreated psoriatic epidermis did not show a similar increase in organic phosphorus concentrations, despite moderately increased activity of PK, suggests that elevations of PK which occur without enhanced levels of phosphorylation are not associated with clinical psoriasis. Alternatively, it is possible that other factors, in addition to increased PK activity, must be present before enhanced phosphorylation reactions occur in uninvolved skin of untreated psoriasis. We surmise that these factors, among others, include trauma (Koebner phenomenon),[12] or the presence of the allergen or cognate antigen,[7] which must precede activation of the T-cell receptor. T-lymphocyte proliferation is intimately involved in the psoriatic process.[12-18] The essential reaction following binding of the cognate antigen[12] to the T-cell receptor is signal transduction, initiated by calcium influx and followed by protein phosphorylation. It must be pointed out that although the cytosolic extract consisted predominantly of epidermal cells, lymphocytes trapped between keratinocytes were also assayed. T-cell activation, with resultant macrophage activation, appears to be a necessary accompaniment to the psoriatic process, as TGF-α, produced by activated macrophages, is the natural ligand for EGF-R.[32] We believe that these additional factors may play a role in induction of the proliferative component of the psoriatic process.

In conclusion, our study demonstrates the presence of increased epidermal glycogenolysis and increased phosphorylation reactions associated with enhanced levels of PK, a calmodulin-containing enzyme. Our data also show that a fall in PK levels may be important in initiating the healing phase of the disease. An elevation in PK activity in uninvolved skin of patients with active psoriasis could be an important factor in predisposing these patients to eruptive lesions. Increased enzyme activity in both involved and uninvolved skin of active/untreated psoriasis suggests that increased PK levels may be pathogenetically significant in the disease. Our

study using proliferative controls suggests that PK elevations are not a necessary phenomenon in non-psoriatic proliferative processes. It is unclear at this time, however, whether the elevated PK levels in psoriasis are a result of increased gene expression or defective homeostatic regulation due to a structural defect in the enzyme.

References

1 Kruegar GG. Psoriasis: current concepts of its etiology and pathogenesis. In: *Year Book of Dermatology* (Dobson RL, Thiers BH. eds). Chicago: Year Book Medical Publishers, 1981; 13–70.

2 Van Scott EJ, Ekel TM. Kinetics of hyperplasia in psoriasis. *Arch Dermatol* 1963; 88: 373–81.

3 Weinstein GD, McCullough JL. Cytokinetics in diseases of epidermal hyperplasia. *Annu Rev Med* 1973; 24: 345–52.

4 Gommans JM, Bergers M, van Erp PE. Studies on the plasma membrane of normal and psoriatic keratinocytes. I. Preparation of material and morphological characterization. *Br J Dermatol* 1979; 101: 407–12.

5 Marcello CL, Voorhees JJ. Cyclic nucleotides, prostaglandins and polyamines in psoriasis. *Pharmacol Ther* 1980; 9: 297–310.

6 Hammerström S, Hamberg M, Samuelsson B et al. Increased concentrations of nonesterified arachidonic acid, 12L-hydroxy-5,8,10,14-eicosatetraenoic acid, prostaglandin E2, and prostaglandin F2 alpha in epidermis of psoriasis. *Proc Natl Acad Sci USA* 1975; 72: 5130–4.

7 Heng MCY, Kloss SG, Kuehn CS, Chase DG. Significance and pathogenesis of basal keratinocyte herniations in psoriasis. *J Invest Dermatol* 1986; 87: 362–6.

8 Heng MCY, Moy RL, Lieberman J. α_1-antitrypsin deficiency in severe psoriasis. *Br J Dermatol* 1985; 112: 129–33.

9 Heng MCY, Kloss SG. Electron microscopic features in psoriatic patients with α_1-antitrypsin deficiency. *J Invest Dermatol* 1986; 87: 59–64.

10 Stuttgen G, Hoffman G, Simich N. Die proteolyse normaler und pathologische veranderter Haut durch Endopeptidasen. *Arch Klin Exp Derm* 1959; 205: 381–8.

11 Fräki JE, Hopsu-Havu VK. Human skin proteases. Fractionation of psoriasis scale proteases and separation of plasminogen activator and a histone hydrolyzing protease. *Arch Dermatol Res* 1976; 256: 113–26.

12 Heng MCY, Allen SG, Haberfelde G, Song MK. Electron microscopic and immunocytochemical study of the sequence of events in psoriatic plaque formation after tape-stripping. *Br J Dermatol* 1991; 125: 548–56.

13 Heng MCY, Allen SG, Chase DG. High endothelial venules in involved and uninvolved psoriatic skin: recognition by homing receptors on cytotoxic T lymphocytes. *Br J Dermatol* 1988; 118: 315–26.

14 Heng MCY, Kloss SG, Kuehn CS, Chase DG. The sequence of events in psoriatic plaque formation after tape-stripping. *Br J Dermatol* 1985; 112: 517–32.

15 Bos JD, Hulsebosch HJ, Krieg SR et al. Immunocompetent cells in psoriasis. In situ immunophenotyping by monoclonal antibodies. *Arch Dermatol Res* 1983; 275: 181–9.

16 Hammar H, Gu S-Q, Johannesson A et al. Subpopulations of mononuclear cells in microscopic lesions of psoriatic patients. Selective accumulation of suppressor/cytotoxic T cells in epidermis during the evolution of the lesion. *J Invest Dermatol* 1984; 83: 416–20.

17 Bjierke JR, Krogh H-K, Matre R. Characterization of mononuclear cell infiltrates in psoriatic lesions. *J Invest Dermatol* 1978; 71: 340–3.

18 Baker BS, Swain AF, Fry L, Valdimarsson H. Epidermal T lymphocytes and HLA-DR expression in psoriasis. *Br J Dermatol* 1984; 110: 555–64.

19 Barker JNWN, Karabin GD, Stoof TJ et al. Detection of interferon-γ mRNA in psoriatic epidermis by polymerase chain reaction. *J Dermatol Sci* 1991; 2: 106–11.

20 Nickoloff BJ, Karabin GD, Barker JNWN et al. Cellular localization of IL-8 and its inducer TNF-α in psoriasis. *Am J Pathol* 1991; 138: 129–40.

21 Tucker WFG, Mac Neil S, Bleehen SS, Tomlinson S. Biologically active calmodulin levels are elevated in both involved and uninvolved epidermis in psoriasis. *J Invest Dermatol* 1984; 82: 298–9.

22 Forster S, Ilderton E, Norris JFB et al. Characterization and activity of phospholipase A_2 in normal epidermis and in lesion-free epidermis of patients with psoriasis or eczema. *Br J Dermatol* 1985; 112: 135–47.

23 Cheung WY. Calmodulin. *Sci Am* 1985; 246: 48–56.

24 Cohen P. The role of cAMP-dependent protein kinase in the regulation of glycogen metabolism in mammalian skeletal muscle. *Curr Top Cell Regul* 1978; 14: 117–96.

25 Krebs EG. Phosphorylation—dephosphorylation of enzymes. *Annu Rev Biochem* 1979; 48: 923–59.

26 Cohen P. The role of protein phosphorylation in neural and hormonal control of cellular activity. *Nature* 1982; 296: 613–20.

27 Cohen P. The role of protein phosphorylation in the neural and hormonal control of intermediary metabolism. In: *Cellular Controls in Differentiation* (Lloyd CW, Rees DA, eds). London: Academic Press, 1981; 81–105.

28 Hennings H, Michael D, Cheng C et al. Calcium regulation of growth and differentiation of mouse epidermal cells in culture. *Cell* 1980; 19: 245–54.

29 Menon GK, Elias PM. Ultrastructural localization of calcium in psoriatic and normal human epidermis. *Arch Dermatol* 1991; 127: 57–63.

30 Dale BA, Haugen Scolfield JA, Hennings H et al. Identification of filaggrin in cultured mouse keratinocytes and its regulation by calcium. *J Invest Dermatol* 1983; 81 (Suppl.): 90s–95s.

31 Heng MCY, Heng SY, Allen SG. Expression of the L-fucose moiety on epidermal keratinocytes in psoriasis induced by the Koebner phenomenon: a sequential study. *Br J Dermatol* 1992; 126: 575–81.

32 Wong ST, Winchell LF, McCune BK et al. The TGF-α precursor expressed on the cell surface binds to the EGF-receptor on adjacent cells, leading to signal transduction. *Cell* 1989; 56: 495–506.

33 Marquardt H, Hunkapiller MW, Hood LE et al. Transforming growth factors produced by retrovirus-transformed rodent fibroblasts and human melanoma cells: amino acid sequence homology with epidermal growth factor. *Proc Natl Acad Sci USA* 1983; 80: 4684–8.

34 Coffey RJ Jr, Derynck R, Wilcox JN et al. Production and auto-induction of transforming growth factor-α in human keratinocytes. *Nature* 1987; 328: 817–20.

35 Elder JT, Fischer GJ, Lindquist PB et al. Overexpression of TGF-α in psoriatic epidermis. *Science* 1989; 243: 811–14.

36 Nanney LB, Stoschek CM, Magid M, King LE Jr. Altered [^{125}I] epidermal growth factor binding and receptor distribution in psoriasis. *J Invest Dermatol* 1986; 86: 260–6.

37 O'Keefe EJ. Payne RE Jr. Modulation of the epidermal growth factor receptor of human keratinocytes by calcium ion. *J Invest Dermatol* 1983; **81**: 231–5.

38 Cohen P. Phosphorylase kinase from rabbit skeletal muscle. *Methods Enzymol* 1983; **99**: 243–50.

39 Means AR. Dedman JR. Calmodulin—an intracellular calcium receptor. *Nature* 1980; **285**: 73–7.

40 Klee CB, Crouch TH, Richman PG. Calmodulin. *Annu Rev Biochem* 1980; **49**: 489–515.

41 Cochet C. Gill GN. Meisenhelder J *et al*. C-kinase phosphorylates the epidermal growth factor receptor and reduces its epidermal growth factor-stimulated tyrosine protein kinase activity. *J Biol Chem* 1984; **259**: 2553–8.

42 Krebs EG. Phosphorylation and dephosphorylation of glycogen phosphorylase: a prototype for reversible covalent enzyme modification. *Curr Top Cell Regul* 1981; **18**: 401–19.

43 Heng MCY. Heng MK. Beta-adrenoceptor antagonist-induced psoriasiform eruption. *Int J Dermatol* 1988; **27**: 617–27.

44 Lowe NJ. Ridgeway HB. Generalized pustular psoriasis precipitated by lithium carbonate. *Arch Dermatol* 1978; **114**: 1788–9.