

# A Highly Decreased Binding of Cyclic Adenosine Monophosphate to Protein Kinase A in Erythrocyte Membranes is Specific for Active Psoriasis

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A cyclic adenosine monophosphate binding abnormality in psoriatic erythrocytes that could be corrected by retinoid treatment has been reported. It was tested whether this binding abnormality is specific for psoriasis and the effects of treatment were compared with etretinate, cyclosporine A, or anthralin on 2-<sup>3</sup>H-8-N<sub>3</sub>-cyclic adenosine monophosphate binding to the regulatory subunit of protein kinase A in erythrocyte membranes. One hundred and fifteen individuals were evaluated, including: (i) 34 healthy persons; (ii) 15 patients with nonatopic inflammatory skin diseases (eczema, erythroderma, tinea, Grover's disease, erysipelas, urticaria); (iii) eight with other dermatoses mediated by immune mechanisms (systemic lupus erythematosus, lichen planus, necrotizing vasculitis, erythema nodosum, systemic sclerosis); (iv) 14 with generalized atopic dermatitis; and (v) 44 with psoriasis vulgaris clinically assessed by Psoriasis Area and Severity Index. In psoriasis, the course of the binding of 2-<sup>3</sup>H-8-N<sub>3</sub>-cyclic adenosine monophosphate to erythrocytes was measured

in nine patients during a 10 wk treatment with etretinate, in 21 patients during a 10 wk treatment with cyclosporine A, and one patient under topical treatment with anthralin for 4 wk. We found the following femtomolar binding per mg protein: (i) healthy persons (1064 ± 124, mean ± SD); (ii) nonatopic inflammatory skin diseases (995 ± 103); (iii) immune dermatoses (961 ± 92); (iv) atopic dermatitis (960 ± 110); and (v) psoriasis (645 ± 159; p < 0.0001 compared with nonpsoriatics, Mann-Whitney U test). Treatment of psoriasis with etretinate, cyclosporine A, or anthralin normalized the binding of cyclic adenosine monophosphate, which was inversely correlated to the Psoriasis Area and Severity Index score. It was concluded that the decreased binding of cyclic adenosine monophosphate to protein kinase A in erythrocytes is specific for psoriasis and normalizes after successful treatment. **Key words:** anthralin/cyclosporine A/dermatitis/etretinate/Psoriasis Area and Severity Index. *J Invest Dermatol* 119:160-165, 2002

In psoriasis, abnormalities of the cyclic adenosine monophosphate (cAMP) system of metabolic regulation have been known for 30 y (Voorhees and Duell, 1971). In particular, a significant decrease in cAMP was found in involved psoriatic epidermis when compared with uninvolved and control epidermis (Voorhees *et al*, 1972a, b). In many cell types, cAMP activates protein kinase A (PKA), which in turn phosphorylates various substrate proteins. Because cAMP regulates glycogen metabolism and promotes cell differentiation while limiting proliferation, it was thought that the cAMP system might play a part in the pathogenesis of psoriasis (Voorhees *et al*, 1972a, b). Later on it was reported that cAMP was not decreased in psoriatic epidermis compared with normal epidermis (Adachi *et al*, 1980). Moreover, a decline in the response of cAMP to β-adrenergic stimulation has been established (Yoshikawa *et al*, 1975; Eedy *et al*, 1990); however, no difference in PKA activity between psoriatic

epidermis and normal epidermis has been found (Nemoto *et al*, 1983). Clinically, it has been well known that β-adrenergic antagonists as well as lithium salts or antimalarials, i.e., drugs that reduce the intracellular accumulation of cAMP (Voorhees *et al*, 1975) are able to exacerbate psoriasis (summarized in Greaves and Weinstein, 1995). Attempts to exploit therapeutically an altered cAMP metabolism in psoriasis, however, have not proven fruitful. Taken together, the pathophysiologic meaning of cAMP and PKA in psoriasis remains unclear.

Two isoenzymes of PKA, PK1 and PK2, have been isolated. PK1 and PK2 are inactive tetramers consisting of two identical catalytic subunits and two regulatory subunits, RI and RII having molecular weights of 47 and 54 kDa, respectively; in the absence of cAMP binding to RI or RII, the substrate binding sites on the catalytic subunits are blocked. Recent studies have indicated that the levels of RI and RII are decreased in the cytosol of psoriatic fibroblasts and that RI in erythrocyte membranes from psoriatic patients is lower than from normal subjects correlating with the severity of the cutaneous manifestations (Brion *et al*, 1986). In erythrocytes, PK1 is the only cAMP-dependent protein kinase (Dreyfuss *et al*, 1978). A cAMP binding abnormality to the RI regulatory subunit has been found in psoriatic erythrocytes; the ability of RI to bind the cAMP analog 8-azido-<sup>32</sup>P-cAMP in erythrocyte membranes has been found to be lower in psoriasis

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Abbreviations: PASI, Psoriasis Area and Severity Index; PKA, protein kinase A; PK1, protein kinase 1; PK2, protein kinase 2; RI, regulatory subunit I of protein kinase A.

compared with control individuals, including 12 patients with other forms of dermatitis (Raynaud *et al*, 1989). Furthermore, a negative correlation between disease severity and the cAMP analog-binding defect has been found that could be corrected after long-term treatment with oral retinoid (Raynaud *et al*, 1989). Oral administration of acitretin induced a rapid increase in the ability of RI regulatory subunit of erythrocytes to bind cAMP (Raynaud *et al*, 1993). *In vitro* exposure of erythrocytes from psoriatic patients to retinoid also promoted an increase in binding of cAMP to RI (Raynaud *et al*, 1993). In psoriatic fibroblasts, a direct interaction of retinoic acid with RI and RII subunits of PKA has been shown (Tourmier *et al*, 1996). The effects of other forms of treatment have not been examined; therefore, it was unclear whether retinoid treatment was specific for correcting the cAMP binding abnormality.

In order to test whether a diminished binding of cAMP to the regulatory subunit RI of PKA in red blood cells is specific for psoriasis, we measured the binding of the cAMP analog 2-<sup>3</sup>H-8-N<sub>3</sub>-cAMP (azido-cAMP) to the RI subunit of PKA in erythrocyte membranes in a large number of patients with psoriasis, compared with healthy persons, and patients with atopic dermatitis and other forms of dermatitis. Moreover, we compared the course of the azido-cAMP binding the RI subunit of PKA in red blood cells during systemic treatment of psoriasis with etretinate, the immunosuppressant cyclosporine A, or topical treatment with anthralin. This study's findings indicate that a decreased binding of azido-cAMP to erythrocyte membranes is specific for patients with psoriatic skin lesions. Moreover, after successful treatment with different modalities, the lower binding of cAMP to RI of PKA normalizes.

## MATERIALS AND METHODS

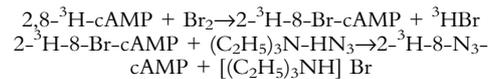
**Subject** The following subjects were studied: (i) 34 healthy individuals; (ii) 15 patients with nonatopic inflammatory skin diseases, including generalized eczema, nonpsoriatic erythroderma, tinea corporis, Grover's disease, erysipelas (bacterial cellulitis), and urticaria; (iii) eight with other dermatoses mediated by immune mechanisms termed "immune dermatoses" (including lupus erythematosus, generalized lichen planus, necrotizing vasculitis, erythema nodosum, systemic sclerosis); (iv) 14 with generalized atopic dermatitis; and (v) 44 with psoriasis vulgaris. None of the patients had received any systemic retinoids, corticosteroids, or other immunosuppressants 4 wk before, or topical treatment other than skin emollients 1 wk before examination. All individuals (115) were in the age range of 18–75 y; the sex distribution was approximately equal between males and females.

Nine patients with psoriasis were treated for 10 wk with a daily dose of 50 mg etretinate, and 21 patients with 2.5–5.0 mg cyclosporine A per kg body weight daily (Sandimmun, Novartis Pharma, Nuremberg, Germany). These patients were enrolled in a treatment study, the details of which have been published before (Schopf *et al*, 1992). The Institutional Review Board approved this study. One psoriatic patient was treated topically for 4 wk with anthralin-petrolatum compounds in increasing anthralin concentrations starting with 1/16% anthralin; the anthralin concentrations were doubled every 5–7 d up to 1%. The severity of psoriasis was determined by the Psoriasis Area and Severity Index (PASI) (Fredricksson and Petterson, 1978).

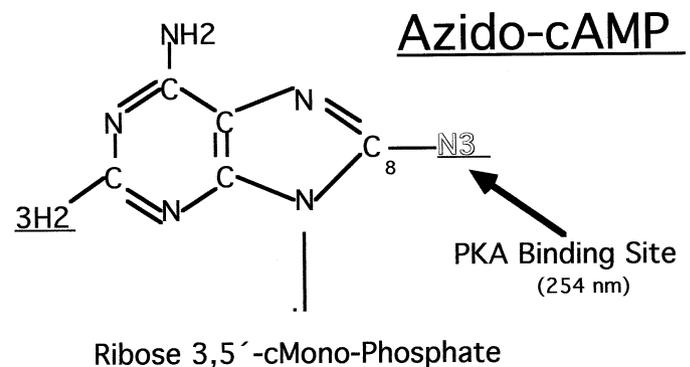
**Isolation of erythrocyte membranes** Erythrocyte membranes were prepared according to the method described by Raynaud *et al* (1989). In brief, 10 ml heparinized blood was collected and immediately spun at 1000 × g for 30 min at 4°C. The erythrocyte pellet was suspended in 10 ml 0.172 mol per liter Tris-HCl (pH 7.6) and washed three times with this buffer. The washed erythrocytes were then lysed by suspension in six volumes of hypotonic 0.011 mmol per liter Tris-HCl (pH 7.6) and the membranes were isolated by centrifugation at 2000 × g for 40 min. The membrane pellet was washed three times with 0.011 mol per liter Tris-HCl (pH 7.6) and stored frozen at –80°C until assay. Chemicals used were purchased from Sigma (St Louis, MO) unless otherwise stated.

In patients under treatment with etretinate and cyclosporine A, blood was drawn at weeks 0, 1, 3, 6, and 10 of treatment. Blood from the psoriatic patient under topical treatment with anthralin was drawn at weeks 0, 1, 2, 3, and 4.

**Synthesis of 2-<sup>3</sup>H-8-N<sub>3</sub>-cAMP (azido-cAMP)** The method according to Walter *et al* (1977) was employed. The reaction has the following course:



One millicurie of 2,8-<sup>3</sup>H-cAMP (specific activity 5 mCi per mmol, New England Nuclear, Dreieich, Germany) was reacted with bromine in 1 ml acetate buffer (0.25 mol per liter, pH 3.9). The reaction was started at 20°C by the addition of 12 mmol bromine (Merck, Darmstadt, Germany). After 15 min the reaction mixture was concentrated to 0.3 ml by the addition of nitrogen gas. It was then separated by thin layer chromatography on silica (GF 254 Merck) in the system 1-butanol/acetic acid/H<sub>2</sub>O (ratio: 5:2:3 vol/vol/vol) employing a nonradioactive 8-Br-cAMP standard (Sigma). Tritiated 8-Br-cAMP was eluted with ethanol/H<sub>2</sub>O (Fluka, Buchs, Switzerland) 1:1 vol/vol and was dried by nitrogen gas in a water bath. The residue was solubilized by diethyl ammonium azide (5 mmol, Merck) in 3 ml dimethyl formamide (Merck) and heated for 10 h at 75°C. The gemisch was concentrated to 1 ml at 37°C in a vacuum rotation vaporizer under the introduction of nitrogen gas. This gemisch was loaded to a column of DEAE-cellulose in HCO<sub>3</sub> form and was eluted with 30 ml triethylammonium bicarbonate in a linear gradient of 0–0.2 mol per liter. A peak of radioactivity only resulted for azido-cAMP. The radioactive-labeled elution fractions next were pooled and vaporized in a vacuum in order to dry, and dissolved in 0.5 ml ethanol and stored in the dark at 4°C. The formula of azido-cAMP is shown here:

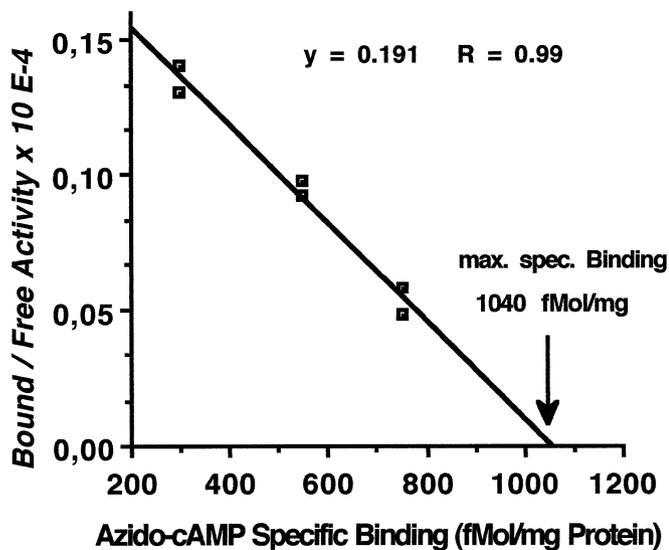


**Binding of azido-cAMP to the subunit RI of PKA of erythrocyte membranes (Walter *et al*, 1977)** The reaction mixture of 1 ml consisted of 2-N-morpholino-ethanol-sulfonate (10 mmol per liter, pH 6.2), magnesium chloride (10 mmol per liter), and cell membrane protein (1 mg). The total binding of azido-cAMP was shown by the three concentrations of 0.1, 0.5, and 2.0 mmol per liter. Duplicate samples were used. The specific binding was determined after saturation of nonspecific binding sites by the addition of cold N<sub>3</sub>-cAMP (2 mol per liter). The reaction solutions were next incubated for 1 h at 4°C in the dark. Next it was photoaffinity labeled at room temperature of 20°C for 20 min under ultraviolet light at 254 nm. The reaction was stopped by the addition of 0.2 ml sodium dodecyl sulfate (2.5%) and mercaptoethanol (5%). Finally, the gemisch was heated for 5 min at 100°C.

The actual experimental cpm [<sup>3</sup>H]8-azido cAMP used were in the order of 15,000 cpm; the radioactivity measuring binding with psoriatic and normal erythrocyte membranes after electrophoresis was 850–4000 cpm; background did not exceed 250 cpm.

**Protein determination** Protein concentration was determined spectrophotometrically employing the Bio-Rad Protein microassay (Bio-Rad, Munich, Germany) according to the manufacturer's instructions.

**Isolation and measurement of RI bound azido-cAMP** Next, the bound azido-cAMP was isolated and measured employing the Phast-System (Pharmacia LKB-Phast Transfer, Freiburg, Germany; Phast System Separation and Control Unit, Protein Blotting 220) on Phast Gel (8–25) and sodium dodecyl sulfate buffer strips. Proteins with the relative molecular mass of 47 kDa and 54 kDa (corresponding to the relative



**Figure 1.** Scatchard plot analysis of the specific binding of azido-cAMP to the regulatory subunit RI of PKA erythrocyte membranes. Dissociation constant  $K_D = (1040 \times 10^{-15} \text{ [mol per liter]} / 0.19 \times 10^{-4}) = 5.4 \times 10^{-8} \text{ [mol per liter]}$ .  $K_D$  values did not change with different specific binding of azido-cAMP to erythrocyte membranes during the course of treatment (data not shown).

molecular masses of subunits RI and RII of PKA) were used. The azido-cAMP binding subunit migrates in the 47 kDa fraction.

Five microliters of the above samples were pipetted to the gels and the electrophoresis was started at a temperature of 15°C. After 30 min, electrophoresis was terminated and protein blotting for 40 min employing the Phast-System on 20  $\mu\text{m}$  thick nitrocellulose membranes was done. The resulting bands on the nitrocellulose were stained with Phast-Green FCF 1804301 (Pharmacia) using 40 parts methanol/10 parts acetic acid/50 parts distilled water as well as 0.1% Phast-Green. Destaining of unbound staining was done with 25 parts methanol/10 parts acetic acid/65 parts distilled water and 0.2 M NaOH. Next, the bands migrating between the stained 47 kDa and 54 kDa on the nitrocellulose membrane were cut out and transferred into 5 ml scintillation fluid. Radioactivity of azido-cAMP was measured in a  $\gamma$ -counter (Beckman  $\beta$ -counter LS 6800).

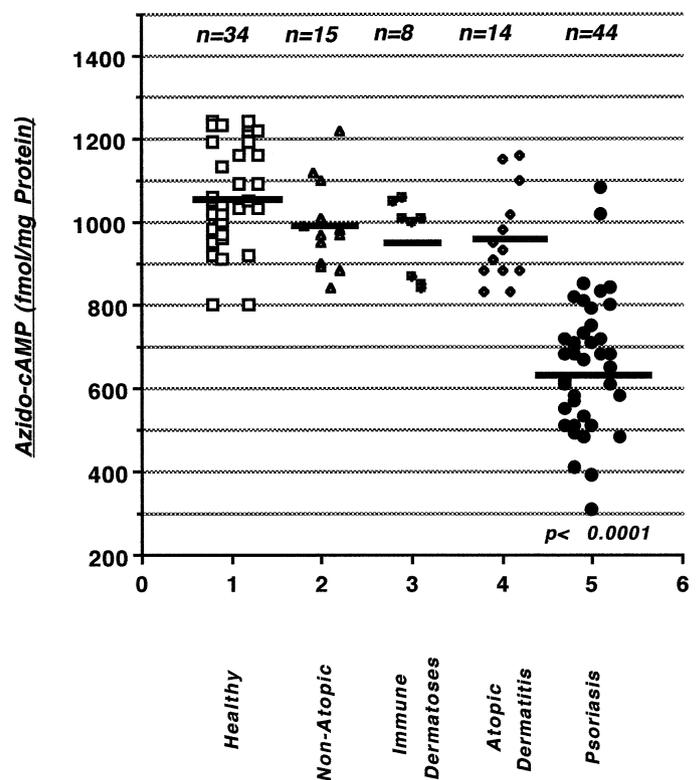
Calculation of the bound azido-cAMP was performed by Scatchard plot employing the  $2 \times 3$  samples. The ratio of the specifically bound and free (unbound) azido-cAMP on the vertical axis was plotted against the specifically bound azido-cAMP (fmol per mg protein) on the horizontal axis. The intersection of the resulting straight line with the horizontal axis indicates the maximal binding of azido-cAMP to the RI subunit of PKA in the erythrocyte membranes.

**Statistical evaluation** The findings were statistically compared by the Mann-Whitney U test employing the statistical program MultiStat 1.13 for Macintosh computers, Biosoft (Cambridge, U.K.).

## RESULTS

**Binding of azido-cAMP to regulatory subunit RI of PKA in erythrocyte membranes** We compared the binding of azido-cAMP in erythrocytes of 34 healthy individuals, 15 patients with nonatopic forms of dermatitis, eight "immune dermatoses", and 14 atopic dermatitis, with 44 patients with psoriatic lesions. A representative Scatchard plot analysis of the cAMP binding to red blood cells is shown in **Fig 1**. The values of the dissociation constant  $K_D$  were in the order of  $5.4 \times 10^{-8}$  [mol per liter] and remained constant in the many samples examined (data not shown).

**Figure 2** summarizes the results of the specific binding comparing patients with controls. Compared with all the control groups, we found a highly diminished binding of azido-cAMP to the regulatory subunit RI of protein kinase in psoriatic erythrocytes ( $p < 0.0001$ ). We concluded that a decreased binding of azido-



**Figure 2.** Specific binding of azido-cAMP to the regulatory subunit RI of PKA in erythrocyte membranes. Horizontal bars denote the means. The p-value compares the psoriatic patients with the different groups of control individuals each employing the Mann-Whitney U test.

cAMP to erythrocytes is specific for patients with psoriatic skin lesions.

**Effects of retinoid treatment on the binding of azido-cAMP** When the severity of psoriasis expressed as PASI was plotted against the binding of azido-cAMP to erythrocyte membranes, we found an inverse relationship between the two parameters as depicted in **Fig 3(i,e)** when psoriasis was more severe, there was less binding of azido-cAMP and vice versa.

Plotting both the PASI score and the binding of azido-cAMP over the time of treatment, X-like curves resulted as depicted in **Fig 4**.

From these results it was concluded that etretinate treatment of psoriasis normalized the decreased binding of cAMP to erythrocytes.

**Effects of cyclosporine A treatment on the binding of azido-cAMP** Similarly, when the binding of azido-cAMP was plotted against the PASI, there was an analogous inverse relationship as shown in **Fig 5**. Moreover, an X-like graph resulted, plotting both PASI score and binding of azido-cAMP over the week of treatment (**Fig 6**).

From these findings it was concluded that treatment with cyclosporine A also normalized the decreased binding of azido-cAMP to the RI regulatory subunit of PKA.

**Effects of topical treatment of psoriasis on the binding of azido-cAMP in erythrocytes** In the psoriatic patient topically treated with anthralin, we also found that the decreased binding of azido-cAMP to erythrocytes before treatment tended towards normal during the course of treatment as shown in **Fig 7**.

From these findings we concluded that both systemic treatment with two different agents as well as topical treatment with anthralin

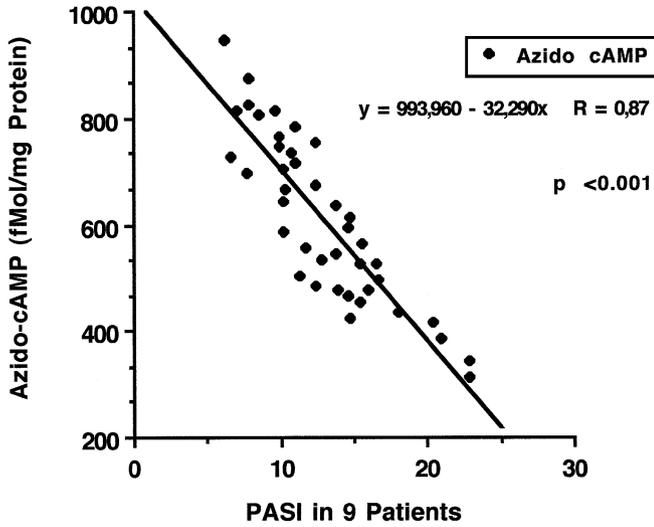


Figure 3. Binding of azido-cAMP to the regulatory subunit RI of PKA in erythrocyte membranes of psoriatic patients during the course of a 10 wk treatment with etretinate.

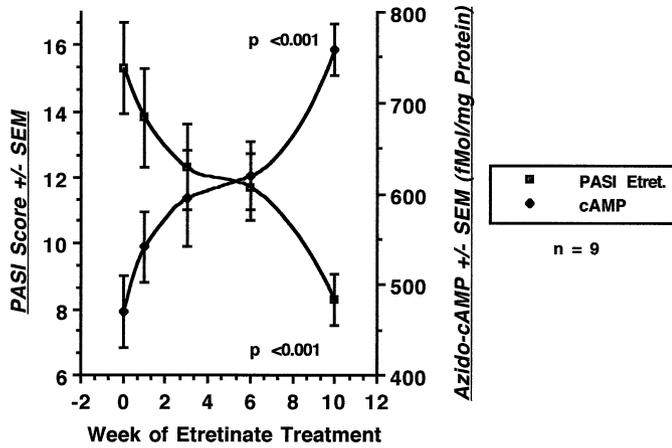


Figure 4. PASI compared with the binding of azido-cAMP to the regulatory subunit RI of PKA in erythrocytes during the course of a 10 wk treatment with etretinate.

normalize the diminished binding of azido-cAMP in erythrocyte membranes.

DISCUSSION

This study clearly indicates that the 44 patients with psoriasis have a markedly lower binding of azido-cAMP to the RI of PKA compared with the 71 controls, including 34 healthy individuals and 37 patients with other forms of dermatitis (including atopic dermatitis), immune dermatoses (such as discoid lupus erythematosus, necrotizing allergic vasculitis, erythema nodosum, systemic sclerosis), and nonatopic inflammatory skin diseases (including generalized eczema, stasis eczema, nummular eczema, deep dermatophytosis, bacterial cellulitis, and urticaria). Although there were slight variations in the level of cAMP binding in the different forms of dermatitis and healthy controls, the psoriasis group clearly exhibited the lowest binding, i.e., approximately 1000 vs 660 fmol protein per mg, respectively, reaching the statistical significance level of  $p < 0.0001$ .

Moreover, we found a highly significant inverse relationship between disease severity and binding of azido-cAMP to the

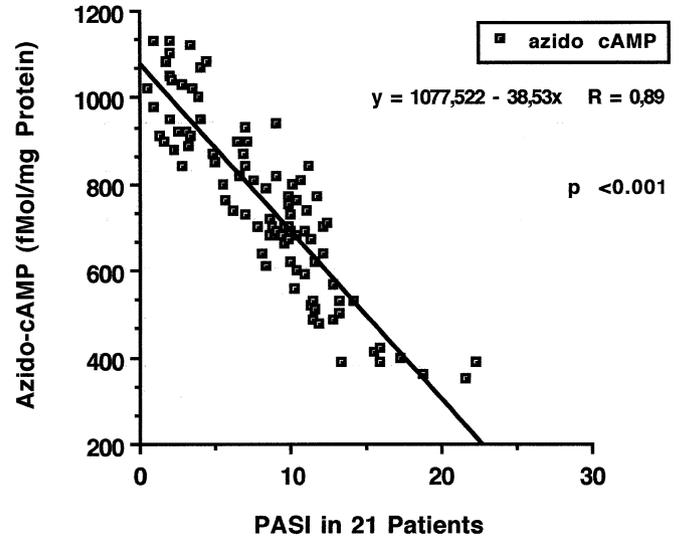


Figure 5. Binding of azido-cAMP to the regulatory subunit RI of PKA in erythrocyte membranes of psoriatic patients during the course of a 10 wk treatment with cyclosporine A (CyA).

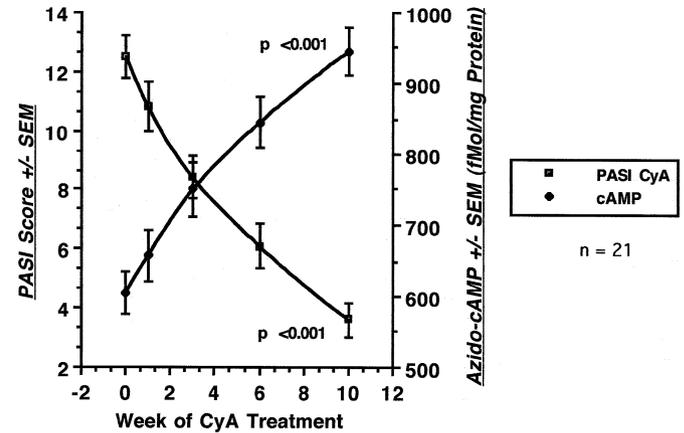
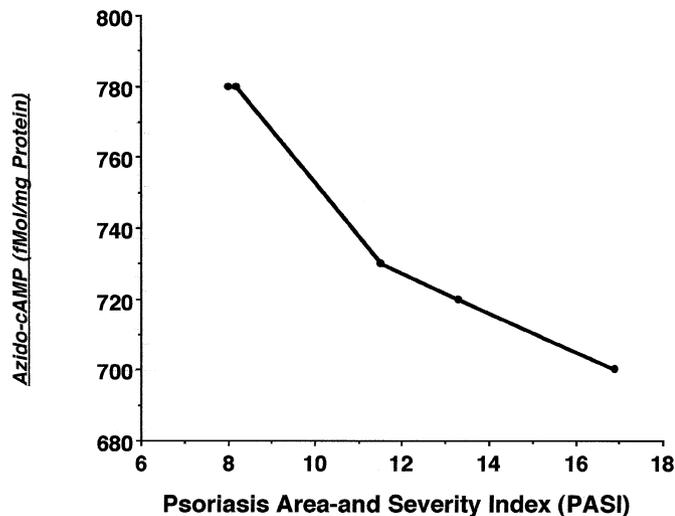


Figure 6. PASI compared with the binding of azido-cAMP to the regulatory subunit RI of PKA in erythrocytes during the course of a 10 wk treatment with cyclosporine A (CyA).

regulatory subunit RI of PKA in psoriasis. This finding offers the opportunity to determine the extent of psoriasis merely from a blood sample. These findings are consistent with previous results by Raynaud *et al* (1989) who had studied a smaller number of patients that had been treated with etretinate only. Our findings in patients who were treated systemically with cyclosporine A or topically with anthralin indicate that the normalization of cAMP binding in erythrocytes of psoriatic patients is not confined to retinoid treatment but rather represents a general phenomenon in these patients.

Interestingly, treatment of psoriatic patients with peptide T, an octapeptide sequence found in human immunodeficiency virus envelope glycoprotein gp120, has been observed to result in an improvement in the psoriatic condition; exposure of psoriatic fibroblasts to peptide T resulted in an increased binding of cAMP to the RI and RII regulatory subunits of PKA (Liapi *et al*, 1998). Erythrocytes have not been examined in this respect. Furthermore, the anti-inflammatory agent adenosine acting at its A(2A) receptor and the selective A(2A) receptor agonist CGS-21680 (known to suppress proinflammatory interleukin-12 and to increase anti-inflammatory interleukin-10 on the monocytic cell line THP-1),



**Figure 7.** Binding of azido-cAMP to the regulatory subunit RI of PKA in erythrocyte membranes during the course of a 4 wk topical treatment of a patient with anthralin-petrolatum.

have been found to stimulate an increase in intracellular cAMP in interleukin-1 and tumor necrosis factor- $\alpha$ -treated cells (Khoa *et al*, 2001). As there is increased tumor necrosis factor- $\alpha$  expression in psoriatic skin lesions (Nickoloff *et al*, 1991) and as the chimeric monoclonal antibody against tumor necrosis factor- $\alpha$ , infliximab, has been described to clear psoriatic skin lesions (Chaudhari *et al*, 2001; Schopf *et al*, in press), it seems likely that the therapeutic effect of this agent also occurs together with an increased binding of cAMP to RI of PKA.

Compared with radioactively labeling the phosphate residue on azido-cAMP with  $^{32}\text{P}$ , which could more easily be cleaved, our method of preparing tritiated azido-cAMP had the advantage of tagging the core of the nucleotide resulting in a very stable label. The fact that the  $K_D$  values of azido-cAMP binding remained unaltered after successful treatment or comparing psoriatic patients with controls indicates that no structural or functional changes of the regulatory unit RI of PKA had occurred in the erythrocyte membranes. Tournier *et al* (1995) who studied modulation of the cAMP-dependent regulatory subunit RI and RII of PKA in fibroblasts from patients with psoriasis reported that the decrease in the binding of azido-cAMP occurred with no change in the level of RII protein suggesting an altered post-transcriptional modification of the cAMP PKII in psoriatic fibroblasts, which could be reversed by exposure of the cells to retinoic acid. Western blot analysis of RI will further support the finding that decreased binding of cAMP to RI represents a quantitative decrease in RI and is not due to alterations in the cAMP binding affinity of RI. We used erythrocyte membranes for the cAMP binding measurement. It would be interesting to examine whether RI/cAMP binding in the cytosol of psoriatic red cells is also decreased. The amount of RI/cAMP binding in the cytosol of fibroblasts, however, was reported to be normal in five of seven psoriatic patients and decreased in two patients; in contrast, membrane-associated levels of RI were found decreased in five patients and normal in two psoriatic patients (Raynaud *et al*, 1987). This may indicate that it is less likely also to find decreased RI/cAMP binding in the cytosol of red blood cells.

Several membrane alterations have been reported in psoriasis. Morsches *et al* (1981) reported that patients with psoriasis exhibit a decreased permeability of erythrocyte membranes for dehydroepiandrosterone. Partsch *et al* (1979) found an increased purine salvage pathway in psoriatic erythrocytes. Semplicini *et al* (1988) reported altered red blood cell membrane permeability for sodium in psoriasis. Moreover, an altered erythrocyte membrane phosphorylation has been reported in psoriasis (Kumar *et al*, 1983);

compared with controls, membranes from psoriatic patients showed less  $^{32}\text{P}$  incorporation into cell membranes. This latter abnormality could well be related to the diminished binding of cAMP to the erythrocyte membranes. Moreover, a decreased ceramide generation in psoriatic cell membranes has been found (Alessandrini *et al*, 2001). This may indicate that the membrane abnormality in erythrocytes is part of a generalized phenomenon. These defects, however, are not permanent but normalize during the clearing of skin lesions, indicating that there is a functional defect and no inherent structural cell membrane abnormality in psoriasis. It should be emphasized here that the change in cAMP binding to RI in psoriatic cells appears to be reversible and may not be caused by a change in cell signaling, such as an increase in the oxidative state of the cell in response to events, such as stress or trauma. As such, the inability to bind cAMP will not be significantly influenced by possible changes in the intracellular level of cAMP. This may explain why attempts to influence intracellular cAMP levels to treat psoriasis would not be highly effective.

An important factor to explain the diminished binding of cAMP to erythrocyte membranes could be increased oxidative stress in psoriasis. Oxidative damage has been found in the dermis of psoriatic skin lesions as measured by carbonylation of macromolecules, an indicator of oxidative stress (Dimon-Gadal *et al*, 2000). Anti-oxidant superoxide dismutase in psoriatic fibroblasts and erythrocytes is significantly higher in psoriatic patients than in normal subjects (Thérond *et al*, 1996). Raynaud *et al* (1997) have reported that free oxygen radicals lead to decreased binding of cAMP in fibroblasts and that oxygen radical scavengers restored the binding of cAMP to normal levels. Moreover, Tournier *et al*, (1995) discovered that oxidative modification of PKA decreased the binding of cAMP while leaving the level of protein unaltered. Previous studies have also reported on the effects of oxidative stress in psoriasis; i.e., mononuclear leukocyte glucose-6-phosphate dehydrogenase, which provides energy to generate oxygen radicals in phagocytes, is enhanced in psoriasis (Schopf *et al*, 1986) and psoriatic phagocytes exhibit an enhanced respiratory burst (Schopf and Straussfeld, 1985). It could well be that the increased oxygen radical generation in psoriasis is related to the diminished cAMP binding to RI of PKA in psoriatic erythrocytes. From clinical experience, however, it is doubtful whether counteracting oxidative stress by oxygen radical scavengers or anti-oxidants will suffice to clear psoriatic lesions. Studies have not yet been published that clearly demonstrate that counteracting oxidative stress by oxygen radical scavengers or anti-oxidants will not have a beneficial effect in the treatment of psoriasis.

The findings of decreased binding of cAMP to PKA raise the question of whether this is merely a specific, temporary biochemical marker of psoriasis or whether this has pathogenic relevance. It is possible that abnormalities of the cAMP system are indeed intrinsically related to pathogenesis as first suggested by Voorhees and Duell, 1971; Voorhees *et al*, 1972a,b. The provocation of psoriasis by agents known to inhibit the intracellular accumulation of cAMP such as  $\beta$ -adrenergic antagonists, lithium, or anti-malarials, also points in that direction (Voorhees *et al*, 1975; summarized by Greaves and Weinstein, 1995). In addition, the induction of major histocompatibility complex class II antigens by  $\beta$ -adrenergic antagonists may play a part (Czernielewski *et al*, 1986) in considering the concept that psoriasis is a T lymphocyte mediated condition (Gottlieb *et al*, 1995). Moreover, it may well be that the exacerbation of psoriasis observed in patients with alcohol abuse may be related to the finding that ethanol caused a 43% decrease in the amount of type I regulatory subunit of PKA (Dohrman *et al*, 1996).

To summarize, our findings indicate that in psoriasis there is a highly decreased binding of cAMP to erythrocytes not present in other forms of generalized dermatitis or in healthy controls. Different forms of effective treatment normalize this defect. It remains to be shown whether the decreased binding of cAMP to red blood cells in psoriasis is a biochemical marker alone or whether this defect does have pathogenic relevance.

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