
Protein C Inhibitor is Expressed in Keratinocytes of Human Skin

Michael Krebs, Pavel Uhrin, Anja Vales, Maria J. Prendes-Garcia, Johann Wojta, Margarethe Geiger, and Bernd R. Binder

Department of Vascular Biology and Thrombosis Research, University of Vienna, Vienna, Austria

Protein C inhibitor is a member of the serpin family that inhibits a variety of serine proteases. Protein C inhibitor is present in numerous body fluids and is produced in the liver and by various epithelial cells. To determine if this epithelial serpin is present in skin, immunohistochemical studies were performed that showed strong staining for protein C inhibitor antigen in the epidermis. Protein C inhibitor mRNA was detected in the keratinocyte cell line HaCaT and the epidermoid carcinoma cell line A431 using reverse transcription-polymerase chain reaction suggesting that also in normal skin protein C inhibitor is derived from keratinocytes. Conditioned media from these cell lines were analyzed on immunoblots, which revealed a protein C inhibitor-antigen band that comigrated with protein C inhibitor derived from the hepatoma cell line HepG2. Using an enzyme-linked immunosorbent assay specific for total protein C inhibitor antigen the accumulation of protein C inhibitor in the cell culture

supernatants of HaCaT keratinocytes was found to be 0.3 ng per h per 1 million cells. This is similar to the amount of plasminogen activator inhibitor-1 produced by these cells, which also produce tissue plasminogen activator and urokinase. Fluorescence-activated cell sorter analysis revealed similar expression of intracellular protein C inhibitor antigen in proliferating and confluent HaCaT cells. These findings demonstrate that protein C inhibitor antigen is present in the normal epidermis and that protein C inhibitor is constitutively expressed by keratinocytes in culture. Therefore, protein C inhibitor may provide protease inhibitory activity not only to internal, but also to the external surface of the body. Additionally, protein C inhibitor could contribute to the regulation of retinoid supply in the epidermis, as we have shown recently that retinoic acid binds specifically to protein C inhibitor. *Key words: epidermis/plasminogen activator inhibitor/serine protease inhibitor. J Invest Dermatol 113:32-37, 1999*

Protein C inhibitor (PCI) is a single chain glycoprotein with a molecular weight of 57 kDa. PCI is a relatively unspecific, heparin-binding serine protease inhibitor (serpin), originally described in plasma as an inhibitor of the anticoagulant protease activated protein C (Marlar and Griffin, 1980). Serine proteases inhibited by PCI include thrombin, factor Xa, factor XIa, tissue and plasma kallikreins, acrosin, tissue plasminogen activator (tPA), and urokinase (for review see Suzuki *et al*, 1989; Geiger *et al*, 1996, 1997). PCI antigen is present in various body fluids (Laurell *et al*, 1992), and the tubular epithelium of human kidney (Radtke *et al*, 1994) and epithelial cells in the male reproductive tract (Laurell *et al*, 1992) have been shown to produce PCI. Plasma PCI is thought to be synthesized in the liver, as the human hepatoma-derived cell line HepG2 secretes significant amounts of PCI *in vitro* (Morito *et al*, 1985; Fair and Marlar, 1986) and as PCI plasma levels are decreased in patients with liver diseases (Francis and Thomas, 1984).

Plasma PCI is elevated in survivors of myocardial infarction (Carroll *et al*, 1997). Activated protein C-PCI complexes have been shown in plasma samples from patients with disseminated intravascular coagulation (España *et al*, 1990), and urokinase-PCI complexes in plasma samples from patients receiving urokinase therapy (Geiger *et al*, 1989). These findings suggest that PCI could play a part in the regulation of hemostasis. Most coagulation and fibrinolytic enzymes, however, are inactivated more efficiently by other serpins. Therefore, the precise physiologic role of PCI is still unclear.

Our group has previously shown that tissue kallikrein is efficiently inhibited by PCI (Ecke *et al*, 1992), suggesting that PCI may be a physiologically important endogenous inhibitor of this serine protease. Many components of the kallikrein-kinin system including tissue kallikrein are expressed in human skin, where they are potentially involved in the regulation of proliferation and in the mediation of inflammatory processes (Poblete *et al*, 1991; Schremmer-Danninger *et al*, 1995).^{1,2} So far no epidermal inhibitor for tissue kallikrein has been described. Furthermore, we have recently shown that PCI also belongs to the group of hormone-

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Reprint requests to: Prof. Dr. Margarethe Geiger, Department of Vascular Biology and Thrombosis Research, University of Vienna, Schwarzschanerstrasse 17, A-1090 Vienna, Austria.

Abbreviations: PCI, protein C inhibitor; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator; uPA, urokinase type plasminogen activator.

¹Schremmer-Danninger E, Hermann A, Fink E, Fritz H, Roscher AA: Identification of mRNAs for components of the kallikrein-kinin-system in human skin and skin diseases. *Abstract Book, 15th International Conference on Kinins-Kinin '98 Nara*. Nara, Japan, 1998, p 139 (abstr.)

binding serpins which includes corticosteroid-binding globulin (Hammond *et al*, 1987) and thyroxine-binding globulin (Flink *et al*, 1986): We were able to show that ³H-*all-trans*-retinoic acid bound in a concentration-dependent and specific manner to PCI but not to other inhibitory serpins and binding could be competed by unlabeled *all-trans*-retinoic acid, 9-*cis*-retinoic acid and retinol. Binding of estradiol, progesterone, testosterone, cortisol, and aldosterone to PCI was not detected. We have also preliminary data that PCI might be involved in the delivery of retinoic acid to target cells.³

The facts that (i) tissue kallikrein is expressed in the epidermis, that (ii) the epidermis is an important target organ for retinoids (Fisher and Voorhees, 1996), and that (iii) PCI is expressed in various kinds of epithelial cells lining internal surfaces, prompted us to investigate the possible presence of PCI in human skin and human keratinocyte cell lines. We used the epidermoid cell line A431 and spontaneously immortalized human keratinocytes (HaCaT) in these experiments. HaCaT cells maintain full epidermal differentiation capacity and have been widely used as representative of normal keratinocytes as they share many features with primary cells (Boukamp *et al*, 1988; Reinartz *et al*, 1994, 1996; Bechtel *et al*, 1996; Breitkreutz *et al*, 1998).

MATERIALS AND METHODS

Immunohistochemistry Normal human trunk skin was obtained from patients who underwent plastic surgery. The samples were embedded in Tissue-Tek OCT Compound (Sakura, Tokyo, Japan), snap frozen in liquid nitrogen, and sectioned at 8 µm thickness using a cryostat. The sections were mounted on to Histobond microscope slides (Marienfeld, Germany) and air-dried for 1 h at room temperature. Sections were then fixed for 15 min in 0.5% paraformaldehyde in phosphate-buffered saline (PBS; 2 mmol KH₂PO₄ per liter, 8 mmol Na₂HPO₄ per liter, 140 mmol NaCl per liter, pH 7.4) and permeabilized by sequential treatment with 0.2% and 1% Triton X-100 in PBS (1 × 10 min each). Slides were then rinsed with PBS and incubated with 1% bovine serum albumin (BSA; Behring, Marburg, Germany) in PBS for 30 min. Incubations with 10 µg monoclonal anti-PCI IgG (4PCI) per ml (Ecke *et al*, 1992) or nonimmune mouse IgG diluted in primary antibody diluting buffer (Biomed, Foster City, CA) were carried out in a humid chamber at 4°C over night. The slides were then rinsed with PBS and washed with automation buffer (Biomed) (2 × 5 min). Thereafter the slides were treated sequentially with biotinylated sheep antimouse IgG (Amersham, Little Chalfont, Buckinghamshire, U.K.) diluted 1:100 in PBS and streptavidin-peroxidase conjugate (Amersham) diluted 1:300 in PBS. After each incubation step the slides were washed as described above. Bound peroxidase was visualized using the Liquid DAB-Plus Substrate Kit (Zymed, San Francisco, CA). Thereafter slides were washed with distilled water, tissue sections were counterstained with hematoxylin, rinsed with water, and mounted in Aquatex (Merck, Darmstadt, Germany). Controls included the substitution of the first antibody with monoclonal anti-heparin cofactor II IgG, monoclonal anti-plasminogen activator inhibitor 1 IgG (5PAI) (Technoclone, Vienna, Austria), and monoclonal anti-complement factor D IgG (D10/4) (Connex, Martinsried, Germany). Sets of slides were processed together in duplicates.

Cell culture and collection of conditioned media Human hepatoma cells (HepG2) and the human epidermoid cell line A431 were obtained from American Type Culture Collection (ATCC; Rockville, MD). The spontaneously transformed human keratinocyte cell line HaCaT was kindly provided by Dr. N.E. Fusenig [Deutsches Krebsforschungszentrum (DKFZ), Heidelberg, Germany] (Boukamp *et al*, 1988). The cells were grown under culture conditions (37°C, 5% CO₂, 95% air) in Dulbecco's modified Eagle's media (Sigma, St. Louis, MO) containing 10% supplemented calf serum (Hyclone, Logan, UT), 1 mmol glutamine per liter, and antibiotics (100 µg streptomycin per ml, 100 IU penicillin per ml, 250 ng amphotericin B per ml) (all: JHR Biosciences, Lenaxa, KS). The cells received fresh medium

every 3 d and were subcultured using a split ratio of 1:5 as soon as they reached confluence. Cells were either grown in 75 cm² tissue culture flasks (Iwaki, Japan) in 10 ml medium or in six-well plates (Iwaki) in 2 ml medium per well. After reaching confluence the monolayers were washed twice with Hanks' balanced salt solution (Sigma) and incubated in serum free medium supplemented with 0.5% BSA (Sigma). After appropriate incubation times conditioned media were collected, centrifuged to remove cell debris, and stored at -70°C until analyzed.

RNA preparation and reverse transcription coupled to polymerase chain reaction Cells were grown to confluence in 75 cm² tissue culture flasks, washed twice with Hanks' balanced salt solution (Sigma), and total cellular RNA was isolated using Trizol reagent (Gibco, Grand Island, NY) according to the manufacturer's instructions. Total RNA was subjected to reverse transcription and subsequent polymerase chain amplification to show the presence of PCI mRNA in analyzed cells. For reverse transcription 1 µg of total RNA from HaCaT and A431 cells and 0.2 µg or 0.1 µg of total RNA from HepG2 cells were used. The RNA was preheated at 65°C for 15 min to remove possible secondary structures and then cooled down to 4°C. Reverse transcription reaction was made using a first-strand cDNA synthesis kit for reverse transcription-polymerase chain reaction (Boehringer Mannheim, Mannheim, Germany) at 42°C for 60 min. Following components were included: an anti-sense human PCI sequence specific primer 5'-CCT GTT GAA CAC TAG CCT CTG AGA G-3' at final concentration of 0.2 µmol per liter, 50 mmol Tris-HCl buffer per liter, pH 9.0, 1.5 mmol MgCl₂ per liter, 0.2 mmol of each deoxyribonucleoside triphosphate per liter, 50 U of RNase inhibitor, and 20 U of avian myeloblastosis virus-reverse transcriptase. Subsequently, 10 µl of the reaction mixture were taken and after addition of a sense human PCI sequence specific primer 5'-GGA TCA GTA TCA CTA CCT CCT GGA C-3', polymerase, and other components, the reaction mixture contained 50 mmol Tris-HCl buffer per liter, pH 9.0, 1.5 mmol MgCl₂ per liter, 0.2 mmol of each deoxyribonucleoside triphosphate per liter, 0.2 µmol of each primer per liter, and 1.0 U of DNA polymerase (DynaZyme; Finnzymes Oy, Finland) in a total volume of 25 µl. The mixture was predenatured for 2 min at 94°C and then subjected to 35 step cycles of 94°C (35 s), 58°C (30 s), 72°C (20 s). Amplified DNA fragments exhibited the expected size (442 base pairs) as judged from 1% agarose gel electrophoresis. Their identities were confirmed by sequencing.

Immunoblotting of conditioned media Prior to electrophoresis samples from keratinocytes and HepG2 cells were concentrated and partially purified by affinity chromatography on heparin-Sepharose CL-6B (Pharmacia, Uppsala, Sweden). Ten milliliters of conditioned media from HaCaT, A431, or HepG2 cells, respectively, grown in 75 cm² tissue culture flasks were incubated with 0.5 ml heparin-Sepharose equilibrated in 50 mM Tris-HCl, 100 mM NaCl, pH 7.5 (loading buffer) for 1 h at room temperature under constant shaking. The Sepharose was filled in a small column and washed with loading buffer. Thereafter bound protein was eluted with 1 ml 1 M NaCl in loading buffer. The samples were concentrated to 100 µl using a 10 kDa molecular weight cut-off Ultra Spin Microfilter (Roth, Karlsruhe, Germany), electrophoresed on a 10% sodium dodecyl sulfate-polyacrylamide gel, and electrophoretically transferred to a Hybond-P PVDF membrane (Amersham) using standard protocols. The membrane was blocked with 1.5% skim milk powder in 0.02 mol Tris-HCl per liter, 0.5 mol NaCl per liter, pH 7.5 (blocking buffer) over night at 4°C followed by a 2 h incubation with 10 µg per ml of specific rabbit anti-PCI IgG diluted in blocking buffer. The antibody used was prepared following standard protocols by immunizing a New Zealand white rabbit with highly purified urinary PCI (Ecke *et al*, 1992). The blot was washed with 0.02 mol Tris-HCl per liter, 0.5 mol NaCl per liter, pH 7.5, containing 0.05% Tween 20 (3 × 5 min) and twice with the same buffer without Tween 20 followed by a 1 h incubation with peroxidase linked anti-rabbit IgG (Amersham, Little Chalfont, Buckinghamshire, UK) diluted 1:1000 in blocking buffer. The membrane was washed as described and bound peroxidase was detected using chemiluminescence (ECL; Amersham). Prestained sodium dodecyl sulfate-standards (BioRad, Richmond, CA) were used for estimation of M_s.

Quantitation of PCI antigen in conditioned media PCI antigen in the conditioned media was quantitated by a specific enzyme-linked immunosorbent assay (ELISA) as described previously (Priglinger *et al*, 1994), using acid-treated monoclonal anti-PCI IgG (4PCI) as catching antibody and peroxidase-labeled immunopurified rabbit anti-PCI IgG as detecting antibody.

Assays for plasminogen activator inhibitor 1 (PAI-1) antigen, tPA antigen, and urokinase type plasminogen activator (uPA)

²Hermann A, Kresse H, Neth P, Fink E: Expression of components of the kallikrein-kinin system in human cell lines. *Abstract Book, 15th International Conference on Kinins-Kinin '98 Nara*. Nara, Japan, 1998, p 133 (abstr.)

³Jerabek I, Krebs M, Geiger M, Binder BR: Binding and delivery of retinoic acid by the inhibitory serpin protein C inhibitor (PCI). *Abstract Book, 15th International Conference on Kinins-Kinin '98 Nara*. Nara, Japan, 1998, p 67 (abstr.)

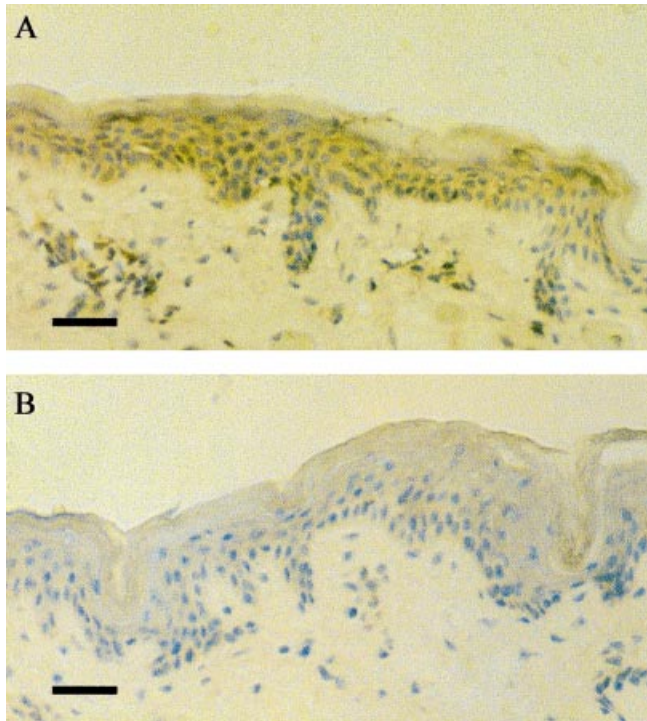


Figure 1. PCI antigen is present in normal human skin. Frozen sections of normal human skin were stained with monoclonal anti-PCI IgG (4PCI) (A) or nonimmune mouse IgG (B) as described in *Materials and Methods*. Scale bar: 50 μ m.

antigen PAI-1 in conditioned media was quantitated by a specific ELISA based on monoclonal antibodies, which allows determination of active and latent PAI-1 as well as PAI-1 in complex with tPA (Technoclone, Vienna, Austria). tPA antigen and uPA antigen were measured with specific ELISA according to the instruction of the manufacturer (Technoclone, Vienna, Austria).

Fluorescence-activated cell sorter (FACS) analysis HaCaT cells were grown in six-well plates in Dulbecco's modified Eagle's media supplemented with 10% supplemented calf serum. Forty-eight hours after reaching confluence the cells were harvested with trypsin-ethylenediamine tetraacetic acid, washed with PBS, pH 7.4, and centrifuged at $450 \times g$ for 5 min. Alternatively, HaCaT cells were seeded at low concentrations to obtain scattered single cells, incubated for 48 h in Dulbecco's minimal essential media containing 10% supplemented calf serum, and trypsinized as described above. One-half of the pelleted cells was fixed with 0.5% paraformaldehyde in PBS, permeabilized with 0.025% saponin (Sigma) in PBS. The other half was used unpermeabilized. Unspecific binding sites were blocked by incubating the cells with 1% BSA in PBS. Thereafter cells were washed with PBS, pH 7.4, containing 1% BSA and 0.025% saponin and centrifuged at $450 \times g$ for 5 min. Subsequently cells were incubated with rabbit anti-PCI IgG at a concentration of 20 μ g per ml in PBS containing 1% BSA and 0.025% saponin for 2 h at room temperature. After washing as described above the cells were incubated with fluorescein-labeled goat anti-rabbit IgG (Vector Laboratories, Burlingame, CA) diluted 1:50 in PBS containing 1% BSA and 0.025% saponin. Control cells were only incubated with the fluorescein-labeled antibody. Then the cells were washed once as described above and once with PBS, pH 7.4. Analysis was performed by a FACS Scan flow cytometer using CellQuest 3.1 software (Becton Dickinson, San Jose, CA).

RESULTS

An immunohistochemical analysis with monoclonal anti-PCI IgG was performed to determine whether or not PCI is present in normal adult skin. Pronounced uniform staining for PCI antigen was detected in the keratinocytes throughout the epidermis (**Fig 1A**). In control sections stained with nonimmune mouse IgG (**Fig 1B**) and monoclonal antibodies to heparin cofactor II, plasminogen activator inhibitor 1 (PAI-1), or complement factor D no signal was observed (data not shown). *In situ* hybridization studies using specific sense

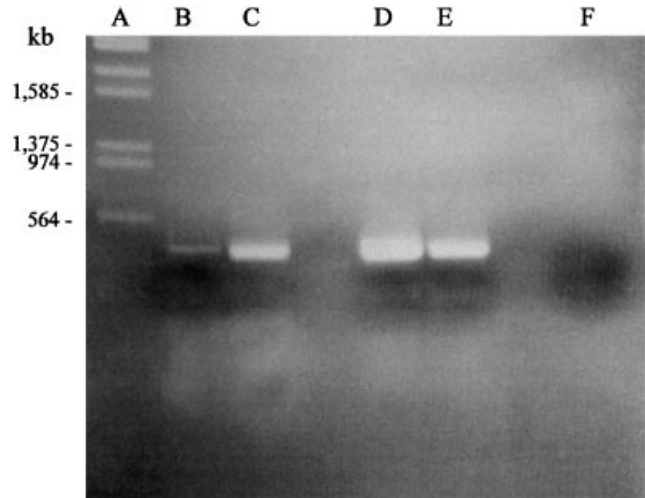


Figure 2. HaCaT, A431, and HepG2 cells produce PCI mRNA. Total RNA obtained from HaCaT (1 μ g, lane B), A431 (1 μ g, lane C), and HepG2 (0.2 μ g, lane D and 0.1 μ g, lane E) cells was subjected to reverse transcription-polymerase chain reaction using primers specific for a 442 bp fragment of PCI-cDNA as described in *Materials and Methods*. The fragments obtained were loaded on a 1% agarose gel and visualized after electrophoresis by ethidium bromide staining. The control in lane F is a template free reaction. Lane A contains molecular weight markers (λ EcoRI-HindIII digest).

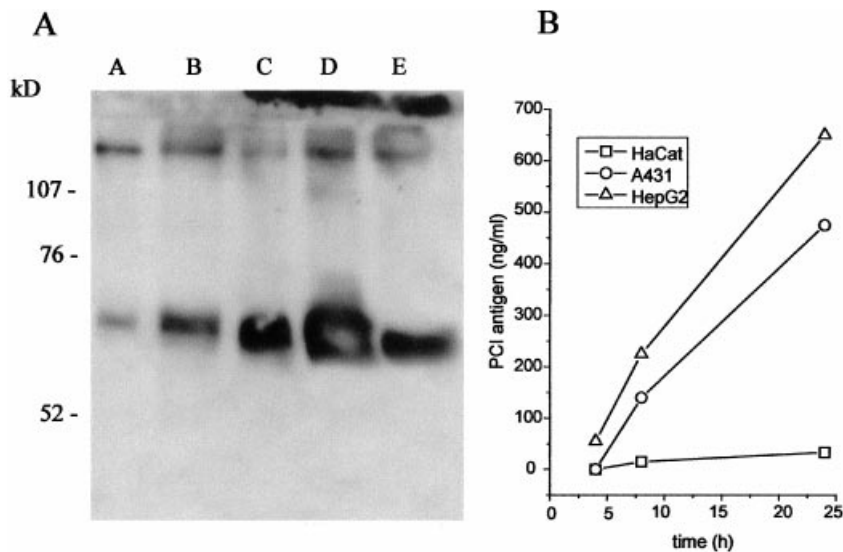
and anti-sense digoxigenin labeled RNA-probes for PCI were performed. No PCI mRNA was apparent in human skin (data not shown), however, presumably because the method was not sensitive enough to detect the low levels of PCI mRNA present in normal keratinocytes (see below).

In order to confirm the results obtained with the immunohistochemical studies of normal skin, PCI synthesis by keratinocytes was analyzed. PCI mRNA expression in spontaneously immortalized human keratinocytes (HaCaT) and epidermoid carcinoma cells (A431) was analyzed using reverse transcription-polymerase chain reaction. RNA prepared from human hepatoma cells (HepG2) was used as a positive control. **Figure 2** demonstrates that PCI mRNA was present in all three cell lines. A strong signal, that was dependent on the amount of RNA used, was obtained with HepG2 cells. In RNA prepared from A431 cells a pronounced band for PCI mRNA was detected, whereas the signal for PCI mRNA in HaCaT cells was rather weak.

To determine whether the PCI mRNA produced in these cells is translated and the protein is secreted into the supernatant, conditioned media were analyzed in immunoblots and ELISA. **Figure 3** demonstrates that PCI antigen accumulated over time in the cell culture supernatants from HaCaT, A431, and HepG2 cells. PCI in conditioned media was partially purified and concentrated using heparin-Sepharose and analyzed in immunoblots (**Fig 3A**), developed with specific polyclonal antibodies. These blots revealed a protein band with a M_r of ≈ 60 kDa in the conditioned media of HaCaT and A431 cells, which comigrated with PCI derived from HepG2 cells. Specificity of the reactions was validated with control blots incubated only with the second, peroxidase-labeled antibody. No bands were detected in these blots (data not shown). The amount of PCI obtained from conditioned media of A431 and HepG2 cells was much higher than that of HaCaT cells, reflecting the rate of PCI secretion. These data were confirmed by a specific antigen ELISA (**Fig 3B**), showing that PCI antigen accumulated much faster in the supernatants from A431 and HepG2 as compared with HaCaT cells. The concentrations of PCI antigen in conditioned media of HaCaT, A431, and HepG2 cells were 33 ng per ml, 475 ng per ml, and 650 ng per ml, respectively, after a conditioning time of 24 h.

The accumulation of PCI was compared with that of uPA, tPA, and PAI-1 in conditioned media of HaCaT cells. **Figure 4**,

Figure 3. (A) PCI antigen is present in conditioned media from keratinocytes. Conditioned media from HaCaT (lanes A and B) and A431 (lanes C and D) cells were collected after incubation times of 24 h (lanes A and C) or 48 h (lanes B and D). Conditioned media (24 h) from HepG2 cells (lane E) were used as positive control. The samples were run on a 10% sodium dodecyl sulfate–polyacrylamide gel and transferred on to a PVDF membrane. Rabbit anti-PCI IgG and peroxidase linked anti-rabbit antibodies were used to identify PCI related protein bands as described in *Materials and Methods*. **(B) PCI antigen accumulates in conditioned media of HaCaT, A431, and HepG2 cells.** Conditioned media were collected from confluent monolayers after incubation times of 4, 8, and 24 h and PCI antigen was determined by a specific ELISA. Data presented are the mean values of two determinations.



demonstrates that the secretion rates of the serpins (PCI and PAI-1) were similar (approximately 0.3 ng per h per 1 million cells), whereas considerably more uPA than tPA was produced.

FACS analysis was used to compare the expression of intracellular PCI antigen in proliferating and confluent HaCaT cells. As can be seen from **Fig 5**, no difference in PCI antigen between proliferating and nonproliferating cells was observed. The PCI detected in this assay was located mainly intracellularly as only a weak signal for PCI antigen was observed in nonpermeabilized cells.

DISCUSSION

In the present study we have shown that PCI is present in normal human epidermis and that PCI mRNA and antigen are expressed by cultured keratinocytes. The immunohistochemical localization experiments for PCI revealed that PCI was expressed in the basal, proliferating as well as in the more superficial, differentiating keratinocytes of the epidermis. The notion that PCI is constitutively expressed by keratinocytes is supported by the FACS data showing that intracellular PCI is equally present in proliferating and confluent, growth arrested HaCaT.

It has been shown that keratinocytes can produce the plasminogen activators uPA and tPA as well as the plasminogen activator inhibitors PAI-1 and PAI-2 in culture and that the pattern of expression of these proteins depends on the state of differentiation (Jensen *et al*, 1990, 1995; Chen *et al*, 1993; Reinartz *et al*, 1996). HaCaT cells secrete twice as much PAI-2 into the supernatant as compared with PAI-1 (Reinartz *et al*, 1996). This is in accordance to previous studies showing that in contrast to PAI-1, PAI-2 can be detected in normal epidermis by mRNA, antigen, and activity assays (Lyons-Giordano *et al*, 1994). These results indicate that PAI-2 (for review see Belin, 1993) is the predominant PAI in keratinocytes *in vivo* as well as *in vitro* (Jensen *et al*, 1995). The distribution of PCI in the epidermis is similar to that of PAI-2: PAI-2 mRNA and antigen have been detected throughout the epidermis, whereas PAI-1 was localized predominantly to the keratinocytes of the basal layer (Chen *et al*, 1993; Lyons-Giordano *et al*, 1994). Receptor-bound urokinase is thought to play an important part in proliferation and differentiation of keratinocytes (Reinartz *et al*, 1994), and PAI-2 has been implicated in the regulation of uPA activity in the epidermis (Reinartz *et al*, 1996). The second order rate constants for the interaction of PCI with urokinase ($1-8 \times 10^3$ per M per s without heparin and $4 \times 10^3-9 \times 10^4$ per M per s with heparin) are much lower than those for the interaction of urokinase with PAI-1 ($>10^7$ per M per s) or PAI-2 (2×10^6 per M per s) (Thorsen *et al*, 1988; Geiger *et al*, 1989). Therefore, it is unlikely that PCI plays a major part in the regulation of epidermal urokinase activity.

It has been postulated that keratinocytes elaborate pro-

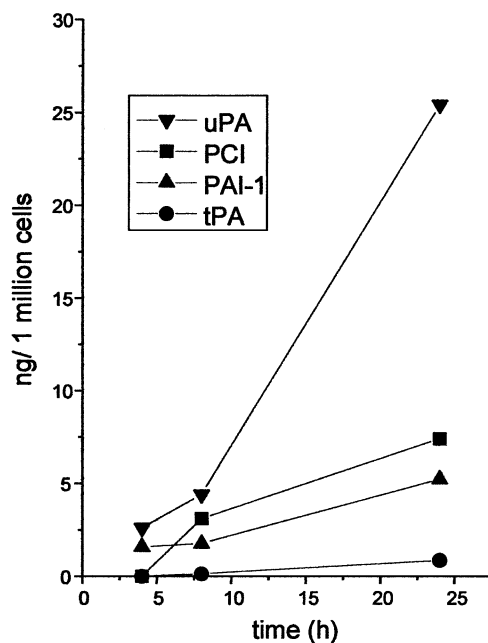


Figure 4. HaCaT cells secrete uPA, tPA, and similar amounts of PCI and PAI-1. Conditioned media were collected from confluent monolayers after incubation times of 4, 8, and 24 h and the concentrations of the respective antigens were determined by specific ELISA.

inflammatory mediators including bradykinin in response to diverse stimuli and thereby initiate cutaneous inflammation (Barker *et al*, 1991). The main components of the kallikrein-kinin system (reviewed in Scicli and Carretero, 1986), including tissue kallikrein, are present in human skin (Poblete *et al*, 1991). As PCI is a potent inhibitor of tissue kallikrein (Ecke *et al*, 1992) and as no other inhibitor of this serine protease has been described in skin, PCI might be a physiologically important inhibitor of tissue kallikrein in the epidermis.

PCI seems to be a protein, associated with internal as well as external body surfaces, as it has been identified in numerous body fluids and in various epithelial cells (Laurell *et al*, 1992; Radtke *et al*, 1994). This protease inhibitor is unusual in that it inhibits chymotrypsin as well as or even better than trypsin, suggesting that it has a broad reactivity with various serine proteases (Suzuki *et al*, 1984; Cooper and Church, 1995). It has been suggested that PCI in the male reproductive tract might function as a scavenger of prematurely activated acrosin, a serine protease stored as proacrosin

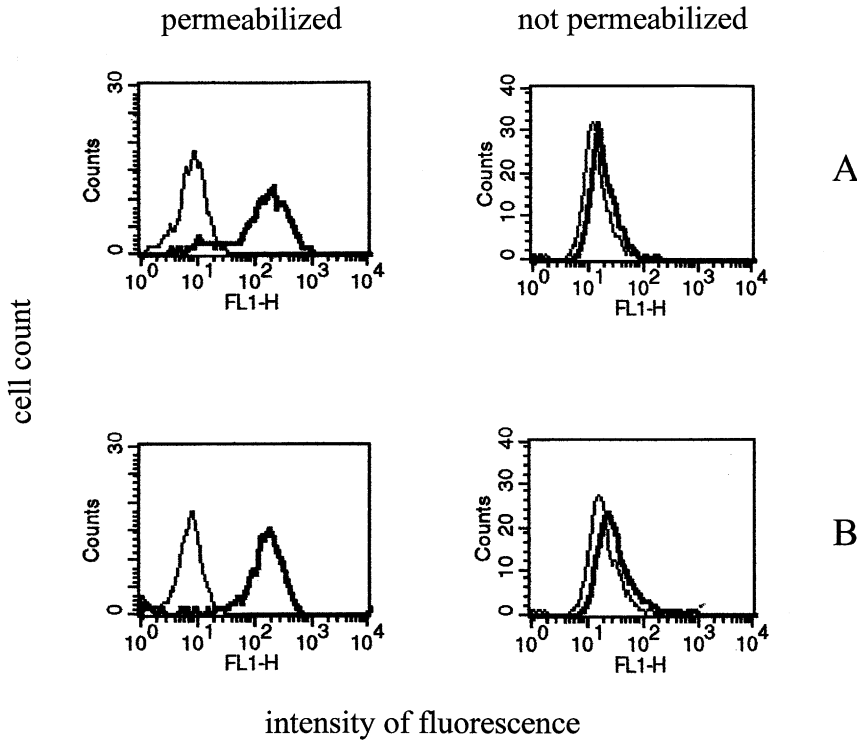


Figure 5. Proliferating and non-proliferating HaCaT cells express similar amounts of intracellular PCI. PCI antigen was detected in permeabilized disperse growing and confluent cells using rabbit anti-PCI IgG (thick line). The thin line represents the fluorescence of control cells treated only with the second, fluorescein-labeled antibodies. In panel A the pattern of fluorescence of confluent, growth arrested cells and in panel B the intensity of fluorescence of scattered, proliferating cells is shown. The FACS analyzes for permeabilized cells are shown on the left and the ones for nonpermeabilized cells are presented on the right.

in the acrosome of spermatozoa, and thereby protect the male reproductive system from proteolytic damage by this protease (Zheng *et al*, 1994). Similarly PCI might protect other body surfaces including the skin from proteolysis by providing unspecific protease inhibitory activity.

PCI is a member of the family of serine protease inhibitors (serpins) (for review, see Harper and Carrell, 1994). During the course of evolution, two members of this group, corticosteroid-binding globulin (Hammond *et al*, 1987) and thyroxine-binding globulin (Flink *et al*, 1986), have apparently exchanged their proteinase inhibitory properties for a new role as carriers of insoluble hormones. We have recently shown that *all-trans*-retinoic acid bound in a specific and concentration-dependent way to PCI but not to other serpins, suggesting an additional role of PCI as retinoic acid delivering serpin.³ The growth and differentiation of the epidermis and other epithelia is critically dependent upon undisturbed delivery of retinoids (Fisher *et al*, 1996).

Therefore epidermal PCI might not only serve as a protease inhibitor being involved in the regulation of the kallikrein-kinin system and/or the protection of the skin from various proteases, but might also take part in the regulation of retinoic acid supply in the skin.

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