Differential Expression of Urokinase-Type Plasminogen Activator and Its Type-1 Inhibitor During Healing of Mouse Skin Wounds

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The expression of urokinase-type plasminogen activator (u-PA) and its type-1 inhibitor (PAI-1) was examined in vivo in mouse wounds by in situ hybridization and immunohistochemistry. u-PA mRNA was present in both basal and suprabasal keratinocytes in the regenerative epithelial outgrowths at the edge of the wounds. In the same area, PAI-1 mRNA was only present in the basal keratinocytes. u-PA protein was detected in keratinocytes in several layers of the epithelial outgrowth, whereas PAI-1 protein was confined to the basal keratinocytes and to the area of the basal membrane. The two proteins and their mRNA were not detected in normal epidermis or in normal-looking epidermis adjacent to the wounds. Fibroblast-like cells and fairly large stellate cells (possibly macrophages) in the granulation tissue underneath the wound contained both the two proteins and their mRNA. The large stellate cells, showing a strong hybridization signal for PAI-1 mRNA, were especially abundant at the border between the necrotic wound and the newly formed granulation tissue. The specificity of these results was supported by the use of two different non-overlapping antisense probes, sense mRNA probes, antibody preparations preabsorbed with purified proteins, and Northern analysis of tissue extracts. The localized and regulated expression of u-PA and PAI-1 seen in this study may reflect that plasminogen activation plays a role in the migration of keratinocytes and connective tissue cells during reepithelialization and tissue remodeling in wound healing. *J Invest Dermatol 97:803–811, 1991*

Wound healing involves extensive cellular migration and tissue remodeling during the processes of reepithelialization, wound contraction, and angiogenesis [1,2]. Interactions between cells and the extracellular matrix are central to these processes and are likely to require both degradation and rebuilding of matrix molecules.

The plasminogen activation system is a general proteolytic system participating in extracellular matrix degradation and thrombolysis (for reviews see [3–5]). Activation of plasminogen leads to formation of plasmin capable of degrading a number of proteins present in the extracellular matrix such as fibronectin, laminin, and fibrin. In addition, plasmin activates procollagenases. Activation of plasminogen is catalyzed by two different plasminogen activators, urokinase-type (u-PA) and tissue-type (t-PA). t-PA has primarily been associated with thrombolysis, whereas u-PA is involved in tissue remodeling and cell migration under normal and pathologic conditions, including cancer and inflammatory reactions.

Recent results have shown that plasminogen activation is regulated in time and space by two specific plasminogen activator inhibitors, PAI-1 and PAI-2, both glycoproteins of the SERPIN (serine protease inhibitor) type (for reviews see [6–8]) and derived from different genes located on different chromosomes [9,10]. In addition, the urokinase pathway of plasminogen activation is regulated by a specific cell-surface receptor for u-PA (u-PAR) ([11–14]; for a recent review see [15]).

PAI-1 has been found in a number of cultured cell lines such as endothelial cells [16] and HTC rat hepatoma cells [17]. PAI-1 is a fast and specific inhibitor of both u-PA and t-PA, and its activity in cell cultures is regulated by a variety of hormones, cytokines, and growth factors [8], e.g., the activity is increased by glucocorticoids [18], interleukin 1 [19], and TGF-β [20], and down-regulated by the polypeptide hormones FSH and LH [21]. PAI-1 has been found in the intact organism in plasma [22], in platelets [23], in adrenal medulla [24], and in the murine Lewis lung carcinoma [25].

Cultured keratinocytes produce plasminogen activators [26,27] and plasminogen activator inhibitors [28]. In vitro, u-PA has been found in migrating keratinocytes and endothelial cells at the edge of experimental wounds [29,30]. However, cultured cells are not necessarily representative of the cells in the intact organism with re-

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Abbreviations:

GAPDH: glyceraldehyde-3-phosphate dehydrogenase
PAI-1: plasminogen activator inhibitor-type 1
PBS: phosphate-buffered saline
PFA: paraformaldehyde in 0.1 M PBS
TBS: Tris-buffered saline
t-PA: tissue-type plasminogen activator
u-PA: urokinase-type plasminogen activator

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spect to production of components of the plasminogen activation system (see [4]). We have therefore previously studied the occurrence of u-PA in keratinocytes during healing of murine and human wounds by immunohistochemistry [31]. These studies indicated that u-PA is involved in the reepithelialization process. To further investigate the role of plasminogen activation in wound healing, we have now localized u-PA and PAI-1 mRNA and protein by in situ hybridization and immunohistochemistry during wound healing in mice.

MATERIALS AND METHODS

Materials The following materials were obtained from the indicated sources: T7, T3, and SP6 polymerase, pBluescript KS(+) plasmid vector, and poly(A)quik Oligo dT-columns (Stratagene, CA); pGEM5z plasmid vector, RNasin, and DNase I (Promega, WI); K5 autoradiographic emulsion (Ilford, Cheshire, U.K.); Tissue-Tek (Miles, IN); swine anti-rabbit IgG and rabbit peroxidase anti-peroxidase complexes (Dakopatts, Copenhagen, Denmark); deoxycytidine 5′-[α-^{32}P] triphosphate (3000 Ci/mmol) (The Radiochemical Centre, Amersham, U.K.); ^{35}S-UTP (Dupont–New England Nuclear, MA); random priming DNA labeling kit, di-thiothretiol, and restriction endonucleases (Boehringer Mannheim, Germany). All other materials were those described previously [20,32].

Animals and Tissue-Treatment Procedures Ten- to twelve-week-old female Balb/c mice were anesthetized with diethylether and shaved, and a full-thickness 15-mm–long cut was made on the mid-dorsal skin with a scalpel. The wounds were neither sutured nor dressed. Control animals were only anesthetized and shaved. Each mouse was then caged separately and anesthetized with diethylether immediately before use. Thirty animals used for RNA isolation (10 control animals, 10 animals studied 12 h, and 10 animals 48 h after wounding) were perfused intracardially with 20 ml 4°C 0.01 M sodium phosphate, 0.14 NaCl, pH 7.4 (PBS). The wound, surrounded by a 1-mm rim of skin (total weight approximately 100 mg) was removed surgically.

Nineteen animals used for in situ hybridization and immunohistochemistry (four control animals, one animal studied 12 h, two animals 48 h, and twelve animals 96 h after wounding) were treated similarly, except that perfusion with cold PBS was followed by intracardial perfusion-fixation with 4% (w/v) paraformaldehyde in 0.1 M PBS (PFA) [33]. The wounded area was then removed and specimens were cut in 3-mm slices perpendicular to the length of the wound. These specimens were postfixed for 16 h in 1% (w/v) PFA, rinsed for 6 h in 0.1 M sodium phosphate, pH 7.4, 20% (w/v) sucrose, frozen in melting Freon 22, and stored at −80°C until further use.

Preparation of RNA Probes Fragments of mouse u-PA cDNA [34], rat PAI-1 cDNA [35], and mouse type-III procollagen [36] were subcloned using standard techniques [37], preparing the following subclones: *pMUPA07,* EcoRI(608)-PstI(1642) fragment in pGEM5z; *pMUPA09,* XbaI(37)-PstI(428) fragment; *pRPAI106,* PstI(325)-ApaI(1051) fragment; *pRPAI107,* EcoRI(0)-PstI(325) fragment; and *pCol-III02,* 500 bp XbaI fragment containing exon 2, all in pBluescript KS(+). Pure plasmid preparations were prepared by banding in CsCl gradients and before transcription the plasmids were linearized using the following restriction endonucleases: pMUPA07, PstI or EcoRI; pMUPA09, BamHI; pRPAI106, PstI or ApaI; pRPAI107, HindIII; pCol-III02, HindIII or SacI. One microgram of the linearized plasmid was extracted with phenol and with chloroform/isoamylalcohol (25 : 1), precipitated with ethanol, and redissolved in water. Each transcription reaction contained the following: linearized DNA template (1 μg), RNasin (40 U), 40 mM Tris-Cl, pH 7.6, 6 mM $MgCl_2$, 10 mM NaCl, 2 mM Spermidine, 10 mM DTT, 1 mM GTP, 1 mM ATP, 1 mM CTP, 4 μM ^{35}S-UTP, and the relevant polymerase (SP6, T3, or T7) (40 U). The transcription was performed for 120 min at 37°C. The template DNA was removed by addition of RNase-free DNase I (1 U), yeast tRNA (20 μg), RNasin (20 U), and continuing incubation at 37°C for 15 min. After extraction with phenol and chloroform/isoamylalcohol (25 : 1) RNA was precipitated by ethanol by centrifugation at 15,000 × *g*, 4°C, for 10 min. After addition of ammonium acetate (final concentration 2 M), RNA was redissolved in 10 mM DTT. The RNA was hydrolyzed in 0.1 M sodium carbonate buffer, pH 10.2, containing 10 mM DTT, to an average size of 100 bp. Hydrolysis time was calculated as described [38]. After hydrolysis, the reaction was neutralized by addition of an equal amount of 0.2 M sodium acetate buffer, pH 6.2, containing 10 mM DTT and the RNA was precipitated twice with ethanol as above. The RNA probe was redissolved in 10 mM DTT and radioactivity measured using scintillation counting. Probe preparations always contained more than 2×10^6 cpm/μl, and the amount of TCA-precipitable material was usually above 90%. The activity of the two corresponding RNA probes transcribed from the opposite strands of the same plasmid template was adjusted to the same radioactivity concentration by addition of 10 mM DTT, and deionized formamide was added to a final concentration of 50%. Probes were stored at −20°C until use.

In Situ Hybridization In situ hybridization was performed using a method described in detail elsewhere [39]. Briefly, 5-μm cryostat sections on chrome-alum gelatine slides, fixed in 4% (w/v) PFA and acid treated in 0.2 M HCl, were incubated in 5 μg/ml Proteinase K in 50 mM Tris-Cl, pH 8.0, with 5 mM EDTA. Slides were fixed in 4% (w/v) PFA and immersed in 100 mM triethanolamine, 0.2% (v/v) acetic acid anhydrid. The hybridization solution contained RNA probe (approximately 80 pg/μl), deionized formamide (50%), dextran sulphate (10%), tRNA (1 μg/μl), Ficoll 400 (0.02% [w/v]), polyvinylpyrrolidone (0.02% [w/v]), BSA Fraction V (0.02% [w/v]), 10 mM DTT, 0.3 M NaCl, 0.5 mM EDTA, 10 mM Tris-Cl, and 10 mM NaH_2PO_4 (pH 6.8).

After hybridization overnight at 47°C, sections were washed at 50°C in a mixture similar to the hybridization solution except that probe, dextran sulphate, DTT, and tRNA were omitted, and subsequently in 0.5 M NaCl, 1 mM EDTA, 10 mM Tris-Cl (pH 7.2) (NTE) with 10 mM DTT at 37°C. Sections were treated with RNase A (20 μg/ml) in NTE followed by washing in NTE at 37°C and in 15 mM sodiumchloride, 1.5 mM sodiumcitrate, pH 7.0 with 1 mM DTT at room temperature. Sections were then dehydrated in graded solutions of ethanol, all containing 300 mM ammonium acetate until 99% ethanol, and air-dried. Autoradiographic emulsion was applied and sections developed after 1–2 weeks of exposure.

Immunohistochemical Staining Polyclonal rabbit IgG against mouse u-PA [40] and polyclonal rabbit antibodies against human PAI-1 [41] were purified on Protein-A Sepharose as described [42]. The antibodies against human PAI-1 cross-reacts with mouse PAI-1 [25].

Immunohistochemical staining was done by the peroxidase-anti-peroxidase method of Sternberger [43], as described in detail previously [31], both with and without blocking of endogenous peroxidase activity by methanol/hydrogenperoxide. Controls were performed by omitting the specific antibodies or by substituting the specific antibodies with antibody preparations that had been absorbed with the appropriate antigen by passage through Sepharose columns [25].

Cell Culture Mouse Lewis lung H122 carcinoma cells were obtained from Dr. G. Vaes, Brussels, Belgium [44]. NIH 3T3 cells were obtained from Dr. F. Blasi (Institute of Microbiology, University of Copenhagen). Both cell lines were cultured in Dulbecco's modified Eagles medium, supplemented with 10% (v/v) fetal calf serum. The cells were grown to confluency and then washed with 3 × 20 ml of 0.01 M sodium phosphate, pH 7.4, containing 0.15 M NaCl, 1 mM Ca_2Cl_2, and 0.5 mM $MgCl_2$. The cells were maintained in serum-free medium for 48 h and were harvested as described [20].

RNA Isolation Total RNA isolation was done by the acid guanidine-thiocyanate-phenol-chloroform method [45]. Poly(A)$^+$ RNA was isolated by chromatography of 400 μg of total RNA using

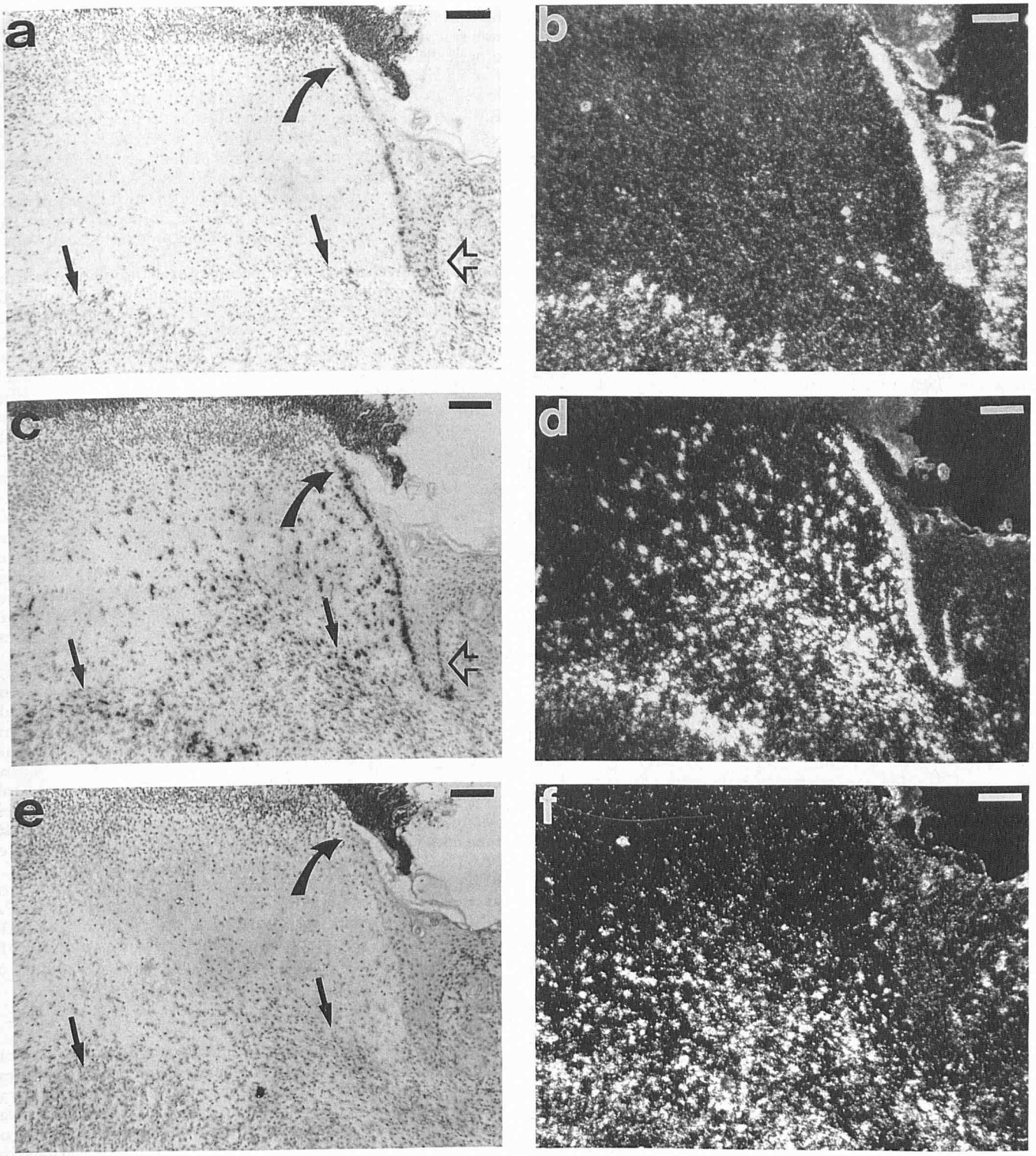

Figure 1. Distribution of u-PA and PAI-1 mRNA in mouse skin 48 h after wounding, as detected by in situ hybridization. Sections were hybridized using anti-sense u-PA RNA *(a,b)*, anti-sense PAI-1 RNA (*c,d*), and anti-sense type-III procollagen RNA *(e,f)* as indicated in the *Materials and Methods* section. Tissue sections were viewed using ordinary *(a,c,e)* or dark-field *(b,d,f)* microscopy. *Curved arrows,* leading front of keratinocytes; *straight arrows,* border of granulation tissue; *open arrow,* area shown at higher magnification in Fig 2. *Bar,* 100 μm.

columns of oligo (dT) cellulose according to the manufacturer's instructions. The concentration of poly(A)$^+$ RNA in the samples as determined spectrophotometrically, assuming that OD-260 = 1.00 corresponds to [RNA] = 40 μg/ml.

Northern Blots and Hybridizations Northern blots were made as described [20], by blotting agarose-separated poly(A)$^+$ RNA to nitrocellulose membranes and hybridizing the membranes with ^{32}P "random prime" labeled linearized cDNA probes. Filters were washed at 65°C for 1.5 h in 2 × SSC, 1% SDS and 1.5 h in 0.2 × SSC, 1% SDS and exposed to Kodak AR 5 films at −80°C for 3–5 d.

The plasmids used for the Northern blots were pMUPA07 and pRPAI106 (see preparation of RNA probes). As a control probe, the plasmid pGAPDH 5, containing a full-length cDNA for the rat glyceraldehyde 3-phosphate dehydrogenase inserted into the PstI site of the pUC 19 vector, was used [46].

RESULTS

Epidermis In situ hybridization of sections from 12-h-old wounds with anti-sense mouse u-PA and rat PAI-1 RNA probes showed that u-PA mRNA was present in both basal and suprabasal keratinocytes in the regenerative epithelial outgrowths at the edge of the wounds. A distinct but weak signal for PAI-1 mRNA was found in the basal keratinocytes. At 48 h and 96 h after wounding, the distribution of both u-PA and PAI-1 mRNA was similar, but the hybridization signal for both the messengers had increased in intensity. In all cases, u-PA mRNA was present in both basal and suprabasal keratinocytes of the epithelial outgrowths (Figs 1*a,b* and 2*a,b*). In contrast, PAI-1 mRNA was exclusively detected in the basal cells (Figs 1*c,d* and 2*e,f*).

No hybridization signals for u-PA and PAI-1 mRNA were observed in epidermis from normal mice or in normal-looking epidermis adjacent to the wound area.

Immmunohistochemical staining of 48-h- and 96-h-old wounds with polyclonal rabbit anti-mouse u-PA IgG showed that u-PA was present in keratinocytes in several layers of the epithelial outgrowths. In some specimens, virtually all of the keratinocytes in this area were positive; in others, only a proportion of the cells were stained (Fig 3*a*). Staining of adjacent sections with polyclonal rabbit anti-human PAI-1 IgG showed that PAI-1 was present in some basal keratinocytes of the epithelial outgrowths and extracellularly in the area of the basal membrane underneath this epithelium. In some cases, PAI-1-positive material was only detected extracellularly in the area of the basal membrane (Fig 3*c*). No u-PA or PAI-1 immunoreactivity was detected in epidermis from normal mice or in the normal-looking epidermis adjacent to the wounds.

Dermis and Subcutis Twelve hours after wounding, a weak signal for u-PA and PAI-1 mRNA was seen in fibroblast-like cells in the fatty tissue underneath the skin muscle. In the upper dermis, a weak signal for u-PA mRNA and a stronger signal for PAI-1 mRNA were seen in fibroblast-like cells in the granulation tissue underneath and adjacent to the wounds. Most of the fibroblast-like cells contained mRNA for type-III procollagen, as shown by hybridization of adjacent sections with an anti-sense RNA probe (Fig 1*e,f*). Larger stellate cells in the granulation tissue, resembling macrophages in morphology, were also positive for u-PA mRNA and showed a strong signal for PAI-1 mRNA. These cells were especially abundant at the edge of the newly formed granulation tissue (Figs 1*c,d* and 2*e,f*). Forty-eight hours and 96 h after wounding, the number of all three types of cells had increased (Figs 1 and 2).

Immunohistochemical stainings showed both u-PA and PAI-1 in cells, with morphology and localization similar to the aforementioned fibroblast-like and stellate cells in the dermis and subcutis, which contained the corresponding mRNA. Figure 3*e* and *f* show u-PA and PAI-1, respectively, in the fibroblast-like cells of the granulation tissue.

Sections of normal skin hybridized with anti-sense mouse u-PA RNA probes showed a very weak but definite signal in a few fibroblast-like cells in the fatty tissue underneath the skin muscle. Immunohistochemistry failed to show any signal for u-PA in these or other cells in normal skin. A weak signal for PAI-1 mRNA was found in a small number of fibroblast-like cells in the upper and lower dermis. Immunohistochemical staining demonstrated a weak PAI-1 immunoreactivity in these cells (results not shown).

Twelve, 48, and 96 h after wounding, u-PA mRNA (Fig 1*a,b* to the right of the epidermal wedge) and protein were found in keratinocytes of the hair follicles in the wound area. No PAI-1 mRNA or protein were found in these keratinocytes. Hair follicle keratinocytes farther from the wound area and from normal skin were negative for both u-PA and PAI-1 mRNA and proteins.

Control Experiments The specificity of all the in situ hybridizations was controlled by using the corresponding sense probes for mouse u-PA, rat PAI-1 mRNA, and type-III procollagen mRNA, all with the same radioactivity (cpm/μl) as the respective anti-sense transcripts. As exemplified in Fig 2*c,d,g,* and *h*, these sense probes gave a diffuse signal, reflecting unspecific binding. This background was always much less pronounced than the signals described above with the anti-sense RNA probes. In addition, the specificity of the signals obtained with the anti-sense u-PA RNA probe (pMUPA07) and the anti-sense PAI-1 RNA probe (pRPAI106) was confirmed by using anti-sense probes covering non-overlapping parts of the mouse u-PA cDNA (pMUPA09) and the rat PAI-1 cDNA (pRPAI107), respectively. In all cases, identical hybridization results were obtained with the two different probes for the same mRNA. Immunohistochemical controls, including the use of antibody preparations preabsorbed with purified antigen, were performed; all of these were negative. Blocking of endogenous peroxidase activity was omitted in the immunohistochemical experiments shown in Figs 1 and 2. Control experiments including the blocking procedure gave identical results.

Northern Analysis Total RNA was isolated from normal mouse skin, wound tissue, Lewis lung tumor cells, and NIH 3T3 cells. Poly (A)$^+$ RNA was prepared as described in *Materials and Methods,* and the mRNA was analyzed by hybridizing Northern blot membranes with the plasmids employed for in situ hybridization, containing mouse u-PA cDNA, rat PAI-1 cDNA, or a cDNA for rat glyceraldehyde-3-phosphate dehydrogenase. This analysis showed expression of u-PA mRNA in normal skin and an increase of the 2.8-kb band both 12 and 48 h after wounding (Fig 4*a*). Weak expression of PAI-1 mRNA was detected in normal skin and, after wounding, a strong increase of the 3.8-kb band was seen (Fig 4*b*). The electrophoretic mobilities were similar to the ones found for u-PA mRNA from Lewis lung tumor cells and PAI-1 mRNA from NIH 3T3 cells (Fig 4). Rehybridization of the filters with a rat GAPDH cDNA probe showed that the lanes contained equal amounts of mRNA.

DISCUSSION

The localization of u-PA and PAI-1 mRNA was done by hybridization with anti-sense RNA probes generated from cDNA subclones. For detection of u-PA mRNA, a mouse u-PA cDNA fragment was used as template for probe generation, and a rat cDNA fragment was used as template for generation of the probe used for detection of PAI-1 mRNA. The latter fragment shows 93% nucleic acid identity with the corresponding mouse cDNA sequence [35,47]. Equal amounts of sense RNA probes transcribed from the other strand of these plasmids were applied to adjacent sections in all experiments, and the experimental protocol included treatment of all sections with RNase A. In order to test whether the anti-sense hybridization was caused by the presence in the tissue section of a mRNA with a strong homology to a part of the u-PA or PAI-1 mRNA, hybridizations were carried out using two different transcripts covering two non-overlapping parts of each of the cDNA for both mouse u-PA and rat PAI-1. Hybridization signals over cells with similar localization in adjacent sections were in all cases seen with the two respective probes. Furthermore, the increase in u-PA and PAI-1 mRNA

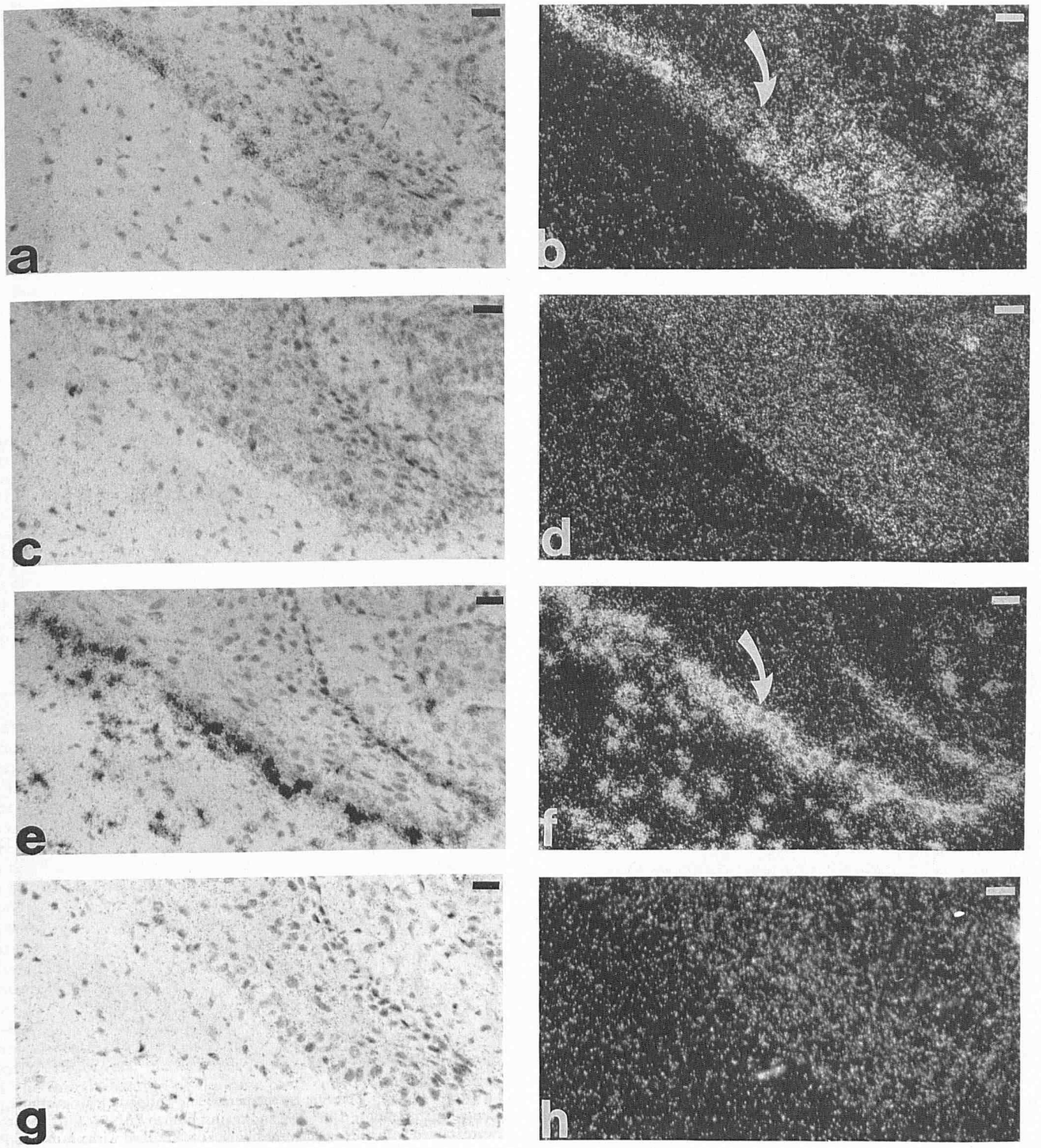

Figure 2. Localization of u-PA mRNA and PAI-1 mRNA in mouse skin epidermis 48 h after wounding by in situ hybridization. Higher magnification of area shown in Fig 1. Sections were hybridized using anti-sense u-PA RNA *(a,b)*, sense u-PA RNA *(c,d)*, anti-sense PAI-1 RNA *(e,f)*, and sense PAI-1 RNA *(g,h)*. Sections are viewed using ordinary *(a,c,e,g)* or dark-field *(b,d,f,h)* microscopy. Note that u-PA mRNA is found in both suprabasal and basal keratinocytes in the epithelium. In contrast, PAI-1 mRNA is confined to the keratinocytes in the basal layer. *Arrow* in *b*, several layers of keratinocytes positive for u-PA mRNA; *arrow* in *f*, only the basal layer of keratinocytes positive for PAI-1 mRNA. *Bar*, 25 μm.

content detected by the in situ hybridization is in agreement with results of the Northern blotting experiments (Fig 4).

The specificity of the immunostainings was supported by the staining controls, e.g., by the demonstration that staining was abolished by absorption of the antibody with purified preparations of the respective antigens. In addition, the polyclonal antibody preparations against u-PA [25,31,33,48] and PAI-1 [24,25] have been used previously for immunolocalization studies. In Kristensen et al [25], immunoblotting control for PAI-1 was included. These studies have not revealed any indication of cross-reaction with other proteins.

The validity of both the in situ hybridizations and the immuno-

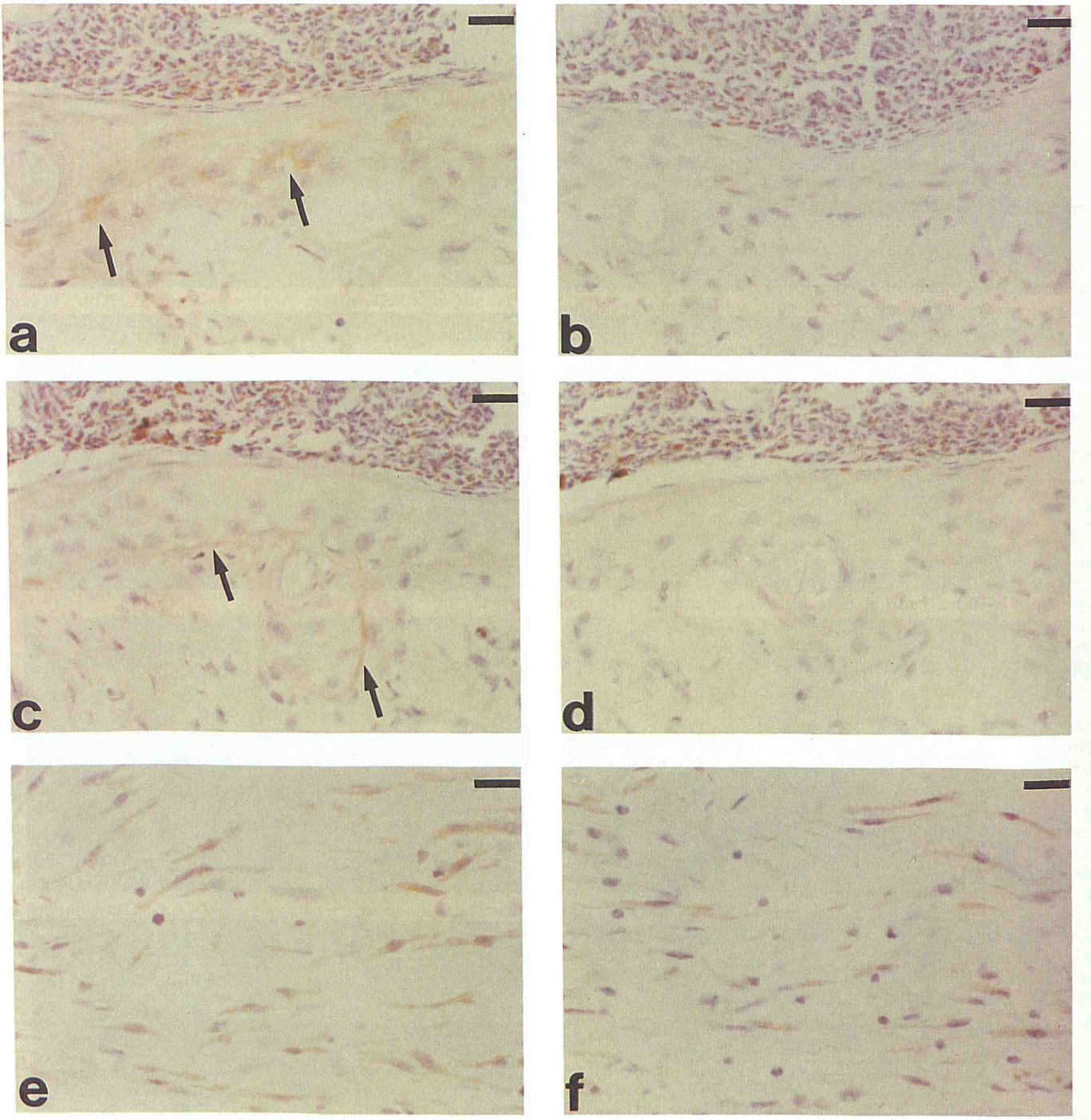

Figure 3. Immunohistochemical staining for u-PA and PAI-1 in mouse skin 48 h after wounding. Staining by the peroxidase-antiperoxidase method, as described in the *Materials and Methods* section using polyclonal rabbit IgG antibodies raised against mouse u-PA *(a,e)* and against human PAI-1 *(c,f)*. The latter antibodies cross-react with mouse PAI-1 (Kristensen et al, 1990). Adjacent sections were stained with the respective antibodies preabsorbed with purified u-PA *(b)* and PAI-1 *(d)*. u-PA immunoreactivity is found in both basal and some suprabasal keratinocytes in the regenerating epithelium (*arrows* in *a*), whereas PAI-1 immunoreactive material in this section is found predominantly below the basal keratinocytes in an area corresponding to the basal membrane (*arrows* in *c*). u-PA *(e)* and PAI-1 *(f)* immunoreactivity is also found in dendritic fibroblast-like cells in the granulation tissue. *Bar,* 20 μm.

stainings is also supported by the intrinsic accordance between the results obtained with the different methods; for both u-PA and PAI-1, the respective mRNA and protein in virtually all cases are detected in the same cell types, the only exception being some fibroblast-like cells underneath the skin muscle in normal mice. These cells showed a weak but definite signal for u-PA mRNA, but no detectable u-PA immunoreactivity. It cannot be determined whether this finding means that the u-PA mRNA is not translated in these cells, or whether the u-PA protein is produced but is present in a concentration below the detection limit.

On the basis of the above results, we conclude that it is very likely that for both u-PA and PAI-1 the signals obtained by in situ hybridization and immunohistochemistry represent the presence of the authentic mRNA and proteins, respectively. Except for the fibro-

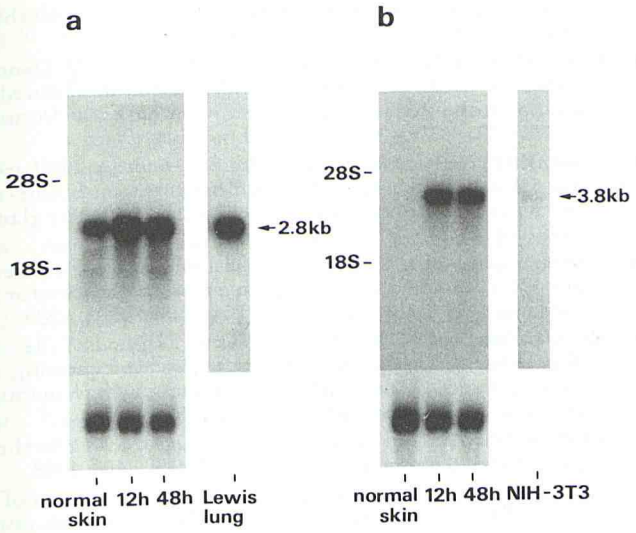

Figure 4. Northern blot analysis of mRNA for u-PA *(a)* and PAI-1 *(b)* in extracts of skin from normal mice or skin wounds at the indicated hours after wounding. mRNA from extracts of cultured Lewis lung carcinoma and NIH-3T3 cells were included as controls. In each *lane*, 2.5 μg of poly(A)$^+$ RNA were electrophoresed in 1.5% agarose gels under denaturing conditions and blotted onto a nitrocellulose membrane. The membranes were hybridized to randomly primed mouse u-PA cDNA *(a)* or rat PAI-1 cDNA *(b)* probes. After probe stripping, both membranes were rehybridized with GAPDH cDNA (lower part of *a* and *b*), to test whether the same amounts of poly(A)$^+$ RNA were transferred to the membranes in each lane. The positions of ribosomal RNA are indicated to the left, and the apparent size of the u-PA and PAI-1 mRNA to the right.

blast-like cells containing u-PA mRNA in normal subcutis, the findings indicate that all the mRNA-containing cells produce the respective proteins.

In the epidermis, u-PA is produced in both basal and suprabasal keratinocytes during wound healing, and the protein is found in many of the keratinocytes migrating under the wound clot as previously reported [31]. In contrast, PAI-1 is exclusively produced in the basal keratinocytes, and this protein is predominantly located in the area of the basal membrane and in some of the basal keratinocytes. It has been suggested that migration of mammalian keratinocytes under the wound clot occurs by a process by which the marginal cells roll over one another, the suprabasal cells rolling or climbing over the basal cells and being immobilized when they encounter the extracellular matrix of the wound bed [49,50]. In this model, the suprabasal keratinocytes may use plasmin formed by the u-PA pathway of plasminogen activation for detachment before they move. Subsequently, when the moving cells reach the provisional extracellular matrix, their secretion of PAI-1 may play a role in the immobilization of the now basal cells by inhibition of the plasminogen activation.

This is probably a very simplified model. It appears likely that the u-PA receptor is also involved in the process. Cultured keratinocytes have been reported to contain binding sites for u-PA that may be identical to u-PAR [64]. As discussed above, cultured cells are often not representative for the corresponding cells in the intact organism with respect to production of the various components of the plasminogen activation system. However, preliminary studies with a recently isolated cDNA for mouse u-PA receptor have shown that u-PA receptor mRNA is present in extracts from mouse skin wounds, although its histologic localization has not yet been determined (J. Rømer and P. Kristensen, unpublished results). u-PA receptor in some cell types localizes pro-u-PA to cell-cell and focal cell-substratum contact sites [51-53]. It is possible that an activation of pro-u-PA on some, but not all, of these sites on the same cell may provide a directional proteolysis involved in opening of the contacts during cell movement. PAI-1 may be involved in the regulation of such a process, because PAI-1 does not bind to pro-u-PA [40], but is an effective inhibitor of active u-PA, even when u-PA is receptor bound [54,55].

Another role for plasminogen activation, during the dissection of the epithelial wedge of keratinocytes under the wound clot, could be involvement in the degradation of extracellular matrix proteins (e.g., fibrin, fibronectin, and collagens), either directly by plasmin or through an activation of procollagenases.

Both u-PA and PAI-1 are found in fibroblast-like cells and in large stellate cells (possibly macrophages) in the granulation tissue and at the border of the newly formed granulation tissue, pointing to a role of plasminogen activation in the migration of these cells or in tissue degradation and remodeling in the necrotic area.

The localized and differential expression of u-PA and PAI-1 observed in this study suggests that there is a regulation of the plasminogen activation during wound healing. Growth factors such as TGF-β, PDGF, and FGF, released from platelets and activated macrophages and involved in inflammation and granulation tissue formation [56-59], are potential candidates as mediators in this regulation. These and other growth factors have profound effects on expression of u-PA and PAI-1 in various cell types in vitro [8,60]. In addition, it has been shown that endogenous basic FGF was required for migration of endothelial cells from the edge of a denuded area [61], and that EGF has chemotactic and mitogenic effects on epithelial cells and seems to be involved in the reepithelialization process [62,63].

These studies indicate that the occurrence and localization of the u-PA receptor in vivo must be investigated to further understand the role of the plasminogen-activation system in wound healing.

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ANNOUNCEMENT

The 18th World Congress of Dermatology will be held June 12–18, 1992 in New York. For information contact Secretariat, 18th World Congress of Dermatology, 875 Kings Highway, West Deptford, New Jersey, 08096. Telephone: (609) 845-7220. Fax: (609) 853-0411.