Methods

Western blot analysis of plg

Wild-type mice first received a standardized burn wound and then were intravenously administered 2 mg of human plg (Omnio AB, Umeå, Sweden). 24 hours after wounding and injection, wounded back skin and unwounded abdominal skin were sampled from each mouse. The samples were homogenized and lysed with lysis buffer (50 mM Tris-HCl buffer pH 8.0 with 120 mM NaCl, 20 mM NaF, 20 mM β-glycerophosphate, 1 mM EDTA, 6 mM EGTA, 1% NP-40 and 1 mM DTT). The total protein concentrations in the lysates were quantified using the Bio-Rad protein assay, according to the user’s manual (Bio-Rad Laboratories, Hercules, CA). Goat anti-plg polyclonal IgG antibody was obtained from Omnio AB (Umeå, Sweden). Anti-β-actin antibodies were obtained from Sigma-Aldrich Sweden AB (Stockholm, Sweden). Western blotting was performed according to the manufacturer’s instructions and visualized using the ECL Plus Western Blotting Detection System (Amersham Biosciences, Uppsala, Sweden).

Quantification of plasminogen accumulation by ELISA

Skin lysates were prepared, and total protein was quantified, as described above. Mouse plg levels were measured using Mouse plg ELISA kit (Kamiya Biomedical Company, Seattle, WA). Human plg level were measured in a sandwich ELISA by using mouse anti-human plg monoclonal antibody (Abcam, Cambridge, UK) as capture antibody and AP conjugated goat anti-plg polyclonal IgG (Omnio AB) as detection antibody. The plg level in the unwounded abdominal skin was used as the plg basal level. The plg levels in the wounded skin and unwounded skin were used to calculate the fold increase.

Immunohistochemical analysis

Wild-type mice in the plg-treated group first received a standardized burn wound and then were intravenously administered 2 mg of human plg (Omnio AB). Mice in the control group were wounded in the same way but not injected with plg. Skin samples were collected 24 hours after wounding and injection. Paraffin embedded wound tissues were sectioned 6 µm thick perpendicular to the wound. Tissue sections were immunohistochemically stained with HRP conjugated Goat anti-human plg antibody
(Omnio AB) and visualized by using ABC staining system (Santa Cruz Biotechnology, Santa Cruz, CA). Finally all sections were counterstained with Mayers hematoxyline (Histolab, Gothenburg, Sweden). Images were taken with a Leica DC300F digital camera attached to a Leica DMLB microscope (Leica, Wetzlar, Germany). Slides incubated with normal goat serum (Dako, Glostrup, Denmark) instead of antibody served as negative controls, and all were found to be negative.

**FACS analysis of plg binding to peripheral leukocytes after injury.**

Human plg was labeled with Alexa Fluor® 488 according to the instructions of the manufacturer (Invitrogen AB, Lidingo, Sweden). The labeling efficiency was 40%. Wild-type mice first received standardized burn wounds, after which 2 mg human plg (containing 0.8 mg fluorescent labeled plg) were intravenously injected. At 24 hours after wounding and injection, blood samples were collected. The erythrocytes were lysed immediately with a solution containing 0.15M NH4Cl for 5 min, and after washing the remaining leukocytes were resuspended in 500 μl PBS. FACS analysis was performed using a Cytomics FC500 (Beckman Coulter).
Figure S1. Detection of plg accumulation in skin samples in wild-type mice
Skin samples were collected at 24 hours after wounding from wounded skin and unwounded skin from non-injected mice and mice injected with 2mg of plg. (A) Western blot analysis was performed to measure plg accumulation in wounded skin and unwounded control skin. Lane 1, unwounded skin from untreated mice. Lane 2, wounded skin from untreated mice. Lane 3, unwounded skin from plg-treated mice. Lane 4, wounded skin from plg-treated mice. (B) ELISA measurements were used to quantify the accumulation of plg in samples. There was a 4.4-fold increase of the plg level in the wound sites of untreated mice (n = 5) and a 19-fold increase of plg in the wound sites of plg-treated mice (n = 3). ** p < 0.01.
Figure S2. Immunohistochemical staining of human plg in wounds from plg-treated and untreated control mice
Skin samples were collected 24 hours after wounding and plg injection. Representative samples from untreated control group (A) and plg-treated group (B) were immunostained with antibodies to human plg. A clear staining of human plg was observed in the plg-treated group at the edge of the wound where the inflammatory cells infiltrate and in the panniculus carnosus region. The magnification is ×50; ×200 (insets).
Figure S3. Detection of exogenous plg bound to peripheral inflammatory cells in wild-type mice.
FACS analyses of peripheral leukocytes collected from untreated wild-type mice (black tracings) and from mice treated with fluorescent labeled plg (red tracings). Neutrophils and monocytes were gated based on their light scatter profile. A clear positive signal was observed when fluorescent plg was injected, indicating that exogenous plg binds to peripheral inflammatory cells after wounding.