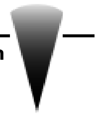

ORIGINAL RESEARCH ARTICLE – BASIC SCIENCE

Mactinin treatment promotes wound-healing-associated inflammation in urokinase knockout mice

Sharon D. Luikart, MD[1,2]; Brett Levay-Young, PhD[3]; Tim Hinkel, MS[1]; Jeffry Shearer, MA[3]*; Charles Mills, PhD[3]; Michael D. Caldwell, MD, PhD[3]; Margaret R. Gyetko, MD[4]; Theodore R. Oegema, PhD[5]

1. Veterans Affairs Medical Center, Minneapolis, Minnesota
2. Department of Medicine and
3. Department of Surgery, University of Minnesota, Minneapolis, Minnesota
4. Veterans Affairs Medical Center and Department of Internal Medicine, University of Michigan, Ann Arbor, Michigan, and
5. Department of Biochemistry, Rush University, Chicago, Illinois

Reprint Requests:
Sharon D. Luikart, MD, Veterans Affairs Medical Center (111E), One Veterans Drive, Minneapolis, MN 55417.
Fax: (612) 725-2149;
Email: Sharon.Luikart@MED.VA.GOV

*Current address: (J.S.) – DVC, LLC, Frederick, MD; (M.D.C.) – Wound Healing Program, Marshfield Clinic, Marshfield, MN

Manuscript received: January 5, 2005
Accepted in final form: September 1, 2005

DOI:10.1111/j.1743-6109.2006.00101.x

ABSTRACT

Mactinin, a 31 kDa fragment from the amino-terminal end of α-actinin, is chemotactic for monocytes and can promote monocyte/macrophage maturation. Macrophages are essential for wound healing, in which they play key roles in debridement, angiogenesis, fibroblast proliferation, and collagen metabolism. We have previously determined that urokinase is necessary to form mactinin from extracellular α-actinin, which may be present at sites of inflammation as a result of cell movement. Thus, urokinase knockout mice are unable to form mactinin and therefore are an ideal model to study mactinin's effects on wound healing. Saline- and mactinin-treated wounds were analyzed in a subcutaneous sponge wound model in both wild-type and urokinase knockout mice. The wounded urokinase knockout mice had markedly decreased leukocyte infiltration compared with wounded wild-type mice. In addition, production of the proinflammatory cytokine, interleukin-12, and of collagen was also decreased in knockouts. Treatment of knockout mice with mactinin resulted in leukocyte infiltration numbers, interleukin-12 levels, and hydroxyproline measurements similar to those in wild-type mice. The results suggest that impaired wound healing in urokinase-deficient mice can be restored by administration of mactinin.

It is known that macrophage function is important for wound healing.[1,2] Thus, wounds made devoid of macrophages by hydrocortisone and anti-macrophage serum[3] or depleted of macrophages by radiation therapy[4] show delayed healing. Furthermore, local injections of macrophages into wounds can accelerate healing.[5] Usually after injury, monocytes, in response to chemotactic factors, arrive at the wound site within 6–8 hours.[6] Once in the tissue, these blood cells can differentiate into macrophages.[7] In the early phase of wound healing, macrophages and the cytokines and enzymes that they produce are important for debridement and angiogenesis.[1] In later stages, they promote fibroblast proliferation and modulate collagen metabolism. Indeed, agents that enhance macrophage function, including glucan[1] and macrophage colony-stimulating factor,[8] have shown beneficial effects on wound healing.

Mactinin is a 31 kDa amino-terminal fragment of the cytoskeletal protein, α-actinin, which promotes monocyte/macrophage maturation[9] and is chemotactic for monocytes.[10] Therefore, it may play a role in recruiting monocytes to areas of inflammation and in enhancing their function. The mactinin fragment can be generated by monocyte-secreted urokinase degradation of extracellular α-actinin.[11,12] α-Actinin is present in focal adhesion complexes[13] and may be left in the microenvironment as a result of cell movement,[11] such as may occur during the inflammatory response. Thus, it is not surprising that we have found mactinin at various sites of inflammation, including in bronchoalveolar lavage fluid from mice after intratracheal challenge with *Pneumocystis carinii*[14] and in synovial fluid from patients with various arthritides.[10]

Urokinase-deficient animals are not able to produce mactinin.[12] These animals also have deficient macrophage recruitment in response to infection[14] and other inflammatory conditions.[15] Approximately 5% of urokinase knockout mice develop nonhealing ulcerations of the eyelids and ears, possibly from scratching against the cage grid.[16] Indeed, the absence of urokinase may have many effects on wound healing. Urokinase mediates arterial neointima

GST	Glutathione S-transferase
IL	Interleukin
uPA	Urokinase-type plasminogen activator

formation during vascular wound healing,[17] and urokinase knockout mice show reduced neointima formation and smooth muscle migration following arterial injury. Vascular smooth muscle cells from urokinase-deficient animals also display a reduced mitogenic response to fibroblast growth factor in vitro.[18] Macrophages derived from urokinase knockouts also display reduced ability to degrade extracellular matrix, an important step in tissue remodeling.[16] The current studies should help establish the role of urokinase-generated mactinin in wound healing.

In these studies, using a sponge wound model,[19–21] we examined wound healing in wild-type and urokinase knockout mice by assessing leukocyte infiltration, histology, cytokine production, and collagen production as measured by hydroxyproline content. By measuring the effects of mactinin treatment in this model, the current studies suggest that mactinin plays an important role in wound healing.

MATERIALS AND METHODS

All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of the Minneapolis Veterans Affairs Medical Center.

Source of mactinin

Because mactinin is difficult to purify from urokinase degradation of 100 kDa α-actinin, a fusion protein containing the chicken actin-binding domain of α-actinin, which differs from mactinin only in containing a few additional amino acid residues on the carboxyl end, and glutathione S-transferase (GST) with an engineered thrombin cleavage site was expressed in *Escherichia coli* and cleaved.[12] The cleavage products were separated by reverse-phase high-performance liquid chromatography on a C-4 column, yielding more than 90% of endotoxin-free α-actinin fragment, with the remaining 10% being GST. This preparation will be referred to as recombinant mactinin and was used both to treat animal wounds and to generate antisera for detecting mactinin.

Antisera production

To generate highly sensitive antisera for detecting mactinin, recombinant mactinin was modified by dinitrophenol coupling and injected into New Zealand white rabbits with complete Freund's adjuvant as described previously.[12] Boosters were performed with fragment and incomplete Freund's adjuvant. Antisera were immunoaffinity purified as described previously.[12]

Animals for wounding

Initial wounding experiments were conducted on Fisher rats (150–200 g, Charles River Breeding Laboratories, Wilmington, MA) to determine the optimal method of mactinin treatment delivery. Transgenic urokinase-type plasminogen activator (uPA)-deficient mice (uPA $-/-$) and background-matched control mice (wild-type) were then studied. The uPA knockout mice were generous gifts from Dr. Peter Carmeliet. They were developed as described previously,[16] and their genotype was confirmed by PCR analysis.[22] Three animals were used per treatment group.

Sponge implantation

Animals were anesthetized with sodium pentobarbital (5 mg/100 g of body weight). The lower back was shaved and the skin cleansed with a 70% solution of isopropanol. In the rats, cellulose vicose sponges (Cellomedia, OY, Turku, Finland) consisting of a cylinder (15 mm long and 10 mm diameter) with a central tunnel (3 mm diameter) were used. Before implantation, silicone rubber septa were stitched on each end to create a stable dead space. In the mice, polyvinyl alcohol sponges (Unipoint Industries, High Point, NC) measuring $\sim$1 cm^2 and 0.5 cm in thickness were used. The sponges were implanted subcutaneously in the dorsum of each animal under sterile conditions.[19] The incision was closed with autoclips. At designated times after implantation of the sponges (1, 3, or 7 days), animals were killed with CO_2 asphyxiation and the sponges were harvested.[20]

Mactinin treatment of wounds

All sponges were soaked overnight in saline alone, 0.2 nM GST in saline, or 2 nM recombinant mactinin in saline before wounding. The GST concentration was one-tenth that of mactinin to control for the contamination of GST in the recombinant mactinin. This concentration of mactinin is above the threshold concentration required for macrophage maturation and chemotactic activity in vitro.[10] In later experiments, an Alzet pump with a catheter was also filled with the appropriate agent before subcutaneous insertion. The other end of the catheter was stitched into the middle of the mouse sponge. The 100 μL pump (Alzet Pumps, Cupertino, CA, catalogue #1007D) then provided continuous infusion of the treatment for 1 or 7 days at a rate of 0.5 μL/hour. Each lot of pumps was tested by the manufacturer to determine exact pumping rate and reservoir volume and to ensure accurate compound delivery.

Sponge harvesting

After sponges were harvested, fluid contained in the sponges was squeezed out with forceps and centrifuged, and the supernatant was frozen at $-80\,^{\circ}C$ until assay. Polyvinyl alcohol sponges from mice were then placed in 5 mL syringe cylinders contained within sterile test tubes and centrifuged for 10 minutes ($400 \times g$) at 4 °C. To maximize cell recovery, the sponge was placed in a sterile Petri dish with 1 mL RPMI media containing 10% fetal calf serum, and gentle pressure was applied several times before removing the fluid with a pipette. This was repeated twice. The liquid from each sponge was pooled with the initial cell pellet for counting. An aliquot of the pooled liquid was centrifuged (Cytospin 2, Shandon, Pittsburgh, PA) onto a glass slide for nonspecific esterase staining.[9]

Results are expressed as mean ± standard deviation, and comparisons were made using a two-tailed unpaired *t* test. To increase sample numbers while using few animals, saline-treated and GST-treated mice were combined as the control group. Some sponges are routinely analyzed for

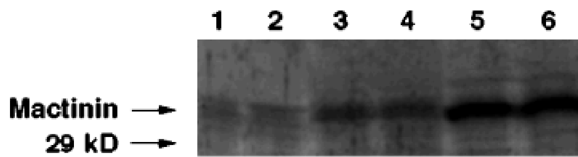

Figure 1. Western blot analysis of rat wound fluid at various time points. Rat wound fluid (10 μL) was harvested from cellulose viscose sponges on day 1 (lanes 1 and 2), day 3 (lanes 3 and 4), and day 7 (lanes 5 and 6) postwounding. Controls with rabbit immunoglobulin G were negative for all samples. The 29 kDa molecular weight marker is indicated.

cell recovery by measuring DNA content after processing by the method of Singer et al.[23]

Sponge histology

At initial harvest, a small piece of each sponge was frozen in liquid nitrogen. The pieces were then sectioned and stained with hematoxylin–eosin for light microscopic analysis.

Wound fluid analysis

Aliquots of wound fluid were subjected to electrophoresis on a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel under reducing conditions. Immunoblot analysis using affinity-purified rabbit antisera raised against recombinant mactinin was performed as described previously.[12] All Western blot analyses were repeated with similar results and also run using rabbit immunoglobulin (Ig)G controls. Wound fluid was also assayed for interleukin (IL)-12 p40 by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN).

Hydroxyproline measurement

Hydroxyproline content of the harvested sponges was determined using the method described by Reddy and Enwemeka.[24] Briefly, sponges were lyophilized and weighed, and then hydrolyzed in 2 N sodium hydroxide. Aliquots of the hydrolyzed samples were reacted with chloramine-T and Erhlich's reagent, and the absorbance of the resulting solution at 550 nm was compared with standard hydroxyproline.

RESULTS

To determine whether mactinin is present in wounds, the cell-free supernatant from centrifuged, harvested saline-soaked control sponges was analyzed by Western blot using affinity-purified antisera to mactinin. Both rats (Figure 1) and wild-type mice (Figure 2, lanes 2–4) had an immunoreactive band corresponding to mactinin in wound fluid 24 hours after wounding. Our rat data suggest that the amount of mactinin increases through day 7. That is, by densitometry, 10 μL of wound fluid on day 1 contained 0.62 $\pm$ 0.17 ng mactinin, 1.26 $\pm$ 0.32 ng on day 3, and 2.21 $\pm$ 0.27 ng on day 7.

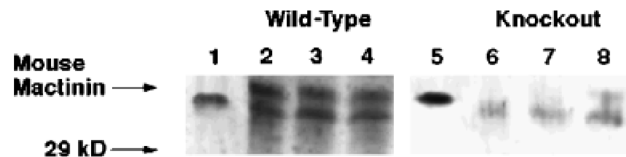

Figure 2. Western blot analysis of murine wound fluid from wild-type and uPA knockout mice. Wound fluid (10 μL) harvested from wild-type (lanes 2–4) and uPA knockout (lanes 6–8) mice 1 day after wounding was analyzed. Control chicken mactinin (0.4 ng) is shown in lanes 1 and 5. In the mouse, the calculated molecular weight of mactinin is 31.8 kDa, which is 820 Da more than in the rat or chicken. The lower band seen in lanes 2–4 and 6–8 is also present in controls with rabbit immunoglobulin G.

Mactinin is not produced by urokinase knockout mice

We have previously reported that urokinase is necessary and sufficient for mactinin formation in vitro and in vivo.[12] In that report, bronchoalveolar lavage fluid from urokinase knockout and wild-type mice was studied after intratracheal challenge with *P. carinii*. Although most (6 of 8) wild-type mice had mactinin in their inflammatory fluid samples, none (0 of 8) of the knockout mice had mactinin. As shown in Figure 2, knockout mice in the sponge wound model also lack mactinin (upper band) in their inflammatory fluid (lanes 6–8) in contrast to wild-type mice (lanes 2–4). There may be a minimal amount of mactinin in one of the knockouts (lane 8), but it is well below the level of in vitro activity. The lower band in the mouse wound fluid lanes is also present in controls run with rabbit IgG as the primary antibody, suggesting that it represents nonspecific cross-reactivity.

Effects of mactinin treatment on wound cellular infiltrate

In initial experiments, soaking the sponges in mactinin overnight before implantation did not appear to be an adequate form of mactinin delivery in rats. That is, there was no difference in the amount of mactinin detected in harvested fluid from mactinin-treated or saline-treated sponges and no difference in markers of wound repair or histology. We felt that this lack of demonstrable mactinin may be due to loss or degradation of mactinin. So, in the present treatment studies in wild-type and uPA knockout mice, osmotic pumps were used to continuously deliver the fragment and replenish the mactinin in the sponges every 6 hours. That is, the sponge was first soaked overnight in 2 nM mactinin, a concentration with in vitro maturation-promoting and chemotactic activity. Then, at an infusion rate of 0.5 microliters per hour and a measured sponge volume of 500 μL, the pump was filled with 333 nM mactinin to fill the sponge with 2 nM every 6 hours.

As shown in Table 1, wounds in uPA knockout mice had significantly reduced total and esterase-positive infiltrating leukocytes compared with those in wild-type mice. However, mactinin significantly increased the number of leukocytes in knockout mice wounds to a level not significantly different from that seen in wild-type mice by day 7. In contrast, a significant increase in white blood cell

Table 1. Mactinin's effects on leukocyte infiltration on day 7

	Wild-type		uPA knockouts	
Treatment	WBC	Esterase +	WBC	Esterase +
Control	19.7 ± 6.6	7.5 ± 2.4	5.0 ± 2.1	2.6 ± 1.0
Mactinin	27.3 ± 12.5	17.5 ± 3.2	14.4 ± 2.6	6.7 ± 0.4

Values ($\times 10^{-5}$) are expressed as the means ± SD and represent total cells per sponge. WBC is an abbreviation of white blood cells. The following comparisons were significantly different by an unpaired, two-tailed *t* test. WBC-control knockouts vs. control wild-types ($p = 0.0078$), mactinin knockouts vs. control knockouts ($p = 0.0032$), mactinin wild-types vs. control knockouts ($p = 0.0167$). Esterase-positive – control knockouts vs. control wild-types ($p = 0.0333$), mactinin knockouts vs. control knockouts ($p = 0.03$), mactinin wild-type vs. control knockouts ($p = 0.0043$). Mactinin wild-types vs. control wild-types ($p = 0.0282$).
All other comparisons are not significant.

infiltrate was not seen on day 1 in mactinin-treated knockouts (300,000 ± 151,300 cells per sponge) vs. saline-treated knockouts (216,700 ± 100,500). This lack of early effect may have been due to the monocytic component of the infiltrate being minor (about 10%) on day 1. The numbers of cells staining positive for nonspecific esterase, which indicates monocytic differentiation, were also significantly increased by mactinin treatment of wounds in uPA knockout mice on day 7 (Table 1), but not day 1 (data not shown). In addition, in the wild-type mice, mactinin treatment increased esterase-positive cells significantly compared with controls.

Effects of mactinin treatment of wounds on IL-12 levels

As shown in Table 2, IL-12, which is produced by phagocytes and plays a key role in cell-mediated immunity,[25] was significantly decreased in wounds of uPA knockout mice compared with those in wild-type mice. Further, mactinin treatment of knockout mice significantly increased IL-12 to a level not significantly different from that seen in wild-type mice. In contrast, as also seen in Table 2, mactinin treatment of wild-type mice had no significant effect, perhaps due to the endogenous production of mactinin.

Effects of mactinin treatment of wounds on collagen production

Collagen production was assessed by measuring hydroxyproline content of the day 7 harvested sponges, as shown in Table 3. The control uPA knockout mice had significantly less hydroxyproline compared with control wild-type mice. Also, mactinin treatment of knockouts significantly increased hydroxyproline content to a level not significantly different from control wild-type animals. Although mactinin did not significantly increase hydroxyproline in sponges from wild-type animals, levels in mactinin-treated wild-type animals were significantly different from mactinin-treated uPA knockouts.

Table 2. Mactinin's effects on IL-12 levels on day 7 (pg/ml)

Treatment	Wild-type	uPA knockouts
Control	1930 ± 210	1352 ± 146
Mactinin	1634 ± 106	2120 ± 196

Values (pg/mL) are expressed as the mean ± SD.
The following comparisons were significantly different by an unpaired, two-tailed *t* test: control wild-type vs. control knockouts ($p = 0.0075$) and mactinin-treated knockouts vs. control knockouts ($p = 0.0053$). All other comparisons are not significant.

Effects of mactinin on wound histology

As shown in Figure 3, light microscopy confirms our quantitative data in Table 1 that mactinin treatment increased the cellular infiltrate in uPA knockout mice (B) compared with saline-treated knockouts (C) and restored it to a level similar to that seen in wild-type mice (A).

DISCUSSION

Urokinase knockout animals are a useful model in which to study wound healing. Urokinase has been implicated in cell migration, angiogenesis, extracellular matrix synthesis, and tissue remodeling,[26] all important steps in wound healing. Indeed, urokinase expression is up-regulated during healing in the tendon healing model[26] and following mechanical wounding of corneal epithelial cells.[27] Consequently, it is not surprising that the uPA knockouts would

Table 3. Mactinin's effects on hydroxyproline production on day 7

Treatment	Wild-type	uPA knockouts
Control	0.40 ± 0.06	0.12 ± 0.03
Mactinin	0.51 ± 0.08	0.32 ± 0.03

Values are expressed as the mean ± SD (μg/mg sponge).
The following comparisons were significantly different by an unpaired two-tailed *t* test: control wild-type vs. control knockouts ($p = 0.0002$), mactinin-treated knockouts vs. control knockouts ($p = 0.0002$), and mactinin-treated knockout vs. mactinin-treated wild-types ($p = 0.0263$). All other comparisons are not significant.

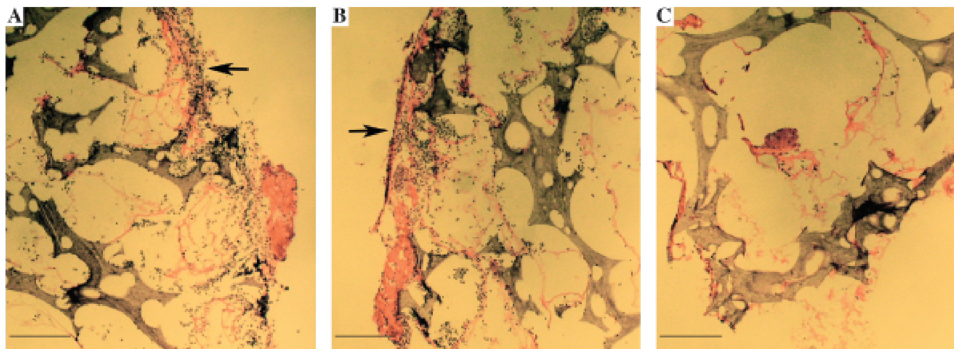

Figure 3. Sponge wound histology. Sponges were harvested on day 7, frozen in liquid nitrogen, sectioned, and stained with hematoxylin–eosin. Photographs were taken from a representative animal of each group. The arrows indicate areas of cellular infiltrate. (A) Saline-treated wild-type mouse; (B) mactinin-treated urokinase knockout mouse; (C) saline-treated urokinase knockout mouse. (Original magnification × 100 with the scale bar representing 0.25 mm)

display decreased inflammatory cell infiltration in the sponge wound model.

In addition, because urokinase is required for the formation of the chemotactic and macrophage maturation-promoting factor, mactinin,[9] the role of mactinin in wound healing can be specifically assessed in this knockout model. Mactinin was able to significantly increase the leukocyte infiltrate and especially the monocyte/macrophage component in the uPA knockout animals. In fact, although the number of animal wounds analyzed was small, mactinin appeared to be able to completely correct the decreased mononuclear phagocyte response seen in the knockouts. This was evident by both cell counts of sponge fluid and by histological examination of the wounds. Mactinin's effects on the leukocyte infiltration in wounded wild-type mice were more modest and only reached statistical significance for the esterase positive component. This may have been due to the endogenous production of mactinin in wild-type animals.

IL-12 is produced by monocytes, macrophages, and neutrophils.[28] It acts on T cells, inducing interferon-γ production and promoting T cell differentiation.[29] It has been shown previously that urokinase knockout mice have decreased IL-12 levels in the lung in response to pulmonary *Cryptococcal neoformans* infection and display impaired antimicrobial activity.[30] In the present study, wounded urokinase knockout mice had decreased IL-12 levels compared with wild-type ones, and mactinin treatment of uPA knockout mice resulted in increased IL-12 levels similar to those seen in wild-type mice. These results indicate that urokinase-deficient animals have an impaired inflammatory response to wounding that is, in large part, corrected by urokinase-generated mactinin. In addition, mactinin appears to promote wound repair, as measured by collagen production, in the urokinase knockout mice. Given the many roles of urokinase, it is likely that there are components of wound healing in the knockouts that are not corrected by mactinin. However, the amount of fluid and sponge material available for analysis in this murine model is limited. Nevertheless, our results show a role for mactinin in wound-associated leukocyte migration, cytokine production, and collagen metabolism, and thus suggest a significant positive effect of mactinin on wound healing.

ACKNOWLEDGMENTS

These studies were supported by the Department of Veterans Affairs.

REFERENCES

1. Browder W, Williams D, Lucore P, Pretus H, Jones E, McNamee R. Effect of enhanced macrophage function on early wound healing. *Surgery* 1988; 104: 224–30.
2. Diegelmann RF, Cohen IK, Kaplan AM. The role of macrophages in wound repair: a review. *Plast Reconstr Surg* 1981; 68: 107–13.
3. Leibovich SJ, Ross R. The role of the macrophage in wound repair. A study with hydrocortisone and antimacrophage serum. *Am J Pathol* 1975; 78: 71–100.
4. Cromack DT, Porras-Reyes B, Purdy JA, Pierce GF, Mustoe TA. Acceleration of tissue repair by transforming growth factor beta 1: identification of in vivo mechanism of action with radiotherapy-induced specific healing deficits. *Surgery* 1993; 113: 36–42.
5. Danon D, Kowatch MA, Roth GS. Promotion of wound repair in old mice by local injection of macrophages. *Proc Natl Acad Sci USA* 1989; 86: 2018–20.
6. Schaffer CJ, Nanney LB. Cell biology of wound healing. *Int Rev Cytol* 1986; 169: 151–81.
7. Andreesen R, Brugger W, Scheibenbogen C, Kreutz M, Leser H-G, Rehm A, Löhr GW. Surface phenotype analysis of human monocyte to macrophage maturation. *J Leukoc Biol* 1990; 47: 490–7.
8. Wu L, Yu YL, Galiano RD, Roth SI, Mustoe TA. Macrophage colony-stimulating factor accelerates wound healing and upregulates TGF-β_1 mRNA levels through tissue macrophages. *J Surg Res* 1997; 72: 162–9.
9. Luikart S, Wahl D, Hinkel T, Masri M, Oegema T. A fragment of α-actinin promotes monocyte/macrophage maturation in vitro. *Exp Hematol* 1999; 27: 337–44.
10. Luikart S, Krug H, Nelson R, Hinkel T, Majeski P, Gupta P, Mahowald M, Oegema T. Mactinin: a modulator of the monocyte response to inflammation. *Arthritis Res Ther* 2003; 5: R310–6.
11. Masri MT, Wahl D, Oegema T, Luikart S. HL-60 cells degrade α-actinin to produce a fragment that promotes monocyte/macrophage maturation. *Exp Hematol* 1999; 27: 345–52.

12. Luikart S, Masri M, Wahl D, Hinkel T, Beck J, Gyetko M, Gupta P, Oegema T. Urokinase is required for the formation of mactinin, an α-actinin fragment that promotes monocyte/macrophage maturation. *Biochim Biophys Acta* 2002; 1591: 99–107.
13. Burridge K, Fath K, Kelly T, Nuckolls G, Turner C. Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. *Annu Rev Cell Biol* 1988; 4: 487–525.
14. Beck JM, Preston AM, Gyetko MR. Urokinase-type plasminogen activator in inflammatory cell recruitment and host defense against *Pneumocystis carinii* in mice. *Infect Immun* 1999; 67: 879–84.
15. Kitching AR, Holdsworth SR, Ploplis VA, Plow EF, Collen D, Carmeliet P, Tipping PG. Plasminogen and plasminogen activators protect against renal injury in crescentic glomerulonephritis. *J Exp Med* 1997; 185: 963–8.
16. Carmeliet P, Schoonjans L, Kieckens L, Ream B, Degen J, Bronson R, DeVos R, van den Oord JJ, Collen D, Mulligan RC. Physiological consequences of loss of plasminogen activator gene function in mice. *Nature* 1994; 368: 419–24.
17. Carmeliet P, Moons L, Herbert JM, Crawley J, Lupu F, Lijnen R, Collen D. Urokinase but not tissue plasminogen activator mediates arterial neointima formation in mice. *Circ Res* 1997; 81: 829–39.
18. Herbert JM, Lamarche I, Carmeliet P. Urokinase and tissue-type plasminogen activator are required for the mitogenic and chemotactic effects of bovine fibroblast growth factor and platelet-derived growth factor BB for vascular smooth muscle cells. *J Biol Chem* 1997; 272: 23585–91.
19. Gartner MH, Benson JD, Caldwell MD. Insulin-like growth factors I and II expression in the healing wound. *J Surg Res* 1992; 52: 389–94.
20. Albina JE, Mills CD, Henry Jr WL, Caldwell MD. Temporal expression of different pathways of 1-arginine metabolism in healing wounds. *J Immunol* 1990; 144: 3877–80.
21. Shearer JD, Coulter CF, Engeland WC, Roth RA, Caldwell MD. Insulin is degraded extracellularly in wounds by insulin-degrading enzyme (EC 3.4.24.56). *Am J Physiol* 1997; 273: E657–64.
22. Gyetko MR, Chen GH, McDonald RA, Goodman R, Huffnagle GB, Wilkinson CC, Fuller JA, Toews GB. Urokinase is required for the pulmonary inflammatory response to *Cryptococcus neoformans*: a murine transgenic model. *J Clin Invest* 1996; 97: 1818–26.
23. Singer VL, Jones LJ, Yue ST, Haugland RP. Characterization of picogreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation. *Anal Biochem* 1997; 249: 228–38.
24. Reddy GK, Enwemeka CS. A simplified method for the analysis of hydroxyproline in biological tissues. *Clin Biochem* 1996; 29: 225–9.
25. Bagby GC Jr, Heinrich MC. Growth factors, cytokines, and the control of hematopoiesis. In: Hoffman R, Benz Jr EJ, Shattil SJ, Furie B, Cohen HJ, Silberstein LE, McGlave P, editors. *Hematology basic principles and practice*. 3rd ed. New York, NY: Churchill Livingston, 2000: 154–202.
26. Xia W, de Bock C, Murrell GA, Wang Y. Expression of urokinase-type plasminogen activator and its receptor is up-regulated during tendon healing. *J Orthop Res* 2003; 21: 819–25.
27. Watanabe M, Yano W, Kondo S, Hattori Y, Yamada N, Yanai R, Nishida T. Up-regulation of urokinase-type plasminogen activator in corneal epithelial cells induced by wounding. *Invest Ophthamol Vis Sci* 2003; 44: 3332–8.
28. Romani L, Puccetti P, Bistoni F. Interleukin-12 in infectious disease. *Clin Microbiol Rev* 1997; 10: 611–36.
29. Gadina M, Ferguson P, Johnston J. New interleukins: are there any more? *Curr Opin Infect Dis* 2003; 16: 211–7.
30. Gyetko MR, Sud S, Chen G-H, Fuller JA, Chensue SW, Toews GB. Urokinase-type plasminogen activator is required for the generation of a type 1 immune response to pulmonary *Crpytococcus neoformans* infection. *J Immunol* 2002; 168: 801–9.