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Differential cytokine activity and morphology during wound healing in the neonatal and adult rat skin

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Abstract

Wound-healing mechanisms change during transition from prenatal to postnatal stage. Cytokines are known to play a key role in this process. The current study investigated the differential cytokine activity and healing morphology during healing of incisional skin wounds in rats of the ages neonatal (p0), 3 days old (p3) and adult, after six different healing times (2 hrs to 30 days). All seven tested cytokines (Transforming Growth Factor (TGF) α, TGFβ_1, -β_2 and -β_3, IGF 1, Platelet Derived Growth Factor A (PDGF A), basic Fibroblast Growth Factor (bFGF) exhibited higher expression in the adult wounds than at the ages p0 and p3. Expression typically peaked between 12 hrs and 3 days post-wounding, and was not detectable any more at days 10 and 30. The neonate specimen showed more rapid re-epithelialization, far less inflammation and scarring, and larger restitution of original tissue architecture than their adult counterparts, resembling a prenatal healing pattern. The results may encourage the use of neonatal rat skin as a wound-healing model for further studies, instead of the more complicated prenatal animal models. Secondly, the data may recommend inhibition of PDGF A, basic FGF or TGF-β_1 as therapeutic targets in efforts to optimize wound healing in the adult organism.

Keywords: wound healing • cytokines • rat • neonatal • adult

Introduction

Wounds in foetal skin heal rapidly, largely free of inflammation and without scar formation. The result is restoration of the original tissue architecture, being indistinguishable from unwounded skin, in contrast to the scar-forming tissue reparation found in adult skin. Scarless wound closure with regeneration and reformation of skin, muscles and bones, including normal long-term postnatal development of face and skull, has been demonstrated after intrauterine surgical corrections of previously created clefts in mice, sheep and monkeys in early gestation [1–4].

Foetal healing properties have been found to be tissue-specific. For example, sheep of middle gestational age demonstrate scar-free healing in skin and bone, a combination of scar-free and scar forming healing in diaphragm muscle and at the same time distinct scar formation after wounding in the gastrointestinal tract [3, 5].

During gestation, a transition phase between foetal and adult healing patterns is observed. The so-called 'transition wound' is characterized by normal

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re-epithelialization and regular reticular collagen deposition, but loss of skin appendages. In large mammals, including man, the transition stage can be found between late second and early third trimenon of gestation (overviews as above). However, small rodents, such as mouse and rat can exhibit foetal healing properties up to the neonatal stage after incisional wounds which is ascribed to their relative tissue immaturity at the time of birth [6]. Therefore, the rat was chosen as animal model in the present study, which enables investigation of foetal healing mechanisms in the early neonatal stage.

Both foetal and adult skin retains its healing properties when transferred into a different milieu. It was concluded that intrinsic, tissue specific factors cause foetal healing patterns and not extrinsic conditions, such as intrauterine sterility or amniotic fluid [7]. A number of factors are suggested to enable the foetal healing pattern, among these specific properties of foetal fibroblasts, platelets and inflammatory cells. Other differences regard composition of the extra-cellular matrix (ECM), and specific transcriptions factors (*i.e.* the homeobox-genes), which are expressed in foetal, but not in adult human skin (overviews in [1, 2]). Furthermore, wide evidence exists for an especially important role of the differential cytokine profile in foetal and adult skin. Cytokines are involved in regulation of merely all steps of the wound-healing cascade, including chemotaxis and cell migration, cell growth and proliferation, deposition of collagen and other ECM-components, and angiogenesis. Most investigated growth factors were shown to have significantly lower expression in foetal compard to adult wounds [8, 9]. Only few data, however, exist on cytokine activity in the early neonatal stage.

In this study we investigated (*i*) the healing morphology, and (*ii*) the differential expression of seven cytokines, which—among others—have been shown to play a role in wound healing and regeneration: TGF-α (mesenchymal cell infiltration and proliferation, neutrophil recruitment), TGF-β_1 and -β_2 (fibroblast chemotaxis and activation; extracellular matrix synthesis), TGF-β_3 (reduction of collagen and fibronectin deposition; reduction of scarring), Insuline Growth Factor 1 (IGF 1) (keratinocyte and fibroblast proliferation; collagen synthesis; cell metabolism; endothelial-cell activation and angiogenesis), Platelet Derived Growth Factor A (PDGF A) (activation of immune cells and fibroblasts; promotion of collagen and proteoglycan synthesis) and basic Fibroblast Growth Factor (bFGF) (endothelial-cell activation and angiogenesis; keratinocyte proliferation and migration) [9].

To the authors knowledge, this is the first study comparing wound healing and cytokine activity at the three ages neonate, p3 and adult at different healing times.

Materials and methods

Animal experiments

Wistar rats of the ages p0 (neonatal), p3 (3 days old) and adult (3 months old, 250–300 g) were used as experimental animals (Charles River, Sulzfeld, Germany). Both male and female rats were used. Anaesthesia was achieved by ether inhalation for p0 and p3 animals, while intraperitoneal injection of a ketamine (100 mg/kg body weight) and rompun (5 mg/kg body weight) was applied for the adult rats. The back of the adult animals was shaved with an electric shaver. In all three ages, one full-thickness incision of 10 mm length was performed in the skin of the animals´ back. The incision was placed in the midline of the back, and extended from the level of the first thoracic vertebra to caudal direction. The complete and standardized cut through the skin could be recognized performed with magnifying glasses by appearance of the muscular fascia underneath the skin. Whereas the control animals did not receive any treatment, the sham controls underwent the same treatment as their experimental counterparts (anaesthesia, shaving of the back in adult animals, post-op analgesia), except for the incision. The sham controls were intended to exclude influence on wound healing and cytokine expression by factors other than the incision, that is the animal´s stress during application of anaesthesia, superficial irritation of the skin through shaving or side effects of the post-op analgesia.

In each of the three age groups, the wounds were allowed to heal for six different times (2 hrs, 12 hrs, 24 hrs, 3 days, 10 days, 30 days), resulting into 18 experimental groups. Each group contained six animals: four experimental animals, one control, one sham control (n total = 108). After the according healing time, the animals were sacrificed, and the skin area containing the lesion was excised.

Use of the laboratory animals was indicated and licensed (HN 2/05) by the local authorities (Regierungspräsidium Tübingen).

Tissue processing and immunocytochemistry

Tissue of the skin excision was fixed in 2% pH neutral formalin for 2 hrs and embedded in paraffin before processing. 8-mM thick sections were mounted on silanized slides and allowed

to dry overnight at 37°C. Sections are deparaffinized 4 x 5 min in Roticlear (Roth, A535.1) and decreasing ethanol, rehydrated and finally washed in Tris-Buffer (pH7.6) for 10 min. For antibody masking sections were pre-treated in microwave oven in citrate buffer (pH 6.0) at 95°C for 20 min as described [10]. Slides were incubated with 3 % hydrogen peroxide for 5 min. Endogenes Biotin was blocked with blocking solution (Dako, Glostrup Denmark, Cat No X0590). Slides were rinsed in buffer (10 mM PBS, pH 7.4). The slides were then incubated overnight at 4°C with the following primary antibodies in normal goat serum (Sigma G 9023) in 10 mM PBS, pH 7.4: Anti-TGF-b1 (rabbit polyclonal IgG, SantaCruz, #sc-90, dilution 1.200), anti-TGF-b3 (rabbit polyclonal IgG, SantaCruz, #sc-82, dilution 1:250), anti-PDGF A (rabbit polyclonal IgG, SantCruz #sc-128), anti-TGF-a (mouse monoclonal IgG, Abcam Co, Cambridge UK, dilution 1:15), anti-bFGF 2 (monoclonal igG, Upstate biomol, Lake placid NY, clon bFM-2, 10 mg/ml, anti-IGF1 (mouse monoclonal IgG, Upsated biomol, Lake placid NY sm1.2, 10 mg/ml).

After two rinses inbuffer, the slides were incubated with a horseradish peroxidase-labelled polymer coupled to secondary antibodies for 30 min. Tissue staining was visualized with diaminobenzidine as substrate-chromogen solution. Slides were counterstained with hematoxylin, dehydrated and mounted. Negative control sections were processed without the primary antibody, but with an irrelevant murine IgG1 supplied with the kit.

For hematoxylin-eosin staining cochleae were thawed and stained for 5 min in Mayers Hemalaun solution (Carl Roth GmbH, Karlsruhe, Germany), rinsed 10 min with running water, washed shortly in A. dest., stained 7 min in 0.1% Eosin G solution in water, washed 5 min in A. dest. and embedded in Moviol. Sections were embedded with Moviol (Hoechst) and photographed performed with an Olympus AX70 microscope equipped with epifluorescence illumination.

The intensity of immunostaining was evaluated semiquantitatively by light microscopy. Microscopic evaluation was performed independently by two observers (authors WW and MW), who were both blinded to the slides. The amount of staining product was classified as showing no (–), slight (+), moderate (++) or strong (+++) immunoreactivity, taking into account both the staining intensity and the size of the stained area in the wound site.

Results

Healing morphology

Two-hr post-wounding (p.w.), incisional gaps filled with blood and fibrin were seen in all age groups (Fig.1). The wounds gaped wider in the p0- and p3-animals than in the adults. There was only little cellular activity perceivable at 2 hrs (immigration of sparse granulocytes in p3 and adult). Beginning immigration of lymphocytes to the wound site was observed 24 hrs p.w. in the p0- and p3-specimen, however, the granulocytes still being predominant. In comparison, presence of granulocytes was lower in the adult stage at that time point, with incomplete infiltration of the fibrin-clots. In general, an inflammatory reaction characterized by immigration of granulocytes, makrophagen und few lymphocytes was observed both at ages p0 and adult, however, that inflammation lasted distinctively shorter at p0 than at adult (ca. 3 days *versus* ca. 30 days).

Re-epithelialization of the wound gap was complete after 1–3 days in the neonate animals, as compared to 3–10 days in the adults. In the p0- and the p3-animals, the newly formed epithelium was thicker than normal at 1 day and at 3 days p.w., but always gained normal thickness in the further course, making the border between lesion and surrounding tissue indistinguishable. In the adult specimen, in contrast, the re-grown epithelium often remained thickened and exhibited calibre steps at the border to the unwounded epithelium (Table 1).

Thirty days p.w., fibrosis (irregular collagen deposition) was absent or minimal in the neonate rats, density of the skin appendages was normal or only slightly rarefied. In contrast, the adult skin showed the expected distinct scar formation with irregular nonreticular collagen deposition and complete lack of appendages in the lesion area. In summary, the neonate skin healed the incisions in a mainly foetal-like healing pattern. The p3-animals represented a transition stage between p0 and adult in all histopathogical aspects described here, with closer similarity to the p0-morphology than to the adult stage.

Cytokine expression

As an overall result, expression of all seven investigated cytokines was distinctly higher in the area of the lesion in the adult animals than at the ages p0 and p3 (Fig. 2 shows expression of TGF-α as example). P0 and p3 did not differ much in their paucity of cytokine expression. The only significant expressions in p0 and p3 were the following: low (to medium)

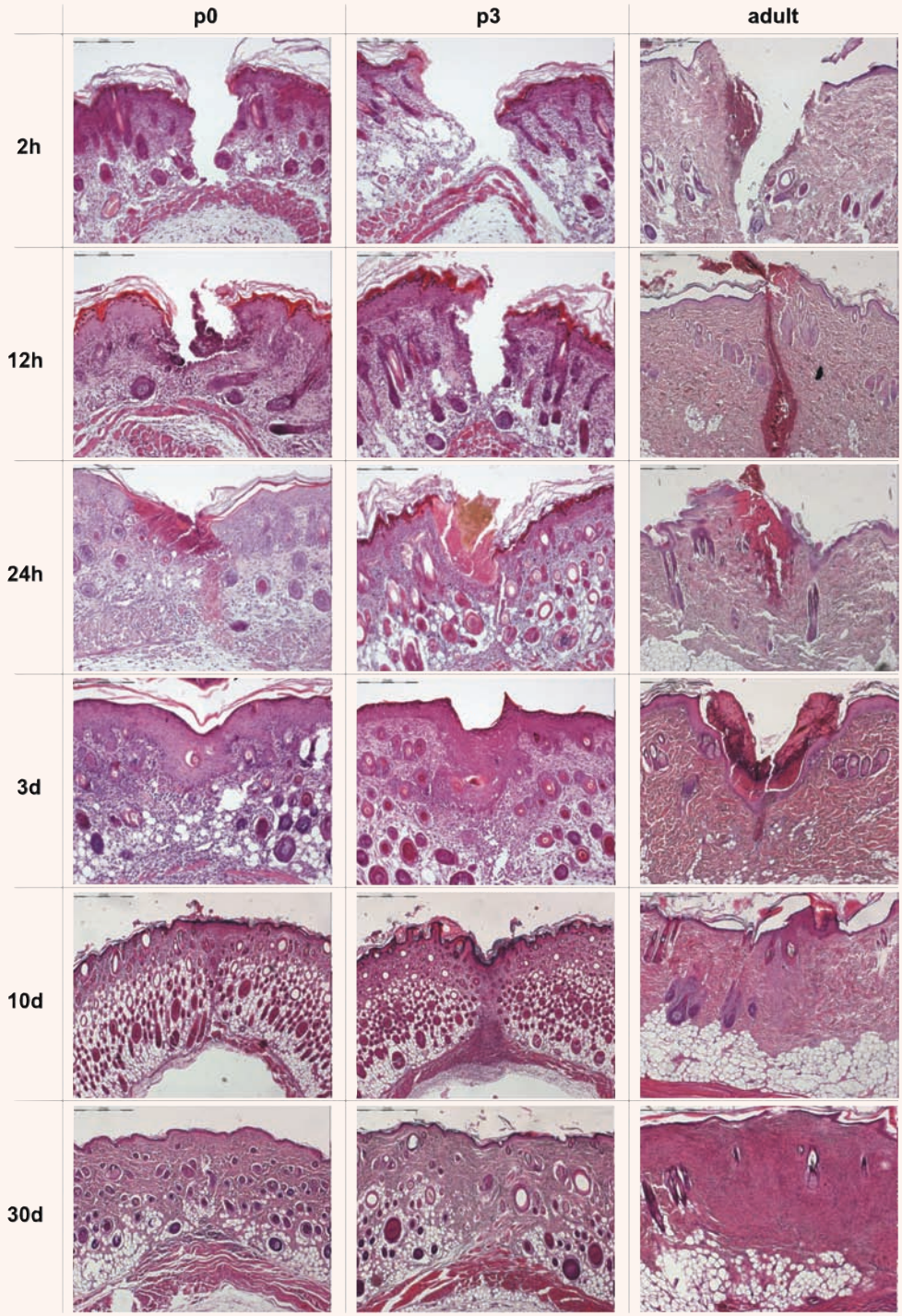

Fig. 1 Incisional wound area after six different healing times (2 hrs, 12 hrs, 24 hrs, 1 day, 3 days, 10 days, 30 days) in neonatal, 3 day old and adult rat skin. Haematoxylin-Eosin-stain. Bar in left top = 200 μm (p0 and p3 at 2 hrs, 12 hrs, 24 hrs and 3 days) or 500 μm (p0 and p3 at 10 days and 30 days, all adult sections).

Table 1 Comparison between important morphologic features in neonatal and adult rat skin during healing of incisional wound

	Neonatal	Adult
Inflammation (Immigration of granulocytes, macrophages, lymphocytes)	Low	High
Duration of inflammation	3 days	30 days (longer?)
Time until re-epithelialization	1-3 days	3-10 days
Morphology of new epithelium	Normal	Often thickened, calibre steps
Skin appendages	Normal or slightly rarefied	Markedly rarefied or missing
Fibrous tissue (fibrocytes) with collagen dispositon	Not or mildly apparent	Moderate or massively apparent

expression of TGF-α in p0 and p3 at 12 hrs, 24 hrs and 3 days p.w., low expression of IGF 1 in p0 at 3 days p.w., low expression of TGF-β_2 in p3 at 3 and 10 days p.w., and low expression of IGF 1 in p3 at 12 hrs and 10 days p.w.. However, expression was higher in all corresponding adult specimen (Figs. 3 and 4).

Expression of most cytokines peaked between 12 hrs and 3 days after the incision. Typically, there was no or very little cytokine stain 2 hrs p.w., and 10 days and 30 days p.w.. Only IGF 1 showed an earlier time course with expression at 2 hrs p.w. in the adult specimen, expression peak at 12 hrs and 24 hrs and diminished detectability 3 days p.w..

In all age groups, intensity of the immunostain (stain per area) was mostly similar in the newly formed epithelium and in the unwounded epithelium surrounding the lesion.

There was no difference in cytokine expression between control animals and sham controls.

Discussion

Healing morphology

To the authors´ knowledge, this is the first study comparing wound healing at the three ages neonate, p3 and adult at different healing times. In the neonate animals, a mainly foetal-like healing pattern was observed. Scar formation was absent or minimal, complete or nearly complete re-growth of skin appendages occurred, and the re-formated epithelium appeared normal. The lesion area was almost indistinguishable from the surrounding tissue. Much more literature data refer to prenatal healing than to healing in the neonatal stage. In large mammals, the transition between foetal and adult healing pattern is observed between second and third trimenon of gestation (see Introduction), whereas mall rodents, such as mice and rats can retain foetal healing properties until the early neonatal stage after wounding of limited extent. In line with our results, Boon *et al.* [6] observed largely identical scarless morphology and content of hyaluronic acid in mice of the late foetal stage and 2 days postpartum. Full-thickness incisions in the upper lip of rats at 19.5 days of gestation (term 21 days) exhibited scarless healing [11]. Hallock *et al.* [12] even reported foetal characteristics of a high percentage of type III collagen relative to type I and low total collagen content as long as 10 to 15 days post- partum in rat skin. Whitby *et al*. [13] compared incision wounds in foetal, neonatal and adult mice. Their study focussed on the differential cytokine activity and not on healing morphology, however, reported time until re-epithelialization in the neonatal animals is well in line with our findings. Studies reporting scar formation in rats in the foetal stage refer to excisional, not to incisional wounds [14, 15]. Excision wounds in rats heal scarlessly until day 16 of gestation (term 21.5. days), while slight scar formation begins at day 18 [16] or day 19 [17]. These findings illustrate that the combination of wound size and gestational age sets the transition threshold between scarfree and adult healing [18]. We conclude from our findings and literature data that relatively small, *e.g.* incisional wounds, can heal in a foetal-like manner in rats until the immediate neonatal stage, while greater lesions such as excisions need to be created prenatally to heal scarlessly. The scar formation with non-reticular collagen deposition, missing

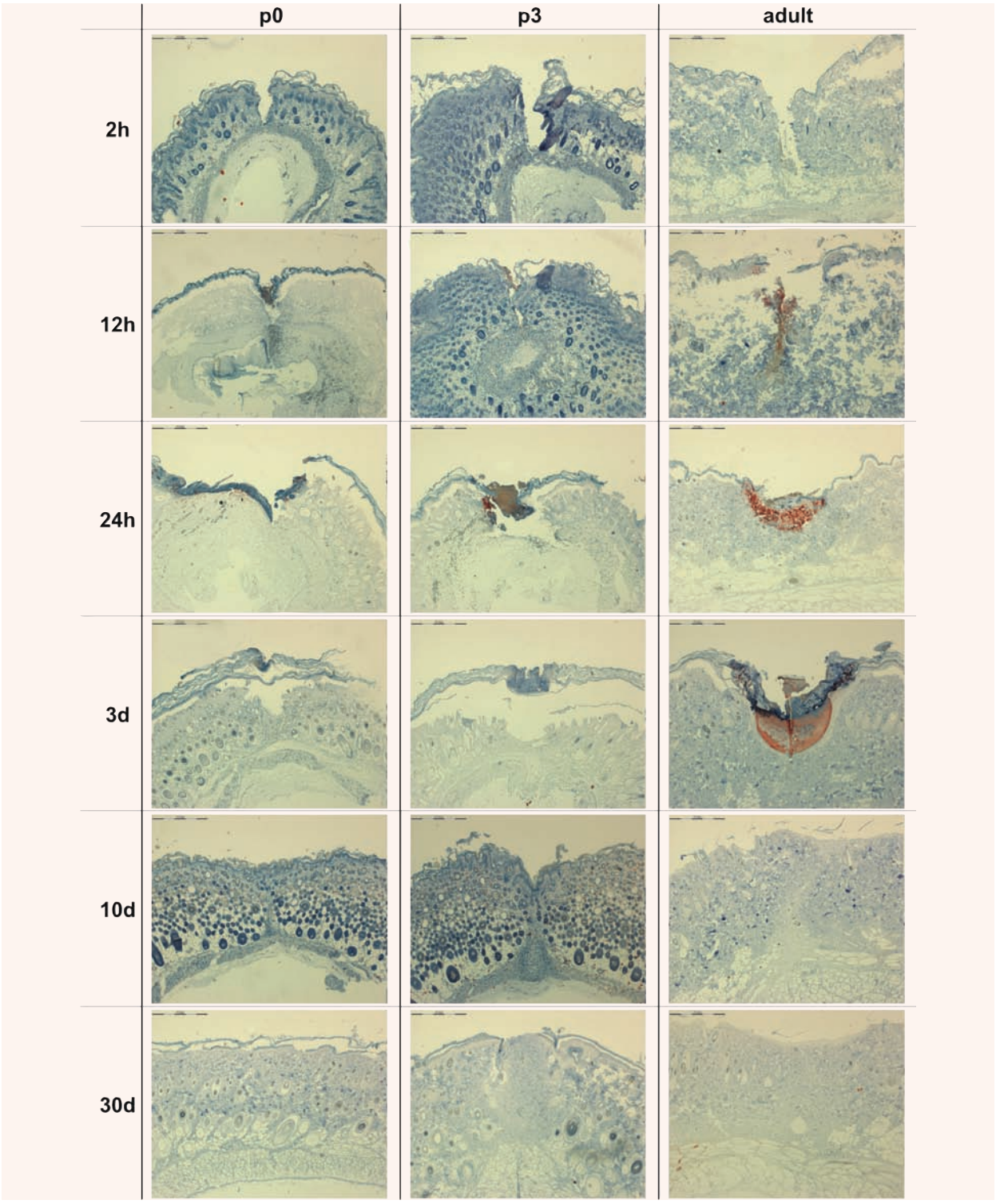

Fig. 2 Immunostain against TGF-α (example). Incisional wound area after six different healing times in neonatal, 3 day old and adult rat skin. Bar in left top = 500 μm (all sections).

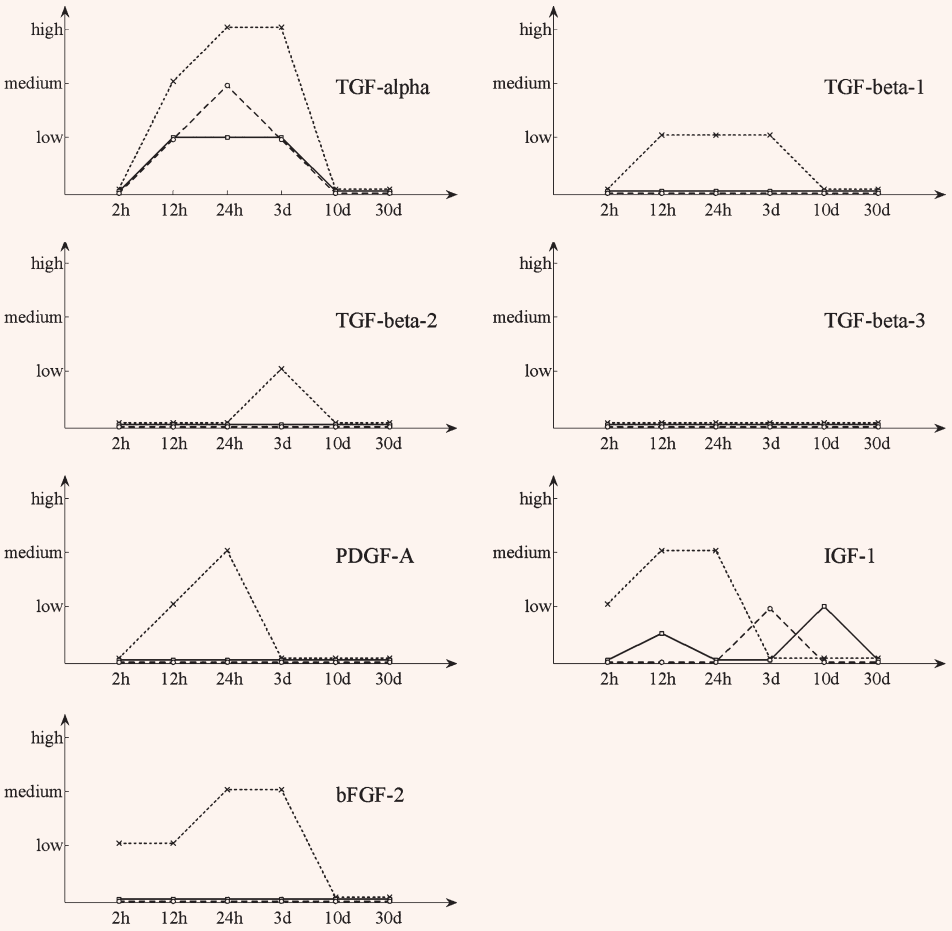

Fig. 3 Temporal distribution of cytokine expression in the wound site. Semi-quantitative evaluation. Abscissa: healing time, Ordinate: expression strength. Lines: P0: – – □ – – ; p3: – –O– –; adult: ▪▪▪X▪▪▪.

skin appendages and longer duration of re-epithelialization in the adult animals was in accordance as with literature. The p3-animals exhibited a transition morphology with closer similarity, however, to the neonatal than to the adult healing properties.

Cytokine expression

The overall findings were: 1. All investigated cytokines showed stronger expression in the adult wound than at p0 and p3, except for TGF-b3, which was not detected in any age group. Cytokine activity was generally low or absent in the neonatal stage. 2. Cytokine expression typically peaked between 12 hrs and 3 days p.w., only IGF 1 displayed an earlier time course. At 10 und 30 days p.w. no cytokine expression was detectable any more.

These findings are in accordance with previous studies. Whitby and Ferguson [23], using immunohistochemistry, reported an increase in PDGF concentration from neonatal to adult during wound healing in mice, similar as was observed in the present study. Also in line with the present results, Whitby and Ferguson reported low or missing post-lesional concentrations of TGF-β and bFGF in the foetal stage, opposed to good detectability of the cytokines in adult animals. In contrast to the presented data, both growth factors were also detectable in the neonates, however, because of the different experimental animal and different healing times, the studies are not directly comparable. Foetal porcine serum was demonstrated to have significantly lower levels of

PDGF AB, TGF-β_1, and TGF-β_2 (presumably released from platelets) compared with adult serum [19]. FGF 5 and the keratinocytes growth factors 1 and 2 exhibit progressively greater expression in healing foetal rat skin with increasing gestational age [20]. The concentration of RNA of Interleukin 6 and Interleukin 8 is distinctly lower in foetal as compared to adult human fibroblasts [21, 22].

TGF-β exhibits a differential expression pattern with opposing concentration of its three isoforms in the foetal and adult stage. At gestational ages associated with scarless healing, low levels of TGF-β_1 and high levels of TGF-β_3 are expressed in rats [23], suggesting that the relative proportion of each isoform may be crucial for repair without a scar, which has also been indicated by therapeutic studies applying addition or neutralization of the different TGF-β isoforms [24]. At the late foetal stage (day 19/21.5 in gestation), TGF-β_3 was no longer detectable [17], which is in accordance with the lacking detectability of that cytokine isoform in our neonate animals. The finding of low expression of TGF-β_1 and TGF-β_2 in foetal compared to adult tissue, and the vice versa finding for TGF-β_3, has also been demonstrated in humans beings [25].

The cited studies are inhomogenous according to species, age groups (different gestational ages/neonatal/adult animals), investigated cytokines, tissue or cell population observed, physiological situation (wounded/unwounded tissue) and examination methods. However, the overall finding is a relative paucity in cytokine activity in the foetal stage and a continuous increase in concentration of most cytokines with aging and maturation.

In the adult wound, inflammatory cells such as polymorphonuclear leukocytes (PMNs) and later macrophages and lymphocytes are recruited to the wound, partially by TGF-β_1 and PDGF released from platelets. These cells again release a number of cytokines, including TGF-α, TGF-β, PDGF, IGF, and bFGF, which attract more PMNs and macrophages into the wound, recruit fibroblasts, and stimulate them to produce collagen and other extracellular matrix molecules. The foetal wound, on the other hand, is characterized by a relative paucity of PMNs and macrophages at the site of injury. Therefore, the cascade of chemotaxis, cytokine secretion and more chemotaxis may not primariliy initiated [2, 3]. As most cytokines act chemotactic, pro-inflammatory and pro-fibrotic, it is plausible to expect an association between paucity in cytokine activity and the inflammation-free and scarless at the foetal and early stage.

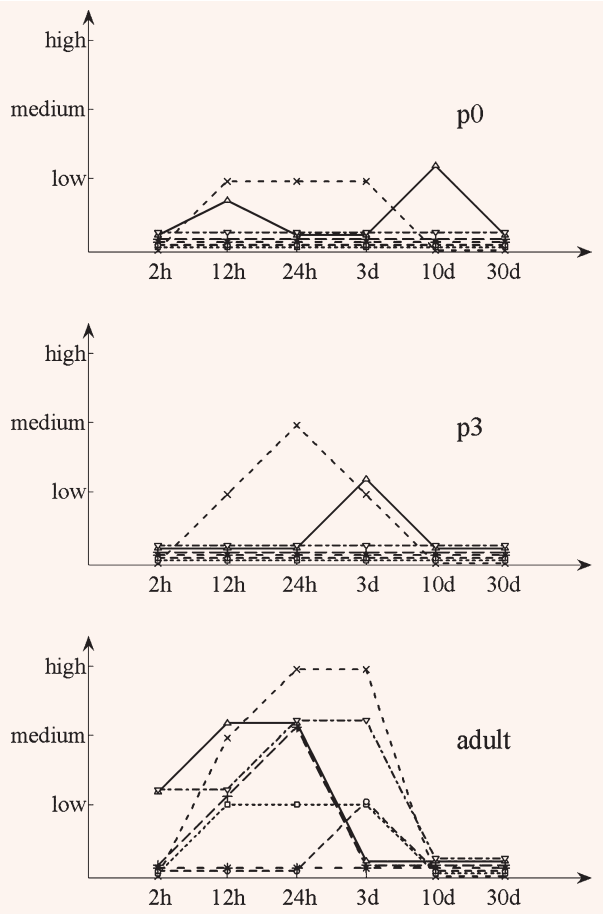

Fig. 4 Temporal distribution of cytokine expression in the wound site. Semi-quantitative evaluation by age group. Lines: TGF-α: ---x---; TGF-β_1: ···□···; TGF-β_2: – –O– –; TGF-β_3: – – ❊ – –; PDGF-A: – –+– –; IGF-1: —Δ—; bFGF: – ▪ –▽– ▪ –.

Outlook

During healing of an incisional wound in neonatal, 3 day old and adult skin in rats, it was demonstrated that neonate rat skin still possesses foetal-like healing properties after this limited kind of wounding, according to both healing morphology and cytokine profile. This encourages the use of neonatal rat skin

as a wound- healing model for further studies, instead of the more complicated foetal animal models. Cytokines are possible therapeutic targets in efforts to optimize wound healing in the adult organism. Candidates may be found in factors that act oppositely in neonate and adult tissue. The results of the present study may help to select such cytokines.

According to the present results, inhibition of TGF-β_1, TGF-α or PDGF A may represent possible strategies. The latter may appear surprising, as PDGF (in its isoform PDGF BB) is yet therapeutically used by means of addition, not substraction. However, that application pursues the goal of wound closure in non-healing chronic wounds, gladly accepting cell immigration and fibrosis. In contrast, cytokine substraction intends to mimic the foetal healing pattern *preventing* fibrosis.

Inhibition of cytokine activity can be achieved by the use of anti-sense-oligonucleotides or neutralizing antibodies. The effectiveness of the topical application of such agents, however, is hampered by problems, such as mechanical loss in the wound site, degradation by proteases, absorption to ECM components and limited availability from eventually used transport vehicles [26]. Some of these problems can be circumvented by gene therapy, which has been shown to be principally effective with regard to cytokine application [27, 28]. Our plans are to use tissue transfection with inhibitory RNA, which is not incorporated in the genome and is therefore regarded an especially safe method [29].

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References

1. **Bullard KM, Longaker MT, Lorenz HP.** Fetal wound healing: Current biology. *World J Surg.* 2003; 27: 54–61.
2. **Clark RAF.** wound repair: overview and general considerations In: Clark RAF, editor. The molecular and cellular biology of wound repair, vol xxiii. New York: Plenum Press; 1996. pp. 3–50.
3. **Dang C, Ting K, Soo C, Longaker MT, Lorenz HP.** Foetal wound healing. Current perspectives. *Clin Plast Surg.* 2003; 30: 13–23.
4. **Samuels P, Tan KW.** Foetal scarless wound healing. *J Otolaryngol.* 1999; 28: 296–302.
5. **Wagner W, Reichl J, Wehrmann M, Zenner H-P.** Neonatal rat cartilage has the capacity for tissue regeneration. *Wound Repair and Regeneration.* 2001; 9: 531–6.
6. **Boon L, Manicourt D, Marbaix E, Vandenabeele M, Vanwijck R.** A comparative analysis of healing of surgical cleft lip corrected in utero and in neonates. *Plast Reconstr Surg.* 1992; 89: 11–7.
7. **Longaker MT, Whitby DJ, Ferguson MW, Lorenz HP, Harrison MR, Adzick NS.** Adult skin wounds in the foetal environment heal with scar formation. *Ann Surg.* 1994; 219: 65–72.
8. **Cross KJ, Mustoe TA.** Growth factors in wound healing. *Surg Clin N Am.* 2003; 83: 531–45.
9. **Rumalla VK, Borah GL.** Cytokines, growth factors, and plastic surgery. *Plast Reconstr Surg.* 2001; 108: 719–33.
10. **Sasaki M, Ikeda H, Haga H. Manabe T, Nakanuma Y.** Frequent cellular senescence in small bile ducts in primary biliary cirrhosis: A possible role in bile duct loss. *J Pathol.* 2005; 205: 451–9.
11. **Goss AN.** Intrauterine healing of foetal rat oral mucosal, skin and cartilage wounds. *J Oral Pathol.* 1977; 6: 35–43.
12. **Hallock G, Merkel JR, Rice DC, DiPaolo BR.** The ontogenetic transition of collagen deposition in rat skin. *Ann Plast Surg.* 1993; 30: 239–43.
13. **Whitby DJ, Ferguson MWJ.** Immunohistochemical localization of growth factors in foetal wound healing. *Dev Biol.* 1991; 147: 207–15.
14. **Hasan W, Zhang R, Liu M, Warn JD, Smith PG.** Coordinate expression of NGF and α-smooth muscle actin mRNA and protein in cutaneous wound tissue of developing and adult rats. *Cell Tissue Res.* 2000; 300: 97–109.
15. **Koizumi M, Matsuzaki T, Ihara S.** The subsets of keratinocytes responsible for covering open wounds in neonatal rat skin. *Cell Tissue Res.* 2004; 315: 187–95.
16. **Beanes SR, Hu FY, Soo C, Dang CM, Urata M, Ting K, Atkinson JB, Benhaim P, Hedrick MH, Lorenz HP.** Confocal microscopy analysis of scarless repair in the fetal rat: defining the transition. *Plast Reconstr Surg.* 2002; 109: 160–70.
17. **Soo C, Beanes SR, Hu FY, Zhang X, Dang C, Chang G, Wang Y, Nishimura I, Freymiller E, Longaker MT, Lorenz HP, Ting K.** Ontogenetic transition in foetal wound transforming growth factor-beta regulation correlates with collagen organization. *Am J Pathol.* 2003; 163: 2459–76.
18. **Cass D, Bullard KM, Sylvester KG, Yang EY, Longaker MT, Adzick NS.** Wound size and gesta-

tional age modulate scar formation in fetal wound repair. *J Pediatr Surg.* 1997; 32: 411–5.

19. **Olutoye OO, Yager DR, Cohen IK, Diegelmann RF.** Lower cytokine release by fetal porcine platelets: a possible explanation for reduced inflammation after fetal wounding. *J Pediatr Surg.* 1996; 31: 91–5.
20. **Dang CM, Beanes SR, Soo C, Ting K, Benhaim P, Hedrick MH, Lorenz HP.** Decreased expression of fibroblast and keratinocyte growth factor isoforms and receptors during scarless repair. *Plast Reconstr Surg.* 2003; 111: 1969–79.
21. **Liechty KW, Crombleholme TM, Cass DL, Martin B, Adzick NS.** Diminished interleukin-8 (IL-8) production in the fetal wound healing response. *J Surg Res.* 1998; 77: 80–4.
22. **Liechty KW, Adzick NS, Crombleholme TM.** Diminished interleukin 6 (IL-6) production during scarless human fetal wound repair. *Cytokine.* 2000; 12: 671–6.
23. **Hsu M, Peled ZM, Chin CS, Liu W, Longaker MT.** Ontogeny of expression of transforming growth factor-beta1 (TGF-beta 1), TGF-beta 3 and TGF-beta receptors I and II in fetal rat fibroblasts and skin. *Plast Reconstr Surg.* 2001; 107: 1787–94.
24. **Shah M, Foreman DM, Ferguson MWJ.** Neutralisation of TGF-beta1 and TGF-beta2 or exogenous addition of TGF-beta3 to cutaneous rat wounds reduces scarring. *J Cell Science.* 1995; 108: 985–1002.
25. **Chen W, Fu X, Ge S, Sun T, Zhou G, Jiang D, Sheng Z.** Ontogeny of expression of transforming growth factor-beta and its receptors and their possible relationship with scarless healing in human fetal skin. *Wound Repair Regen.* 2005; 13: 68–75.
26. **Davidson JM, Krieg T, Eming S.** (2000) Particle-mediated gene therapy of wounds. *Wound Repair Regen.* 2000; 8: 452–9.
27. **Braun-Falco M.** Gentherapeutische Konzepte zur Förderung der Wundheilung. *Hautarzt.* 2002; 53: 238–43.
28. **Davidson JM, Whitsitt JS, Pennington B, Ballas CB, Eming S, Benn SI.** Gene therapy of wounds with growth factors. *Curr Top Path.* 1999; 93: 111–21.
29. **Sullenger BA, Gilboa E.** Emerging clinical applications of RNA. *Nature.* 2002; 418: 252–8.