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The Rate of Wound Healing is Increased in Psoriasis

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Abstract

Background—Psoriasis shares many features with wound healing, a process that involves switching keratinocytes from growth to differentiation. Ca²⁺ is known to regulate this process. The N-methyl-D-aspartate receptor (NMDAR), an ionotropic glutamate receptor found on keratinocytes, is expressed abnormally in psoriasis *in vivo*.

Objectives—The goals of this study are to determine whether the rate of healing in the skin of psoriatic individuals differs from that observed in normal skin and whether the keratinocyte hyperproliferation found in psoriasis correlates with expression of specific NMDAR subunits.

Methods—Three mm punch biopsies were performed on the skin of normal, as well as, involved and uninvolved skin of subjects with psoriasis. On day 0, as well as, on day 6 after the biopsy, photographs were taken and the size of the wounds determined using ImageJ. Using immunohistochemistry, the biopsy material was stained for NMDAR and its subunits.

Results—Involved and uninvolved skin of individuals with psoriasis shows significantly more rapid healing than normal. The NR2C subunit of NMDAR is down-regulated in the basal cell layer of involved and uninvolved epidermis of psoriatic subjects compared to controls. By contrast, cells in the basal cell layer of the uninvolved epidermis showed a significantly greater percent strong staining for NR2D compared to those cells in normal epidermis.

Conclusions—Wound healing is significantly accelerated in psoriasis compared to normal. Immunohistochemistry showed that the relative intensity of strong immunostaining for subunits of the NMDAR is altered in the basal cell layer in psoriatic skin compared to normal controls. We suggest that these alterations may contribute to the increased rate of wound healing in psoriasis.

Keywords

wound healing; psoriasis; NMDA-receptor; keratinocyte proliferation

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Conflict of Interest Disclosures: None

Introduction

Psoriasis is a common skin disease characterized by abnormal growth and differentiation of keratinocytes, increased vasodilation with local release of nitric oxide (NO), and skin infiltration by leukocytes.^{1,2} The etiology of this disease is unknown, although an abnormal immune response seems to play a key role.^{3,4} Thus, tumor necrosis factor alpha, dendritic cells and T lymphocytes appear to be involved in the disease.⁵ Family studies have provided evidence for a genetic predisposition and several putative psoriasis susceptibility loci have been identified, including one in the major histocompatibility complex region on chromosome 6.^{6,7}

Psoriasis demonstrates many of the features of a healing wound. For example, clinically both psoriasis and wound healing are characterized by erythema and scale.⁸ The antimicrobial protein REG3A that regulates keratinocyte proliferation and differentiation after skin injury also is expressed in psoriasis.⁹ Furthermore, similar immunohistochemical staining patterns using antibodies against involucrin, transglutaminase, and filaggrin are found in both abnormalities *in vitro*.¹⁰ Moreover, compared to normal skin, keratins 1 and 10 are reduced in wound healing and psoriasis, whereas, keratins 6 and 16 are up regulated in both conditions.¹¹ Finally, in contrast to normal skin, the skin in a psoriatic plaque constitutively expresses antimicrobial peptides (AMP) as does wounded skin.^{12,13,14}

The *N*-methyl-*D*-aspartate (NMDA) receptor (NMDAR), is an ionotropic glutamate receptor that is an heteromeric, integral membrane protein originally described in neurons.¹⁵ The receptor is comprised of several subunits, NR1, that appears to be obligatory, as well as subunits NR2A, NR2B, NR2C, NR2D and NR3A.¹⁶ Many of the functional properties of the receptor are determined by the subunit composition, which varies across cell types. In neurons, NMDAR regulates Ca²⁺ influx into the cells. Recently, the NMDAR has been documented in keratinocytes where it also regulates Ca²⁺ entry.^{17,18} Ca²⁺ is known to regulate keratinocyte growth and differentiation, a process that is abnormal in psoriasis,¹⁹ and it has been documented recently that the expression of the NR1 subunit of this receptor is reduced in the basal layer of psoriatic epidermis.²⁰ Furthermore, in rats, the expression of NR1 is decreased in keratinocytes during re-epithelialization of wounded skin.²¹ L-glutamate, the ligand for NMDAR, is present in large concentrations in normal human epidermis.²² Thus, NMDAR may play a role in the keratinocyte hyperproliferation seen in wounds, as well as, in psoriasis.

The group of genes ("psoriogene") resulting in the psoriatic phenotype is common (about 2%) in the human population, suggesting that these genes may convey a survival benefit.^{10,23} Given the many reports of similarities between wound healing and psoriasis, we hypothesized that wound healing might be accelerated in psoriasis, which could give individuals with this disease a survival advantage.²⁴ Further, we examined the expression of NMDAR subunits in psoriasis and normal epidermis, to determine if differential expression of these receptor subunits was associated with psoriasis, a difference that could relate to the increased keratinocyte proliferation seen in this disease.

Materials and Methods

Biopsies

After obtaining approval from Scripps Research Institute's Human Subjects Committee, subjects were enrolled and consented. An attempt was made to enroll age and sex matched controls. On day 0, after injecting subcutaneous anesthesia consisting of 1% lidocaine containing 1:100,000 epinephrine and buffered with sodium bicarbonate, 3 mm punch biopsies were performed using standard techniques on subjects with normal skin and subjects with psoriasis. For subjects with psoriasis, biopsies were obtained from epidermis in both uninvolved and psoriasis involved regions. All the normal biopsies and biopsies of uninvolved epidermis of psoriasis subjects were obtained from the buttocks area about 3 cm from the sacral crease. Except for two biopsies from psoriasis involved epidermis, all the other involved biopsies were taken from the same general area of the body. Two subjects did not demonstrate psoriasis in the buttock area. Therefore, these subjects' biopsies of involved skin were obtained from the extremities (one area on the arm) and one of these sites was sutured. The sutured sites were not measured. Pressure was used to control hemostasis. After hemostasis had occurred, Aquaphor[®] ointment and a band aid(s) was applied. Subjects were given a tube of Aquaphor[®] ointment and band aids and instructed to apply the ointment under a band aid each day after showering.

The subjects were asked to return on day 6 after the biopsy for evaluation of their wound(s) or suture removal. The wounds were photographed at a standard distance from the wound, with a digital camera on day 0, as well as, day 6. A total of 19 biopsies were allowed to heal by secondary intention.

In vitro measurement of wound size

Using digital photographs obtained on both days 0 and 6, the circumference of the wound was outlined and the area measured with the ImageJ software (available on the Internet at <http://rsb.info.nih.gov/nih-image/>). On day 0, the edge of the epithelial surface around the wound was used as the edge of the wound. On day 6, the area of the wound not re-epithelialized, i.e. not yet healed, was outlined and was measured as above.

Immunohistochemistry

The biopsy material was put into a sterile tube containing cold saline and transported to the laboratory on ice. Sections were cut and the slides fixed in cold acetone for 5 min, air dried for 10 min, blocked with 0.3% hydrogen peroxide/PBS and rinsed with 3 changes of PBS. The diluted primary antibodies were applied and the sections were incubated overnight at 4°C. (All antibodies were diluted 1:100 in Dako Antibody Diluent (Dako Inc., Carpinteria, CA). The slides were washed with 3 changes of phosphate buffered saline (PBS)/0.1% Tween. The secondary antibody was applied and incubated for 30 min at room temperature and washed with 3 changes of PBS/ 0.1% Tween. The secondary is conjugated to horseradish peroxidase and was reacted with diaminobenzidine. The slides were then counterstained with hematoxylin. The percent of strong, medium and weak immunostaining was quantified by computer in selected areas of the entire epidermis and in the basal cell layer only using the Aperio software computer program (Aperio Technologies, Vista, CA).

Standardized thresholds for background and strong, medium and weak immunostaining were set and used for the determination of staining level. Strong is defined by: (Strong Threshold) > Intensity > (Black Threshold). Medium is defined by: (Medium Threshold > Intensity > (Strong Threshold). Weak is defined by: (Weak Threshold) > Intensity > (Medium Threshold). Background staining was subtracted from all measurements.^{2,5}

Antibodies

The antibodies used were: a rabbit polyclonal antibody raised against a synthetic peptide (non-phosphorylated) derived from the human NR1 subunit NMDAR in the regions of the phosphorylation site of serine 897 (Abcam, Cambridge, MA); a rabbit polyclonal antibody raised against a synthetic peptide from the second cytoplasmic domain of human NR2A subunit of NMDAR conjugated to an immunogenic carrier protein (Abcam); a rabbit polyclonal antibody raised against a synthetic peptide mapping at the N-terminus of human NR2B subunit of NMDAR; a rabbit polyclonal antibody raised against a N-terminal fusion protein corresponding to amino acids 25–130 of rat NR2C subunit of NMDAR (Abcam); a rabbit polyclonal antibody raised against a synthetic peptide conjugated to the KLH derived from within residues 1300 to the C-terminus of human NR2D subunit of NMDAR (Abcam).

Statistical analyses

Data were combined according to skin phenotype and expressed as mean $\pm$ SEM. Statistical significance was determined by the students *t*-test ($p < 0.05$) or ANOVA followed by the Fisher's PLSD post hoc test ($p < 0.05$).

Results

Wound healing in psoriasis skin

The healing of wounds in individuals with psoriasis and in age and sex matched controls who had no personal or family history of psoriasis were compared on the sixth day after the biopsy was performed. Healing was defined as the re-epithelialization of the denuded surface. The wounds in the psoriatic plaque and uninvolved skin of psoriasis subjects healed significantly more rapidly than did the wounds in the skin of the normal controls ($p < 0.05$) [Fig. 1]. Thus, on day 6, the size of the wounds in the psoriatic plaques was $2.9 \pm 0.3 \text{ mm}^2$ ($n=7$) and in the wounds in uninvolved skin of psoriasis subjects was $3.4 \pm 0.6 \text{ mm}^2$ ($n=4$). There was no significant difference in these rates of wound healing between involved and uninvolved skin ($p = 0.35$). In contrast, the size of the wounds on day 6 in normal controls was $6.2 \pm 0.3 \text{ mm}^2$ ($n=5$), which was significantly larger than the wound size in either the psoriatic plaques ($p < 0.0001$, ANOVA) or uninvolved skin ($p = 0.0002$, ANOVA). For the normal subjects, the mean age was 37 years and the median age was 40 years (range 26–47); for the subjects with psoriasis the mean age was 47 years and the median was 46 years (range 23–69).

Expression of various NMDARs in Involved, Uninvolved and Normal skin

NMDAR has been shown to gate a Ca^{2+} channel in keratinocytes and may be involved in Ca^{2+} flux that regulates growth and differentiation in cells responsible for wound healing in the skin.¹⁸ It has been reported previously that normal skin shows strong immunostaining

with anti-NMDAR1 antibody as does the uninvolved skin of psoriasis subjects.²⁰ Information on the expression of other NMDAR subunits, which are necessary for functionality of the receptor, in either the normal or psoriasis epidermis is limited. Therefore, we determined whether the expression of NR2A, NR2B, NR2C, and NR2D was expressed in normal epidermis and if the expression in the normal epidermis differed from that observed in psoriasis (involved and uninvolved). We also measured the NMDAR subunit expression in the basal cell layer.

Immunostaining with the antibodies against NR1, NR2A, NR2B, NR2C and NR2D demonstrated that all four subunits including NR1 were expressed in all the layers of the normal, involved and uninvolved epidermis [Fig. 2]. Quantification of the extent of strong, medium and weak immunostaining showed differences in the staining pattern for the three skin phenotypes, but most differences did not reach statistical significance ($p < 0.05$) [Fig. 3]. However, the percent strong staining for NR2C was significantly less in the cells in the basal cell layer of involved epidermis compared with the cells in the basal cell layer of normal epidermis ($p < 0.02$, ANOVA) [Fig. 3]. Cells in the basal cell layer of the uninvolved epidermis also showed significantly less percent strong staining for NR2C than the cells in the basal layer of normal epidermis ($p < 0.02$, ANOVA) [Fig. 3]. In contrast, cells in the basal cell layer of the uninvolved epidermis showed a significantly greater percent strong staining for NR2D compared to cells in the basal cell layer of normal epidermis ($p < 0.001$, ANOVA). Thus, the basal cell layer of both the involved and uninvolved epidermis showed differences in the level of NMDAR subunit expression compared to the basal cell layer of normal epidermis, a difference that may relate to the increased rate of wound healing found in both the involved and uninvolved skin in psoriasis.

Discussion

We document here that small wounds in the integument of individuals with psoriasis, both involved and uninvolved skin, heal significantly more rapidly than do those in normal subjects. The mean (37 years) and median (40 years) ages for normal subjects were younger than the mean (47 years) and median (46 years) ages for the psoriatic. Since it has been reported that the rate of wound healing decreases with age, the age(s) of the subjects in the two groups does not appear to be a factor in the results from our studies.²⁶ In the times before the advent of antibiotics, individuals who carry the gene(s) that cause accelerated wound healing would have had a survival advantage. This would explain the prevalence of the genes(s) in the human population.²⁴ We also show that the expression of the NR2C receptor, a subunit of the NMDAR, is significantly down-regulated in the basal cell layer of involved and uninvolved epidermis compared to normal skin, whereas the percent strong expression of NR2D is upregulated in uninvolved skin compared to normal.

It has been known for many years that the keratinocytes in not only the involved but also the uninvolved skin in psoriasis show an increased growth fraction as well as an increased number of cycling cells compared to normal skin.²⁷ The findings reported here that the uninvolved skin heals almost as rapidly as the involved is consistent with those data. Our results showing that the NR2C subunit of the NMDAR is down regulated in the involved, as well as the uninvolved skin of psoriatic individuals raises the possibility for a role for NR2C

in the altered keratinocytes' growth pattern in psoriasis. Genever, et al have documented the expression of NR1, as well as, NR2A/B subunits in normal and wounded rat skin, but in this study, did not determine the presence of NMDA-R2C in these tissues.²¹

Tumor necrosis factor alpha (TNF α), a cytokine that inhibits human keratinocyte proliferation, is found in wounds, as well as, in psoriatic plaques *in vivo*.^{28,29} It has recently been reported that this protein induces the expression of R2C in normal human keratinocytes *in vitro*.³⁰ By contrast, we have recently documented that TNF α does not induce R2C in keratinocytes obtained from psoriatic subjects under the same conditions of culture (manuscript submitted). Thus, the expression of R2C may be regulated differently in normal vs. psoriatic keratinocytes. Intracellular Ca²⁺ concentration is known to regulate keratinocyte growth and differentiation¹⁹ and it has been shown that NMDAR can regulate influx of Ca²⁺ into these cells.¹⁸ The properties of the NMDA receptors are determined by the subunit composition of the receptor.³¹ NMDARs comprised of NR1/NR2C have been studied in expression systems and show smaller Ca²⁺ influx than NMDARs comprised of NR1/NR2A or NR1/NR2B.³² Therefore, the down regulation of NR2C in the involved basal cells is likely to result in a greater proportion of receptors with NR2A and NR2B receptor subunits that are associated with greater Ca²⁺ flux upon NMDAR activation than receptors with NR1/NR2C. Such a shift in receptor subunit composition could result in aberrant keratinocyte Ca²⁺ homeostasis and dysregulation of signaling mechanisms important in keratinocyte proliferation and differentiation. The increased level of NR2D in the uninvolved epidermis may compensate for the reduced NR2C and thereby contribute to differences between the involved and uninvolved epidermis.

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References

1. Krueger JG, Bowcock A. Psoriasis pathophysiology: current concepts of pathogenesis. *Ann Rheum Dis*. 2005; 64(Suppl 2):ii30–ii36. [PubMed: 15708932]
2. Ormerod AD, Weller R, Copeland P, Benjamin N, Ralston SH, Grabowski P, et al. Detection of nitric oxide and nitric oxide synthases in psoriasis. *Arch Dermatol Res*. 1998; 290:3–8. [PubMed: 9522994]
3. Morhenn VB. Is psoriasis a disease of the immune system? *Cutis*. 1984; 34:223–224. [PubMed: 6237885]
4. Uyemura K, Yamamura M, Fivenson DF, et al. The cytokine network in lesional and lesion-free psoriatic skin is characterized by a T-helper type 1 cell-mediated response. *J Invest Dermatol*. 1993; 101:701–705. [PubMed: 7693825]
5. Griffiths CE, Barker JN. Pathogenesis and clinical features of psoriasis. *Lancet*. 2007; 370:263–271. [PubMed: 17658397]
6. Schoen MP, Boehncke WH, Broecker EB. Psoriasis: Clinical manifestations, pathogenesis and therapeutic perspectives. *Discov Med*. 2005; 5:253–258. [PubMed: 20704884]
7. Tsoi LC, Spain SL, Knight J, et al. The identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity. *Nat Genet*. 2012; 44:1341–1348. [PubMed: 23143594]

8. Lai Y, Li D, Li C, et al. The antimicrobial protein REG3A regulates keratinocyte proliferation and differentiation after skin injury. *Immunity*. 2012; 37:74–84. [PubMed: 22727489]
9. Honma M, Minami-Hori M, Takahashi H, Iizuka H. Podoplanin expression in wound and hyperproliferative psoriatic epidermis: regulation by TGF- β and STAT-3 activating cytokines, IFN- γ , IL-6, and IL-22. *J Dermatol Sci*. 2012; 65:134–140. [PubMed: 22189341]
10. Mansbridge, J.; Reusch, M.; Bohr, V.; Hanawalt, P. The wound healing model for psoriasis, the “Psoriogene”, and rational approaches to therapy; Psoriasis Symposium; 1986 Jul.
11. Mansbridge JN, Knapp AM. Changes in keratinocyte maturation during wound healing. *J Invest Dermatol*. 1987; 89:253–263. [PubMed: 2442269]
12. Bardan A, Nizet V, Gallo RL. Antimicrobial peptides and the skin. *Expert Opin Biol Ther*. 2004; 4:543–549. [PubMed: 15102603]
13. Nakatsuji T, Gallo RL. Antimicrobial peptides: old molecules with new ideas. *J Invest Dermatol*. 2012; 132:887–895. [PubMed: 22158560]
14. Frohm M, Agerberth B, Ahangari G, et al. The expression of the gene coding for the antibacterial peptide LL-37 is induced in human keratinocytes during inflammatory disorders. *J Biol Chem*. 1997; 272:15258–15263. [PubMed: 9182550]
15. McLennan H. Receptors for the excitatory amino acids in the mammalian central nervous system. *Prog Neurobiol*. 1983; 20:251–271. [PubMed: 6142499]
16. Stephenson FA. Subunit characterization of NMDA receptors. *Curr Drug Targets*. 2001; 2:233–239. [PubMed: 11554550]
17. Morhenn VB, Waleh NS, Mansbridge JN, et al. Evidence for an NMDA receptor subunit in human keratinocytes and rat cardiocytes. *Eur J Pharmacol*. 1994; 268:409–414. [PubMed: 7805765]
18. Morhenn VB, Murakami M, O’Grady T, et al. Characterization of the expression and function of N-methyl-D-aspartate receptor in keratinocytes. *Exp Dermatol*. 2004; 13:505–511. [PubMed: 15265015]
19. Karvonen SL, Korkiamaki T, Yla-Outinen H, et al. Psoriasis and altered calcium metabolism: downregulated capacitative calcium influx and defective calcium-mediated cell signaling in cultured psoriatic keratinocytes. *J Invest Dermatol*. 2000; 114:693–700. [PubMed: 10733675]
20. Fischer M, William T, Helmbold P, et al. Expression of epidermal N-methyl-D-aspartate receptors (NMDAR1) depends on formation of the granular layer--analysis in diseases with parakeratotic cornification. *Arch Dermatol Res*. 2004; 296:157–162. [PubMed: 15338240]
21. Genever PG, Maxfield SJ, Kennovin GD, et al. Evidence for a novel glutamate-mediated signaling pathway in keratinocytes. *J Invest Dermatol*. 1999; 112:337–342. [PubMed: 10084312]
22. Fischer M, Glanz D, Urbatzka M, et al. Keratinocytes: a source of the transmitter L-glutamate in the epidermis. *Exp Dermatol*. 2009; 18:1064–1066. [PubMed: 19397696]
23. Dobzhansky, TG. *Genetics the Evolutionary Process*. Columbia University Press; 1931. ISBN 0-231-08306-8.
24. Allison AC. Protection afforded by sickle-cell trait against subtertian malarial infection. *Brit Med J*. 1954; 1:290–294. [PubMed: 13115700]
25. Aperio.com Color Devolution Algorithm User's Guide. :13.
26. Gosain A, DiPietro LA. Aging and wound healing. *World J Surg*. 2004; 28:321–326. [PubMed: 14961191]
27. Staiano-Coico L, Gottlieb AB, Barazani L, Carter DM. RNA, DNA and cell surface characteristics of lesional and nonlesional psoriatic skin. *J Invest Dermatol*. 1987; 88:646–651. [PubMed: 2437219]
28. Pillai S, Bikle DD, Eessalu TE, Aggarwai BB, Elias PM. Binding and biological effects of tumor necrosis factor alpha on cultured human neonatal foreskin keratinocytes. *J Clin Invest*. 1989; 83:816–821. [PubMed: 2466051]
29. Nickoloff BJ, Karabin GD, Barker JN, et al. Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. *Am J Pathol*. 1991; 138:129–140. [PubMed: 1702929]
30. Jansen BJH, van Ruissen F, de Jongh G, Zeeuwen PLJM, Schalkwijk J. Serial analysis of gene expression in differentiated cultures of human epidermal keratinocytes. *J. Invest Dermatol*. 2001; 116:12–22. [PubMed: 11168793]

31. Liu Y, Wong TP, Aarts M, et al. NMDA receptor subunits have differential roles in mediating excitotoxic neuronal death both in vitro and in vivo. *J Neurosci.* 2007; 27:2846–2857. [PubMed: 17360906]
32. Krupp JJ, Vissel B, Heinemann SF, Westbrook GL. Calcium-dependent inactivation of recombinant N-methyl-D-aspartate receptors is NR2 subunit specific. *Mol Pharmacol.* 1996; 50:1680–1688. [PubMed: 8967993]

Fig. 1. Measurement of Wound Healing in psoriasis and normal Skin

(a) Photographs of the sites of the biopsies at days 0 and 6 performed in normal, involved and uninvolved skin. (b) Measurement of the size of the wounds on days 0 and 6. Inserts indicate the number of subjects biopsied. The involved and uninvolved biopsies were performed on the buttock of a 23 year old female; The normal biopsy was performed on the buttock of a 26 year old female. Asterisks indicate a significant difference from normal skin.

Fig. 2. Immunohistochemical Staining

Depicted are representative histologic sections of the three different phenotypes, normal, involved and uninvolved, stained with five different antibodies (magnification 10 $\times$).

Fig. 3. Quantitation of the Staining Intensity Using Five Different Antibodies on Three Different Skin Phenotypes

The intensity of the staining expressed as percent of cells staining either strong, medium or weak is depicted for normal, involved and uninvolved skin samples. The background staining was subtracted. Asterisks indicate a significant difference from normal skin.

Table I

Demographics of the Subjects

Subject #	Sex	Age	Phenotype
01	F	26	NI
02	F	30	NI
03	F	27	NI
04	F	43	NI
06	M	45	Pso
07	M	52	Pso
08	M	40	NI
09	M	37	Pso
010	F	46	Pso
011	M	47	NI
012	F	23	Pso
013	M	46	NI
014	F	46	Pso
015	F	69	Pso
016	M	58	Pso