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keratinocyte cDNA library. *Genome Res* 11:341–55

Marshall D, Hardman MJ, Nield KM, Byrne C (2001) Differentially expressed late constituents of the epidermal cornified envelope. *Proc Natl Acad Sci USA* 98: 13031–6

McCarroll SA, Hadnott TN, Perry GH, Sabeti PC, Zody MC, Barrett JC (2006) Common deletion polymorphisms in the human genome. *Nat Genet* 38:86–92

Steinert PM (2000) The complexity and redundancy of epithelial barrier function. *J Cell Biol* 151:F5–8

Steinert PM, Parry DA, Marekov LN (2003) Trichohyalin mechanically strengthens the hair follicle: multiple cross-bridging roles in the inner root sheath. *J Biol Chem* 278:41409–19

Su AI, Wiltshire T, Batalov S, Lapp H, Ching KA, Block D *et al.* (2004) A gene atlas of the mouse and human protein-encoding transcriptomes. *Proc Natl Acad Sci USA* 101:6062–7

Varki A, Altheide TK (2005) Comparing the human and chimpanzee genomes: searching for needles in a haystack. *Genome Res* 15:1746–58

Wang A, Johnson DG, MacLeod MC (2001) Molecular cloning and characterization of a novel mouse epidermal differentiation gene and its promoter. *Genomics* 73:284–90

Zhao XP, Elder JT (1997) Positional cloning of novel skin-specific genes from the human epidermal differentiation complex. *Genomics* 45:250–8

Increased Expression of Carbonic Anhydrase II (CA II) in Lesional Skin of Atopic Dermatitis: Regulation by Th2 Cytokines

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TO THE EDITOR

We have previously performed a microarray analysis of expressed genes in purified epidermal cells from lesional skin of psoriasis and atopic dermatitis (AD) patients (de Jongh *et al.*, 2005). The complete data set revealed more than 180 genes that showed significantly different expression between the two diseases. The observed generalized overexpression of host defense genes in psoriasis was studied in depth at the protein and cellular level (de Jongh *et al.*, 2005). Among the genes that were found to be upregulated in AD skin, carbonic anhydrase II (CA II, HUGO gene symbol: CA2) was upregulated eightfold ($P<10^{-5}$). Similar upregulation of CA II was previously identified independently using microarray analysis of full-thickness skin biopsies (Nomura *et al.*, 2003). No validation at the mRNA or protein level was provided by any of these microarray studies. CA II belongs to the family of metalloenzymes that catalyze the reversible reaction: $CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+$ (Geers and Gros, 2000; Boron, 2004). Fourteen different isoforms are known so far and they all show distinct distribution patterns (Sly and Hu, 1995; Mastrolorenzo *et al.*, 2003). CA II is broadly expressed in a variety of tissues, including kidney, erythrocytes, sweat glands, salivary glands, and skin (Spicer *et al.*, 1982; Briggman *et al.*, 1983; Noda *et al.*, 1986; Mastrolorenzo *et al.*, 2003) and the enzyme is involved in the maintenance of cellular pH, water transport, and ion homeostasis. The importance of CAs in these processes is witnessed by the pharmacological inhibition of these enzymes to reduce intraocular fluid pressure in glaucoma patients (Casini *et al.*, 2000). Firstly, the aim of this study was to validate these preliminary microarray data on CA II at the mRNA and protein level and secondly to examine the cellular source and regulation of CA II gene expression. We therefore examined lesional epidermis of psoriasis and AD patients. Quantification of CA II mRNA by real time PCR (qPCR) revealed an increase in CA II expression in epidermal sheets of lesional skin of AD patients compared with lesional skin of psoriasis patients and healthy controls (Figure 1a), thereby confirming the microarray data. CA II expression in lesional psoriatic epidermis is significantly lower than in normal skin. Statistical analysis of the data depicted in Figures 1a, c, and 2b was performed by analysis of variance followed by a Duncan's multiple range *post hoc* test. To examine whether the changes in CA II mRNA result in changes at the protein level, a second cohort of patients and healthy individuals, which was different from the first study, was analyzed by Western blotting (rabbit CA II antibody; Abcam, Cambridge, UK) and ELISA (rabbit CA II-capturing antibody and a sheep CA II antibody; R&D Systems, Minneapolis, MN). AD patients (extrinsic type, mean age 52 years) were from our in-patient department and had moderate to severe AD, according to the Hanifin criteria. Patients suffered from chronic AD, but were in an active phase of disease, for which they were hospitalized and the lesions were regarded as (sub)acute lesions. Biopsies were taken from lesional skin, but care was taken to exclude vesicles or scratched skin. All psoriasis patients (mean age 60 years) had moderate to severe plaque type psoriasis. Patients were all diagnosed by a dermatologist; the study was approved by the local medical ethical committee and adhered to the Declaration of Helsinki Principles. Written informed consent was obtained. Both

Abbreviations: CA, carbonic anhydrase; AP, atopic dermatitis

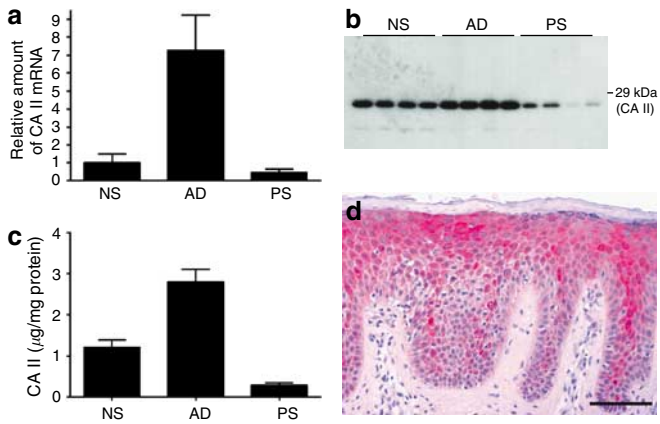

Figure 1. CA II is upregulated in lesional skin of AD patients and is present in differentiated keratinocytes. (**a**) CA II mRNA levels in epidermal sheets were significantly higher ($P<0.0002$) in lesional skin from AD patients ($n=6$) than in normal skin (NS) ($n=5$), whereas it was significantly lower ($P<0.005$) in lesional skin from psoriasis patients (PS) ($n=9$). CA II mRNA levels were determined by qPCR as described previously (Franssen *et al.*, 2005). (**b**) Western blot analysis of epidermal sheets of four individuals in each group showing similar findings for CA II protein levels as found at the mRNA level. (**c**) Quantification of CA II protein levels in the same material using ELISA showed that in AD, CA II protein was significantly higher than in NS ($P<0.0005$), whereas it was significantly lower in PS ($P<0.05$). (**d**) CA II is predominantly expressed in the suprabasal keratinocytes as shown here by immunostaining of lesional AD skin. Bar = 100 μm.

Western blot analysis and ELISA showed that CA II protein expression in epidermis of AD was higher than in normal skin and psoriasis (Figure 1b and c). To analyze if CA II expression was stratum-specific, basal and differentiated keratinocytes from normal skin biopsies were sorted by flow cytometry on the basis of β1-integrin expression (Franssen *et al.*, 2005). CA II mRNA levels were measured by qPCR and revealed that CA II is almost exclusively expressed in the differentiated keratinocytes (data not shown). Immunohistochemistry confirmed the expression in the suprabasal keratinocytes of both normal and diseased skin. Figure 1d shows the cytoplasmic localization in suprabasal cells of AD skin as an example.

AD is characterized by a Th2-mediated immune response, whereas in psoriasis mainly Th1 cytokines are present. To examine if these cytokines influence CA II expression, or whether cell-autonomous differences between AD, psoriasis, and controls determine the CA II expression level, confluent keratinocytes cultured from healthy volunteers and non-affected skin of AD and psoriasis patients were exposed to Th1 or Th2 cytokines. qPCR data revealed that CA II mRNA was not induced by Th1 cytokines in any of the keratinocyte groups, whereas Th2 cytokines increased CA II expression almost fourfold in keratinocytes from AD and psoriasis patients and from healthy volunteers (Figure 2a). The observed induction of CA II mRNA by Th2 cytokines also resulted in an increased CA II protein level as revealed by Western blot analysis (Figure 2b) and ELISA (data not shown). Dose–response experiments revealed that a plateau of CA II expression is reached at a Th2 cytokine concentration of 10 ng/ml (data not shown). At this concentration, a maximum CA II mRNA level was observed after 24 hours of stimulation, whereas a maximum CA II protein level was found after 48 hours (data not shown).

Our findings suggest that relative overexpression of CA II as initially found by microarray analysis of AD and psoriasis skin is probably due to differences in cytokine environment. These data indicate that there is no cell-autonomous (genetically programmed) difference between these groups with respect to CA II upregulation. Although CA II is regarded as a ubiquitously expressed enzyme, our immunohistochemical data show that, in skin, expression is particularly high and inducible in keratinocytes, which may be relevant for understanding epidermal physiology and abnormalities found in inflammatory skin disease. Our findings are at variance with one earlier study on CAs in human skin (Mastrolorenzo *et al.*, 2003), showing CA II in the basolateral membrane of the basal keratinocyte layer, which is unexpected in view of the presumed cytoplasmic localization of this enzyme in other cells. We believe that our results are correct, as we find suprabasal expression at the mRNA and protein level, using various independent techniques.

The (patho)physiological significance of these findings remains to be explored at the moment, but it is tempting to link the differential expression (high in AD, low in psoriasis) to differences in the clinical phenotype of these diseases. The increased level of CA II in AD epidermis could cause a pathological cellular CA II activity in selected keratinocytes that are exposed to Th2 cytokines. CAs are involved in the maintenance of cellular pH and water homeostasis (Nakhoul *et al.*, 1998). *In vitro* studies have shown that pharmacological inhibition of CA II lowers the intracellular pH in a number of cell types (Johanson *et al.*, 1992; Bonanno *et al.*, 1995; Kniep *et al.*, 2006). Elevated skin surface pH values have been reported in AD patients (Eberlein-Konig *et al.*, 2000), and recently it was shown that alkalinization of the stratum corneum leads to an altered barrier function (Hachem *et al.*, 2003). Speculatively, increased expression of CA II in AD epidermis might contribute to abnormalities in intracellular and extracellular pH. Alternatively, the CA II increase could be a compensatory response to the elevated pH as an attempt to restore cellular pH. However, as CA II is induced by Th2 cytokines, we interpret increased CA II expression as an early epidermal response to activated Th2 T lymphocytes, and as such it could contribute to other pathological changes that are characteristic for AD, such as spongiosis. It is of interest to note that skin of AD patients is often colonized with *Staphylococcus aureus* leading to a higher susceptibility of

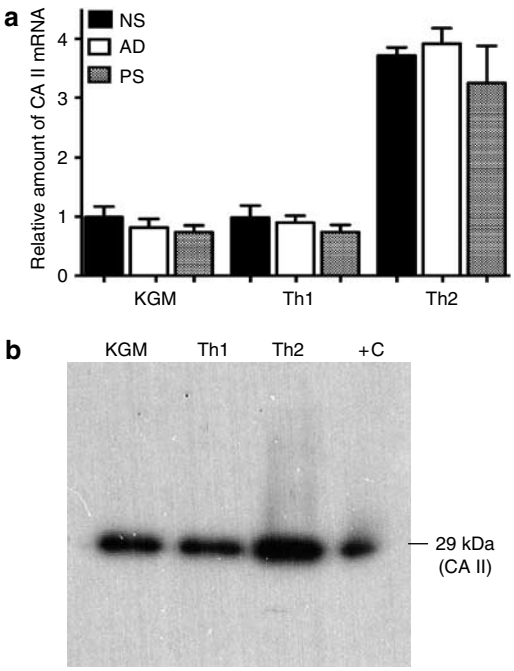

Figure 2. CA II expression is induced by Th2 cytokines but not by Th1 cytokines. (**a**) No significant effect of the diagnosis on CA II expression was found in cultured keratinocytes derived from seven healthy donors (NS) and non-lesional skin of six AD patients and seven PS. Keratinocytes were cultured in keratinocyte growth medium (KGM) and allowed to differentiate by growth factor depletion as described before (Pfundt *et al.*, 1996). Independent of the diagnosis, 48 hours stimulation by Th2 cytokines (50 ng/ml IL-4 and 50 ng/ml IL-13; Peprotech, London, UK) significantly increased CA II mRNA levels ($P<0.0002$) compared with untreated keratinocytes (KGM without growth factors), whereas 48 hours of Th1 cytokine stimulation (30 ng/ml IL-1α (Peprotech), 30 ng/ml tumor necrosis factor-α (Peprotech) and 10 U/ml IFN-γ (Hycult Biotechnology, Uden, The Netherlands)) had no effect on CA II expression levels. CA II mRNA levels were determined as in Figure 1. (**b**) A Western blot representative for all these keratinocyte cultures shows that CA II protein levels were also increased after 48 hours of Th2 cytokine stimulation and not by 48 hours of Th1 cytokine stimulation. Purified CA II (Sigma-Aldrich, St Louis, MO) was used as a positive control (+C).

infections with *S. aureus* (Leung and Bieber, 2003). As *S. aureus* prefers a neutral pH, disturbance of skin-surface pH may affect microbial colonization and infection of the skin. Clearly, the physiological consequences at the cell and tissue level owing to increased CA II expression require further investigation. Future studies will be directed at the role of epidermal CA II in the maintenance of intracellular pH and the regulation of epidermal water transport.

DEPOSITION OF MICROARRAY DATA

Microarray data on gene expression levels in lesional psorasis and AD epidermal cells have been deposited, compliant with MIAME criteria, at http://www.ncbi.nlm.nih.gov/geo/ and are accessible through GEO Series accession number GSE6601.

CONFLICT OF INTEREST

The names of M.K., G. de J., P.Z., and J.S. appear on a patent application submitted by their employer (Radboud University Nijmegen Medical Centre) that covers the use of CA inhibitors in AD.

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REFERENCES

Bonanno JA, Srinivas SP, Brown M (1995) Effect of acetazolamide on intracellular pH and bicarbonate transport in bovine corneal endothelium. *Exp Eye Res* 60:425–34

Boron WF (2004) Regulation of intracellular pH. *Adv Physiol Educ* 28:160–79

Briggman JV, Tashian RE, Spicer SS (1983) Immunohistochemical localization of carbonic anhydrase I and II in eccrine sweat glands from control subjects and patients with cystic fibrosis. *Am J Pathol* 112: 250–7

Casini A, Scozzafava A, Mincione F, Menabuoni L, Ilies MA, Supuran CT (2000) Carbonic anhydrase inhibitors: water-soluble 4-sulfamoylphenylthioureas as topical intraocular pressure-lowering agents with long-lasting effects. *J Med Chem* 43:4884–92

de Jongh GJ, Zeeuwen PL, Kucharekova M, Pfundt R, van der Valk PG, Blokx W *et al.* (2005) High expression levels of keratinocyte antimicrobial proteins in psoriasis compared with atopic dermatitis. *J Invest Dermatol* 125:1163–73

Eberlein-Konig B, Schafer T, Huss-Marp J, Darsow U, Mohrenschlager M, Herbert O *et al.* (2000) Skin surface pH, stratum corneum hydration, trans-epidermal water loss and skin roughness related to atopic eczema and skin dryness in a population of primary school children. *Acta Derm Venereol* 80:188–91

Franssen ME, Zeeuwen PL, Vierwinden G, van de Kerkhof PC, Schalkwijk J, van Erp PE (2005) Phenotypical and functional differences in germinative subpopulations derived from normal and psoriatic epidermis. *J Invest Dermatol* 124:373–83

Geers C, Gros G (2000) Carbon dioxide transport and carbonic anhydrase in blood and muscle. *Physiol Rev* 80:681–715

Hachem JP, Crumrine D, Fluhr J, Brown BE, Feingold KR, Elias PM (2003) pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J Invest Dermatol* 121:345–53

Johanson CE, Parandoosh Z, Dyas ML (1992) Maturational differences in acetazolamide-altered pH and HCO_3 of choroid plexus, cerebrospinal fluid, and brain. *Am J Physiol* 262:R909–14

Kniep EM, Roehlecke C, Ozkucur N, Steinberg A, Reber F, Knels L *et al.* (2006) Inhibition of apoptosis and reduction of intracellular pH decrease in retinal neural cell cultures by a blocker of carbonic anhydrase. *Invest Ophthalmol Vis Sci* 47:1185–92

Leung DY, Bieber T (2003) Atopic dermatitis. *Lancet* 361:151–60

Mastrolorenzo A, Zuccati G, Massi D, Gabrielli MG, Casini A, Scozzafava A *et al.* (2003) Immunohistochemical study of carbonic anhydrase isozymes in human skin. *Eur J Dermatol* 13:440–4

Nakhoul NL, Davis BA, Romero MF, Boron WF (1998) Effect of expressing the water channel aquaporin-1 on the CO_2 permeability of Xenopus oocytes. *Am J Physiol* 274: C543–8

Noda Y, Takai Y, Iwai Y, Meenaghan MA, Mori M (1986) Immunohistochemical study of

carbonic anhydrase in mixed tumours from major salivary glands and skin. *Virchows Arch A Pathol Anat Histopathol* 408: 449–459

Nomura I, Gao B, Boguniewicz M, Darst MA, Travers JB, Leung DY (2003) Distinct patterns of gene expression in the skin lesions of atopic dermatitis and psoriasis: a gene microarray analysis. *J Allergy Clin Immunol* 112:1195–202

Pfundt R, Van RF, Van Vlijmen-Willems IM, Alkemade HA, Zeeuwen PL, Jap PH *et al.* (1996) Constitutive and inducible expression of SKALP/elafin provides anti-elastase defense in human epithelia. *J Clin Invest* 98:1389–99

Sly WS, Hu PY (1995) Human carbonic anhydrases and carbonic anhydrase deficiencies. *Annu Rev Biochem* 64: 375–401

Spicer SS, Sens MA, Tashian RE (1982) Immunocytochemical demonstration of carbonic anhydrase in human epithelial cells. *J Histochem Cytochem* 30:864–73

Skin Corticotropin-Releasing Hormone Receptor Expression in Psoriasis

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TO THE EDITOR

Psoriasis is characterized by keratinocyte proliferation, inflammation, and mast cell activation (Schon and Boehncke, 2005). It is also triggered or exacerbated by acute stress (Katsarou-Katsari *et al.*, 1999; Saraceno *et al.*, 2006); however, this mechanism remains poorly understood. Stress typically results in release of corticotropin-releasing hormone (CRH) from the hypothalamus and regulates the hypothalamic–pituitary–adrenal (HPA) axis (Chrousos, 1995) through activation of CRH receptor-1 (CRH-R1), leading to immunosuppression. CRH is also found peripherally (Chrousos, 1995) and has pro-inflammatory effects through mast cell activation (Theoharides *et al.*, 1998). CRH and CRH-R gene expression has been documented in rodent and human skin (Slominski *et al.*, 2001). In fact, it has been proposed that skin has the equivalent of the HPA axis (Slominski *et al.*, 2000). In mice, CRH is released from nerve endings (Slominski *et al.*, 2001), whereas in humans it is synthesized by skin cells (Slominski *et al.*, 1998), immune cells (Karalis *et al.*, 1997), and human mast cells (Kempuraj *et al.*, 2004).

To study the effect of stress and the role of CRH in psoriasis, we investigated, by quantitative PCR, CRH-R expression in affected and unaffected skin of psoriasis patients ($n=13$) and skin from normal controls ($n=4$), as well as serum CRH levels from psoriasis patients ($n=8$) and controls ($n=4$). The characteristics of the subjects (Table S1) were as follows: male mean age 47.4 ± 7.0 years ($n=7$); female mean age 28.0 ± 5.2 years ($n=6$); normal subjects (one male, three female subjects, mean age 40 ± 15.2 years). All skin biopsies requiring two stitches were collected for diagnostic purposes (Table S1). The Medical Ethics Committee of Attikon Hospital HIRB approved this protocol. All participants gave their written informed consent according to the Declaration of Helsinki Principles. Patients had moderate chronic plaque psoriasis with psoriasis area and severity index (PASI) scores 5–16 and had not received any therapy for psoriasis (topical or systemic) for the past month. The PASI score for males was 11.3 ± 13.5 and for females was 11.5 ± 3.7.

Expression of CRH-R1 mRNA was lowest in affected samples from psoriasis patients (0.27 ± 0.23, $n=13$, $P<0.05$), compared with control patients (Figure 1a). CRH-R1 expression in unaffected skin from psoriasis patients (0.53 ± 0.38) was not statistically different from that of affected samples or controls (Figure 1a). There was no statistically significant difference in CRH-R2 mRNA expression among the control samples, those obtained from affected (0.86 ± 0.51) and from unaffected (0.97 ± 0.65) psoriatic skin (Figure 1a).

The serum CRH level (11.52 ± 6.09 pg/ml) was higher ($n=8$, $P<0.05$) in psoriasis patients than controls (5.42 ± 1.2 pg/ml, $n=8$). There was no apparent correlation between the PASI scores and either CRH-R1 expression or serum CRH levels.

This study provides early evidence that affected psoriatic skin has decreased gene expression of CRH-R1 mRNA than normal controls. One possible explanation is that overstimulation by increased levels of local or systemic (serum) CRH in psoriasis patients, possibly in response to chronic stress, may lead to CRH-R1 downregulation. In fact, CRH protein expression was recently reported to be increased in the affected skin of three patients with active psoriasis than in one control; however, this effect was not quantitated (O'Kane *et al.*, 2006). As non-affected psoriatic skin apparently did not overexpress CRH-R, as shown by our quantitative real-time–PCR data, there was apparently no mechanism in place to lead to downregulation. Increased CRH-R expression in psoriatic skin was also mentioned as ''unpublished observations'' (O'Kane *et al.*, 2006) and it is, therefore, difficult to evaluate it. The reduction in CRH-R1 mRNA expression in affected skin of patients with psoriasis we observed could be due to the intense inflammation seen in plaques without any association with serum CRH levels; how-

Abbreviations: CRH, corticotropin-releasing hormone; HPA, hypothalamic–pituitary–adrenal; CRH-R1, CRH receptor-1; PASI, psoriasis area and severity index