Evidence for Expression of Melanocortin-1 Receptor in Human Sebocytes *In Vitro* and *In Situ*

Markus Böhm, Meinhard Schiller, Sonja Ständer, Holger Seltmann,* Zhuo Li, Thomas Brzoska, Dieter Metze, Helgi B. Schiöth,† Anna Skottner,‡ Kristina Seiffert,* Christos C. Zouboulis,* and Thomas A. Luger

Department of Dermatology and Ludwig Boltzmann Institute for Cell Biology and Immunobiology of the Skin, University of Münster, Münster, Germany; *Department of Dermatology, University Medical Center Benjamin Franklin, The Free University of Berlin, Berlin, Germany; †Department of Neuroscience, Uppsala University, Uppsala, Sweden; ‡Melacure Therapeutics AB, Uppsala, Sweden

Many lines of evidence indicate that the activity of sebaceous glands can be modulated by neuropeptides. Direct evidence in man, however, is still missing. We show that SZ95 sebocytes, an immortalized human sebaceous gland cell line, express receptors for α-melanocyte-stimulating hormone. Reverse transcription polymerase chain reaction with primers against the five melanocortin receptors and immunofluorescence studies using an antibody directed against a peptide corresponding to the amino acids 2–18 of the human melanocortin-1 receptor disclosed specific transcripts and immunoreactivity for melanocortin-1 receptor in these cells. Melanocortin-1 receptor expression was confirmed in sebocytes of normal human skin by immunohistochemistry. In contrast, no immunostaining for the melanocortin-5 receptor could be detected in sebocytes *in situ*, in accordance with the lack of specific transcripts for this melanocortin receptor in SZ95 sebocytes. As cytokines play an important role in the recruitment of inflammatory cells in acne and related disorders and α-melanocyte-stimulating hormone exerts immunomodulatory effects in many other cell types, we investigated the effect of α-melanocyte-stimulating hormone on interleukin-8 secretion by SZ95 sebocytes. Treatment with interleukin-1β resulted in a marked increase in interleukin-8 release that was partially blocked by coincubation with α-melanocyte-stimulating hormone in a dose-dependent manner. Taken together, we show here that the melanocortin-1 receptor is expressed *in vitro* and *in situ* in human sebocytes. By modulating interleukin-8 secretion, α-melanocyte-stimulating hormone may act as a modulator of inflammatory responses in the pilosebaceous unit. *Key words: α-melanocyte-stimulating hormone/cell proliferation/cytokine/interleukin-8/sebaceous gland/SZ95 sebocytes. J Invest Dermatol 118:533–539, 2002*

It is well established that the skin is a target organ and a peripheral source for proopiomelanocortin (POMC)-derived peptides (reviewed in Böhm and Luger, 2000; Slominski *et al*, 2000). One of these peptides is α-melanocyte-stimulating hormone (α-MSH), a tridecepeptide that is generated from POMC by successive endoproteolytic cleavage through the action of distinct prohormone convertases. During the last few years it has become apparent that α-MSH can elicit many biologic effects besides regulation of pigmentation. In monocytes α-MSH induces interleukin-10 (IL-10), a potent suppressor of proinflammatory cytokines (Bhardwaj *et al*, 1996). In mice, α-MSH is capable of suppressing the contact hypersensitivity reaction by inducing hapten-specific tolerance (Grabbe *et al*, 1996). In a mouse model of experimentally induced cutaneous vasculitis, α-MSH also suppresses endothelial damage and expression of distinct adhesion molecules on dermal endothelial cells (Sunderkötter *et al*, 1999). The diversity of these biologic effects of α-MSH are mediated by specific receptors that are known as the melanocortin receptors (MC-Rs) (reviewed in Cone *et al*, 1996). These receptors belong to the super-family of G-protein-coupled receptors with seven transmembrane domains whose engagement by the ligand results in increased levels of intracellular cAMP. Until now, five different MC-Rs have been cloned (reviewed in Cone *et al*, 1996). The individual MC-Rs differ in their tissue distribution and can be discriminated from each other by their relative affinities towards the naturally occurring melanocortins α-, β-, and γ-MSH and adrenocorticotropic hormone (ACTH) as well as the synthetic MSH analog [Nle4, D-Phe7]-MSH (reviewed in Cone *et al*, 1996).

The sebaceous gland has long been recognized as a target for α-MSH (reviewed in Thody and Schuster, 1989; Böhm and Luger, 1998). Thody and coworkers reported that removal of the posterior pituitary in the rat significantly reduces the lipid secretion rates by the preputial gland, a specialized lipid-producing organ (Thody and Schuster, 1972). The lipotrophic substance responsible for this effect was identified as α-MSH (Thody and Schuster, 1973). Further studies have revealed that daily treatment with α-MSH resulted in a dose-dependent increase in sebum secretion of intact rats (Thody *et al*, 1975). By means of *in vivo* binding studies with iodinated [Nle4, D-Phe7]-MSH it was later shown that MSH receptors were widely distributed in numerous glandular organs in rodents (Tatro and Reichlin, 1987). Direct evidence for a biologic activity of α-MSH on sebocytes in man, however, is still missing.

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Reprint requests to: Dr. Markus Böhm, Department of Dermatology, University of Münster, Von Esmarch-Str. 58, D-48149 Münster, Germany. Email: bohmm@uni-muenster.de

Abbreviations: α-MSH, α-melanocyte-stimulating hormone; MC-R, melanocortin receptor.

Table I. Primer sets used in RT-PCR[a]

Gene/Accession number	Gene product	Primer (F, forward; B, backward)	Size	Reference
MC1R/AF326275	MC-1R	F: 5′-GCCACCATCGCCAAGAACC-3′ B: 5′-ATAGCCAGGAAGAAGACCA-3′	416	Hartmeyer *et al* (1997)
MC2R/Z25470[b]	MC-2R	F: 5′-CTGCATTTCTTGGATCT-3′ B: 5′-AAGCTGCACATGGATGC-3′	380	Hartmeyer *et al* (1997)
MC3R/NM019888	MC-3R	F: 5′-CGGTGGCCGACATGCTGGTAAGTG-3′ B: 5′-TGAGGAGCATCATGGCGAAGAACA-3′	366	Hartmeyer *et al* (1997)
MC4R/NM005912	MC-4R	F: 5′-CAATAGCCAAGAACAAGAATC-3′ B: 5′-GACAACAAAGACGCCAATCAG-3′	566	Hartmeyer *et al* (1997)
MC5R/NM005913[b]	MC-5R	F: 5′-CATTGCTGTGGAGGTGTTTCT-3′ B: 5′-GCCGTCATGATGTGGTGGTAG-3′	357	Bhardwaj *et al* (1997)

[a]Sequences as deposited in the National Center for Biotechnology Information (NBCI) database under the given accession number. Size denotes the number of base pairs of the amplification product.
[b]Note that the mRNA sequence for MC2 is a colinear but truncated form of the MC-5R.

In vitro, α-MSH was shown not to affect human sebocyte proliferation (Zouboulis *et al*, 1998).

A major factor that has hampered basic research on human sebocytes until recently is the lack of an easy-to handle sebocyte culture model. Although human sebocyte culture models were introduced in 1989 (Xia *et al*, 1989), the cultured primary cells are predestined to differentiate by accumulating new fat droplets until they burst and die. Recently, we have established a human sebaceous gland cell line, SZ95, that was immortalized by transfecting primary human facial sebocytes with the coding region of the Simian virus-40 large T antigen (Zouboulis *et al*, 1999). This cell line shows the morphologic, phenotypic, and functional characteristics of normal human sebocytes.

By employing SZ95 sebocytes, we have set out to investigate the expression of MC-Rs as a base for testing the hypothesis that human sebocytes are target cells for α-MSH. After having determined the MC-R expression profile in SZ95 sebocytes, MC-1R expression was confirmed in the sebaceous unit of adult human skin. In order to assess the relevance of MC-1R expression in sebocytes we finally investigated whether α-MSH can modulate secretion of IL-8, an important chemoattractant for inflammatory cells.

MATERIALS AND METHODS

Cell culture SZ95 sebocytes were maintained in Sebomed basal medium supplemented with human epidermal growth factor (5 ng per ml), 10% fetal bovine serum (FBS) (all from Biochrom, Berlin, Germany), 1% glutamine, and 1% penicillin/streptomycin (both from BioWhittaker, Walkersville, MD) in a humidified atmosphere of 5% CO_2 at 37°C unless otherwise stated. Normal human neonatal melanocytes and human dermal fibroblasts were purchased from BioWhittaker and were cultured according to the manufacturer's instructions. The melanoma cell line A375 was obtained from ATCC and maintained in RPMI 1640 (Biochrom) supplemented with 10% FBS, 1% glutamine, and 1% penicillin/streptomycin.

RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and sequencing For all experiments, SZ95 sebocytes were maintained in chemically defined Sebomed complete medium to exclude the potential influence of FBS. Total RNA from SZ95 sebocytes and normal human melanocytes was isolated using the RNeasy kit from Qiagen, Santa Clarita, CA. The RNA obtained was routinely digested with DNAse (Promega, Madison, WI) to ensure complete absence of genomic DNA. PCR amplification was performed with REDTaq polymerase (Sigma, Taufkirchen, Germany) and commercially synthesized HPLC-purified primers (Gibco-BRL, Gaithersburg, MD). The sequences of the primer pairs and the sizes of their amplification products are given in **Table I**. The thermocycler protocols were the same as previously reported by us (Bhardwaj *et al*, 1997; Hartmeyer *et al*, 1997). The Px reaction mixture contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.1 mM $MgCl_2$ 0.01% gelatin, 0.05 V REDTaq DNA polymerase (all from Sigma, St. Louis, MO) and 0.2 mM dNTP. Samples of RNA that yielded amplification products without prior reverse transcription were excluded. PCR products were separated electrophoretically on 1.5%–2% TAE-agarose gels, stained with ethidium bromide, and photographed under ultraviolet. The positive controls for MC-2R, MC-3R, MC-4R, and MC-5R were derived from genomic DNA that was prepared from human dermal fibroblasts by routine protocols. The resulting positive bands from SZ95 sebocytes (MC-1R) were purified using a gel extraction kit (Qiagen), cloned into pGEM-T easy vectors (Promega), and sequenced (4base lab, Reutlingen, Germany).

Immunofluorescence SZ95 sebocytes were seeded in Sebomed basal medium into eight-well Laboratory-Tek Permanox chamber slides (Nalge Nunc, Naperville, IL) at a density of 10,000 cells per well. Cells were then grown for an additional 1–2 d to ensure sufficient adherence to the slides. They were fixed with 4% paraformaldehyde for 30 min and nonspecific binding was blocked with 5% donkey serum for 1 h. Then, cells were incubated with the MC-1R antibody (1–10 μg per ml) for 1 h. Slides were washed with phosphate-buffered saline (PBS) and incubated with a donkey anti-rabbit antibody conjugated with Texas Red (1:100; Dianova). All procedures were performed at room temperature. After final washing the slides were mounted in Mowiol (Hoechst, Frankfurt, Germany) and stored until use. Cells were imaged and examined with a fluorescence microscope (Leica DMRD/RXA, Leica Mikroskopie und Systeme, Wetzlar, Germany). Red fluorescence was excited using the TX2 filter (BP 560/40 nm). Emission was measured at 645 ± 75 nm. In the negative controls cells were incubated with preimmune serum or in the absence of the primary antibody.

Generation for an anti-human MC-5R antibody The antibody was raised against the N-terminal region of the human MC-5R by immunizing Swedish loop rabbits by intramuscular injections with thyroglobulin-conjugated peptides in the presence of Freund's adjuvant. The rabbits were given a booster of 1.1 mg of the conjugate in complete Freund's adjuvant. The booster was then repeated twice with a 2 wk interval with 0.8 mg of the conjugate and incomplete Freund's adjuvant. Serum was collected 2 wk after the last booster. Preimmune serum had been obtained before the immunization. The peptide NLSGPN-VKNKSSPC corresponding to the amino acids 20–33 of the N-terminal domain of the human MC-5R was made and conjugated by Medprobe, Oslo, Norway. The specificity of the serum to the peptide was confirmed by enzyme-linked immunosorbent assay (ELISA) against the preimmune serum and a human MC-1R antiserum made by Xia *et al* (1995).

Immunohistochemistry Samples of human adult skin ($n = 6$) derived from patients undergoing routine surgery for therapeutic and diagnostic reasons were examined. They were from various body sites including the face and scalp. The positive control for MC-1R immunohistochemistry consisted of a primary cutaneous human melanoma. For MC-5R immunostaining, human prostate tissue was used as a positive control, kindly provided by the Department of Pathology, University of Münster. Specimens were either cryofixed in liquid nitrogen or fixed in 7% buffered paraformaldehyde, dehydrated, and embedded in parablast. Parablast-embedded sections and cryosections were mounted on Tissue-Tek (Mikrom, Walldorf, Germany). Parablast-embedded sections were deparaffinized, microwave-treated and quenched for endogenous peroxidase activity, and blocked with 2% bovine serum albumin for 30 min at room temperature as described previously (Böhm *et al*, 1999a).

The sections were incubated with the anti-MC-1R antibody at 1 μg per ml, with the individually generated MC-5R antiserum at 1–10 μg per ml, or with one commercially available anti-MC-5R antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at 0.1–2 μg per ml for 45 min. After washing, sections were developed by an indirect immunoperoxidase technique using 3-amino-9-ethylcarbazole (Sigma, Deisenhofen, Germany) as a chromogen. For negative controls the primary antibody was omitted or substituted with preimmune serum.

Determination of IL-8 Cells were trypsinized and seeded into six-well tissue culture plates at a density of 150,000 per well. On the next day, cells were washed with PBS and the culture medium was switched to Sebomed complete medium. After 3 d in culture the medium was removed. The cells were gently washed once with PBS. Two milliliters of fresh Sebomed complete medium supplemented with α-MSH at varying concentrations (10^{-6}–10^{-12} M), IL-1β (50 ng per ml), or both substances were added per well. Cells were then incubated for 2 d. Culture supernatants were collected and passed through a 0.2 μm filter to remove any cell detritus. Aliquots were stored at –70°C until use. IL-8 in the culture supernatants was determined using an ELISA from R&D Systems, Minneapolis, MN. Duplicate wells were used for each individual sample; the detection limit was less than 10 pg per ml. Statistical significance was calculated by the two-sample independent-groups *t* test.

RESULTS

Expression of MC-1R specific transcripts in SZ95 sebocytes We first determined the MC-R expression profile on the RNA level in SZ95 sebocytes by using specific primers against the five known MC-Rs. RT-PCR of total RNA revealed only the expression of MC-1R (**Fig 1*a***), whereas all other transcripts, namely the MC-2R, MC-3R, MC-4R, and MC-5R, were absent (**Fig 1*b***, ***c***). To ascertain that the lack of MC-2R to MC-5R transcripts in SZ95 sebocytes was not caused by insufficient amplification, positive controls (genomic DNA from diploid human dermal neonatal fibroblasts) were included in all reactions. As shown in **Fig 1**(***b***, ***c***), these samples resulted in amplification products of the expected size as previously reported (Bhardwaj *et al*, 1997; Hartmeyer *et al*, 1997). The detected MC-1R amplification product derived from RNA of SZ95 sebocytes (416 bp) (**Fig 1*a***) comigrated with the product derived from RNA of normal human melanocytes, which are known to express MC-1R (Mountjoy *et al*, 1992). As it is known that the MC-1R displays a high polymorphism in man (Rana *et al*, 1999) and the primers used for RT-PCR (nucleotide positions 181–596) span a region with known variants, the amplification product of SZ95 sebocytes was sequenced. It was identical to the original sequence of MC-1R as published by Mountjoy *et al* (1992) (data not shown).

Detection of MC-1R immunoreactivity on the surface of SZ95 sebocytes We next investigated the association of MC-1R expression on the RNA level with surface expression of the receptor in SZ95 sebocytes. Immunostaining of SZ95 sebocytes was performed using an affinity-purified anti-MC-1R antibody directed against the amino acids 2–18 of the N-terminal domain of the human MC-1R (Böhm *et al*, 1999a; 1999b). As a positive control, A375 melanoma cells, which are known to express MC-1R (Siegrist *et al*, 1997), were included in these studies. Cells were fixed with 4% paraformaldehyde without permeabilization. This fixation method confirmed the presence of surface MC-1R immunoreactivity in both A375 melanoma cells and SZ95 sebocytes (**Fig 2*a***, ***c***). Both cell lines exhibited a punctate or granular staining pattern. About 90% of A375 melanoma cells per viewing field exhibited MC-1R immunostaining, whereas the percentage of SZ95 sebocytes staining positively was lower and ranged between 40% and 60%. Interestingly, whereas in SZ95 sebocytes the punctate MC-1R immunostaining was diffuse and occasionally accentuated in the cell periphery (**Fig 2*a***), MC-1R immunoreactivity in A375 melanoma cells was almost exclusively located in focal areas opposing the nucleus (**Fig 2*c***). Preimmune serum or omission of the primary antibody, in contrast, did not result in any immunostaining (**Fig 2*b***, ***d***).

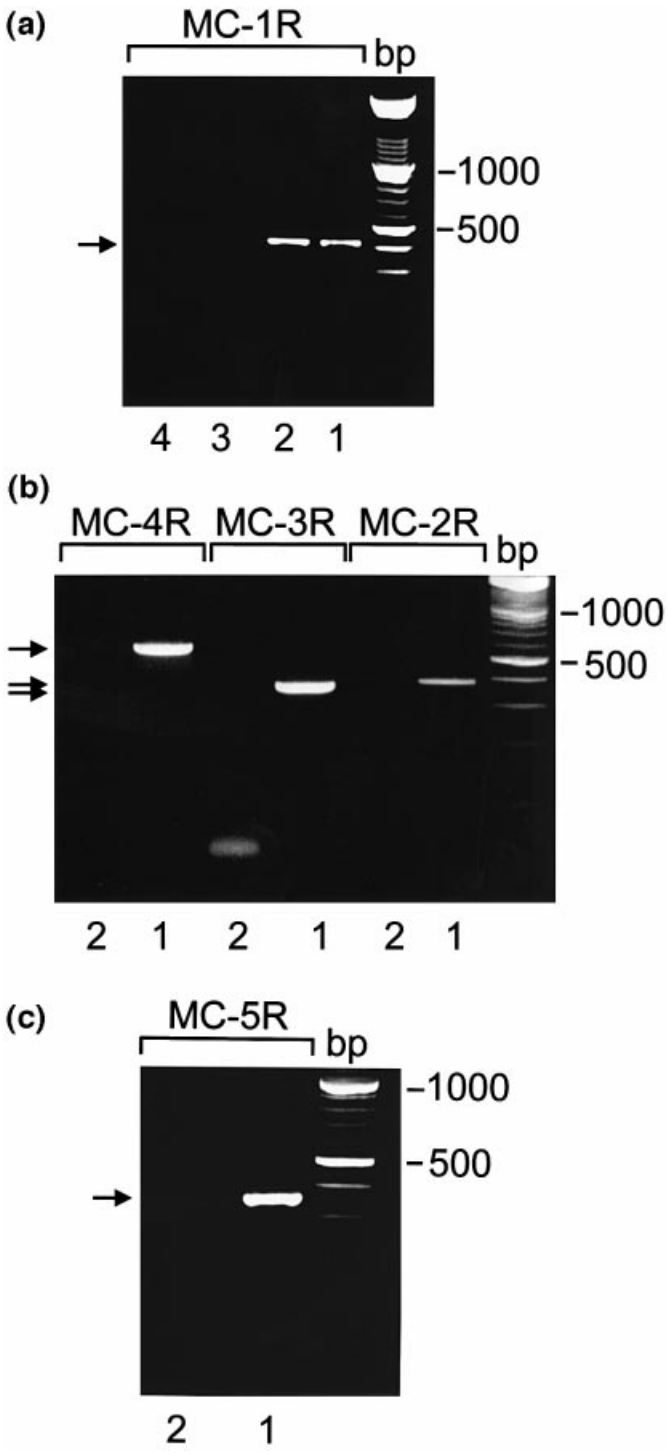

Figure 1. Expression of MC-1R-specific transcripts in SZ95 sebocytes as shown by RT-PCR. Total RNA from SZ95 sebocytes (*lane 2*) was reverse transcribed and amplified with primers against MC-1R (*a*), MC-2R, MC-3R, MC-4R (*b*), and MC-5R (*c*). Positive controls (*lane 1*) consisted of RNA from normal human melanocytes (for MC-1R) and genomic DNA from normal human dermal fibroblasts (for MC-2R, MC-3R, MC-4R, and MC-5R). Negative controls for the MC-1R RT-PCR consisted of a contamination control using total RNA from SZ95 sebocytes without reverse transcription (*lane 3*) and substitution of the template with H_2O (*lane 4*).

Expression of MC-1R but not MC-5R immunoreactivity in sebaceous glands *in situ* To assess the significance of MC-1R expression in SZ95 sebocytes, we performed immunohistochemistry on normal adult human skin. As it has recently been

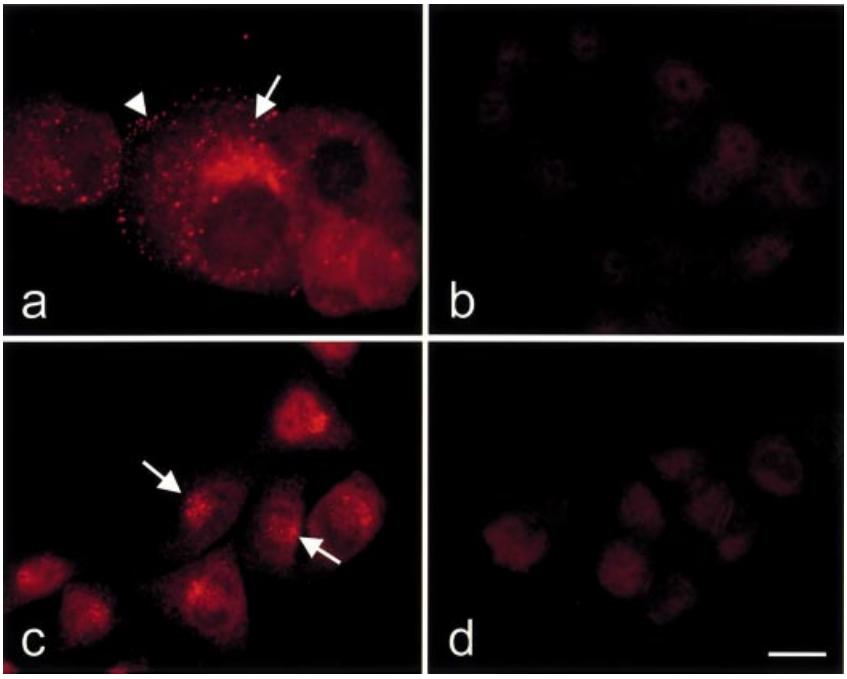

Figure 2. Visualization of MC-1R immunoreactivity on the surface of SZ95 sebocytes by immunofluorescence. Cells were fixed with paraformaldehyde and stained with an anti-MC-1R antibody directed against the amino acids 2–28 of the N-terminal domain of MC-1R. Bound antibodies were visualized using a Texas-Red-conjugated secondary antibody. The staining pattern of SZ95 sebocytes (*a*) was compared with that of A375 melanoma cells, which are known to express MC-1R (*c*). Note the diffuse punctate immunostaining of SZ95 sebocytes (*arrow*) compared to the focal staining pattern of A375 melanoma cells (*arrow*). Note also immunostaining in the cell periphery of SZ95 sebocytes (*arrowhead*). Preimmune serum did not produce any specific staining in SZ95 sebocytes (*b*) or A375 melanoma cells (*d*). *Scale bar*: 10 μm.

demonstrated that MC-5R knockout mice suffer from a defect in water repulsion due to reduced sebaceous sebum secretion (Chen *et al*, 1997), we also performed immunohistochemical studies for the MC-5R. Sebaceous glands displayed MC-1R immunoreactivity in almost all sebocytes, but immunostaining was most prominent in undifferentiated peripheral cells and in the central more differentiated cells immunostaining was much less intense and occasionally absent (**Fig 3*a***). MC-1R immunostaining was mostly detected in the cytoplasm of the sebocytes and, to a lesser extent, on the surface of the cells. In accordance with previously reported findings (Böhm *et al*, 1999a), MC-1R immunoreactivity was also detected in secretory eccrine epithelia and in hair follicles whereas interfollicular epidermis was mostly nonreactive (data not shown). Omission of the primary antibody, substitution with preimune serum, or preincubation of the anti-MC-1R antibody with the immunogenic peptide used for generation of the MC-1R antibody did abrogate staining (**Fig 3*b***). Immunostaining of a primary cutaneous human melanoma that was used as a positive control revealed moderate MC-1R immunoreactivity in the cytoplasm of the tumor cells (data not shown), in accordance with our previous findings (Böhm *et al*, 1999a). We next performed immunohistochemistry of skin sections with two commercially available anti-MC-5R antibodies and one individually generated antibody. MC-5R immunoreactivity could not be detected in cryostat nor in paraffin-embedded sections derived from normal human adult corporal and facial skin (**Fig 3*c***). Our failure to detect MC-5R immunoreactivity in normal adult human skin was not due to the nature of the anti-MC-5R antibody used as prostate tissue known to express MC-5R (Van der Kraan *et al*, 1998) clearly revealed immunostaining within the secretory epithelia (**Fig 3*d***). Preimmune serum, in contrast, did not produce any staining (**Fig 3*e*).**

Modulation of IL-8 secretion by α-MSH in SZ95 sebocytes In order to assess the biologic significance of MC-1R being expressed in SZ95 sebocytes, we determined the effect of α-MSH on the secretion of IL-8. This chemokine is a key chemoattractant for neutrophils and other immune cells, which play a crucial role in the inflammatory response of the pilosebaceous unit in acne and related disorders. All experiments were performed in the absence of FBS and to reduce potential effects of nonspecified substances on IL-8 secretion. In addition to nontreated cells and cells treated with α-MSH alone at varying concentrations (10^{-6}–10^{-12} M), a known inducer of IL-8 (Kristensen *et al*, 1991), namely IL-1, was included. Immunoreactive IL-8 was detected by ELISA in significant amounts (mean ± SD = 1722.5 ± 176.2 pg per ml) in culture media of unstimulated SZ95 sebocytes, indicating constitutive production (**Fig 4*a***). α-MSH alone barely affected IL-8 secretion by SZ95 sebocytes, although the observed inhibitory effect of α-MSH at 10^{-9} M was statistically significant (p-value 0.06) (**Fig 4*a***). As expected, IL-1β treatment upregulated the secretion of IL-8 to 3371.26 ± 366.9 pg per ml (**Fig 4*b***). When α-MSH at 10^{-9} M was coincubated with IL-1β, it significantly suppressed the IL-1β-induced IL-8 release by 28.4% (2415.5 ± 8.5 pg per ml; p-value 0.02) (**Fig 4*b***). In contrast, all other concentrations of α-MSH (whether given alone or in combination with IL-1β) did not affect IL-8 secretion significantly.

DISCUSSION

In this report we have shown that human sebocytes *in vitro* and *in situ* express MC-1R. Our results on MC-1R expression in sebocytes were unexpected as studies in mice have demonstrated that targeted disruption of the MC-5R gene resulted in a defect in water repulsion and thermoregulation due to reduced production of sebaceous lipids (Chen *et al*, 1997). Moreover, *in situ* hybridization in mice revealed MC-5R transcripts in a number of secretory epithelia including those of the preputial gland, prostate, lacrimal gland, and Harderian gland (Chen *et al*, 1997; Van der Kraan *et al*, 1998). Recently, Thiboutot and coworkers also reported that dissected human sebaceous glands express MC-5R as shown by RT-PCR as well as by Western immunoblotting using an MC-R5 antiserum raised in chicken (Thiboutot *et al*, 2000). This antibody was directed against the same epitope of human MC-5R that we chose for generation of our antiserum. By means of their MC-5R antiserum, the latter authors observed MC-5R immunoreactivity in sebaceous glands, sebaceous ducts, hair follicles, epidermis, eccrine glands, endothelial cells, and fibroblasts of cryostat sections from normal human facial skin. In contrast, we were unable to detect MC-5R but MC-1R expression was present in SZ95 sebocytes *in vitro* and sebocytes *in situ*. MC-1R expression was not confined to the RNA level in SZ95 sebocytes but could also be detected by immunofluorescence studies and immuno-

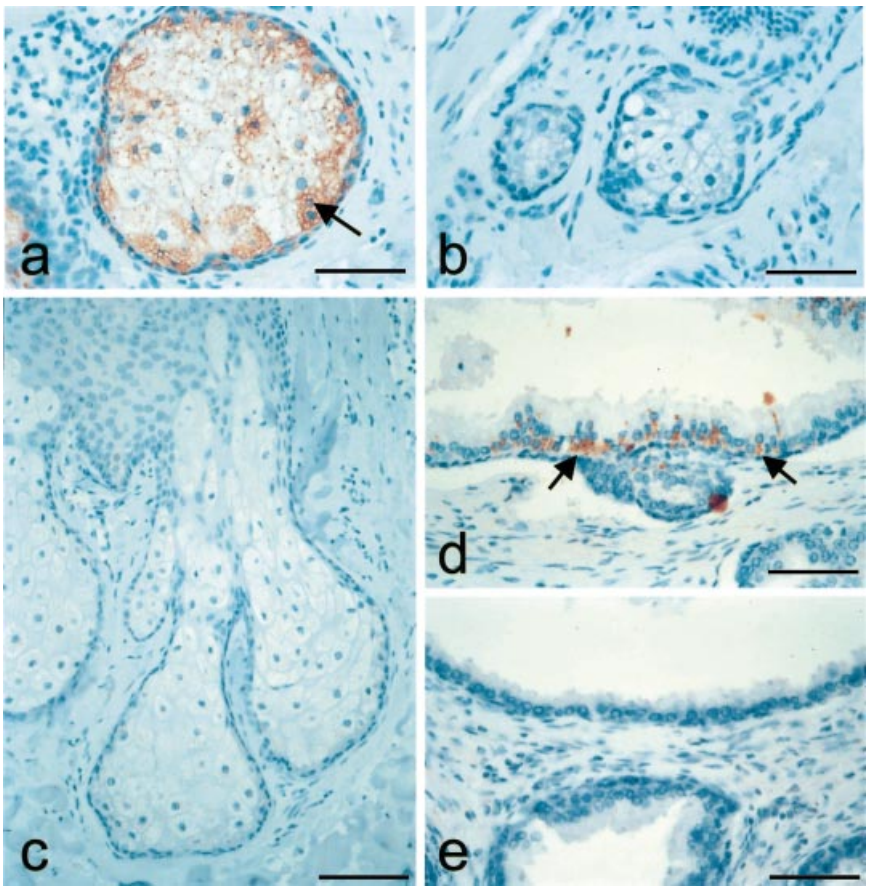

Figure 3. Detection of MC-1R but not MC-5R immunoreactivity in the human sebaceous gland *in situ*. Deparaffinized sections from normal human adult corporal skin and human prostate gland (the latter serving as a positive control for MC-5R) were incubated with antibodies against MC-1R or MC-5R, respectively. Bound antibodies were visualized by the immunoperoxidase technique. Sebaceous gland showing intracytoplasmic MC-1R staining mainly in the undifferentiated peripheral cells (*arrow*), 400× (*a*); negative control of a normal skin section, i.e., preincubation of the primary antibody with the immunogenic peptide in excess, 400× (*b*); lack of MC-5R immunoreactivity in human sebaceous glands, 200× (*c*); human prostate gland; note intracellular MC-5R staining of secretory epithelia (*arrow*), 400× (*d*); negative control using preimmune serum instead of the anti-MC-5R antibody, 400× (*e*). *Scale bars*: 50 μm (*a*, *b*, *d*, *e*) and 100 μm (*c*).

histochemistry in SZ95 sebocytes and in sebaceous glands. Interestingly, the surface MC-1R staining pattern differed between SZ95 sebocytes and A375 melanoma cells. In contrast to SZ95 sebocytes MC-1R staining in A375 melanoma cells was confined to distinct areas, which supports earlier investigations on Cloudman mouse melanoma cells that displayed a nonrandom and patchy distribution of MSH binding sites (Varga *et al*, 1976). It is possible that the latter finding is related to autonomous secretion of α-MSH and ACTH, which both bind to the MC-1R and thus may partly abrogate binding of the anti-MC-1R antiserum. As it has been shown that MSH binding sites correlate with pigmentation (Kameyama *et al*, 1988), the MC-1R staining pattern of the amelanotic A375 melanoma cell line, on the other hand, may also indicate a relatively low expression level of MC-1R. Regarding our immunohistochemical studies immunoreactivity for MC-1R was confirmed in sebocytes *in situ*. Ultrastructural studies by immunoelectron microscopy have further shown that MC-1R immunoreactivity in seboytes *in situ* is located both on the cell surface and intracellularly in tubular endosomes (Ständer *et al*, 2002). Most of the *in situ* MC-1R immunoreactivity in human sebocytes, however, was found within the cytoplasm, suggesting that these cells do not produce POMC or POMC-derived peptides (Slominski *et al*, 1993 and our own unpublished findings). The surprisingly weak immunoreactivity of MC-1R in human interfollicular epidermis as previously reported (Böhm *et al*, 1999a) may indicate that only a limited number of receptors are available for the anti-MC-1R antiserum and most of the MC-1Rs are complexed with MSH or ACTH, which are produced by both keratinocytes and melanocytes. In light of the discrepancies in the detected MC-R expression profile in sebocytes between our study and that by Thiboutot *et al* (2000) it is necessary to dissect the nature of these differences in more detail. First, the primer sets against all examined MC-Rs in our study and the one by Thiboutot *et al* (2000) were different. With regard to MC-1R, Thiboutot *et al* (2000) neither included a positive RT-PCR control in their studies nor continued with immunohistochemical studies on MC-1R. Second, we have used a uniform sebocyte population, whereas the RNA preparation from microdissected human sebaceous glands in combination with RT-PCR as performed by Thiboutot *et al* (2000) does not exclude contamination by other cell types. On the other hand, although we have confirmed the immunoreactivity of our anti-MC-5R antibody by demonstrating immunostaining of prostate epithelia, the MC-5R antibody generated by Thiboutot *et al* (2000) may be more sensitive than our own, especially in combination with the applied metal enhancer kit.

Our studies on chemokine release by SZ95 sebocytes have confirmed our previous reports that sebocytes secrete IL-8 in a constitutive manner.[1,2] IL-8 may serve as the chemokine that is responsible for the attraction of inflammatory cells to the sebaceous gland and the initiation of the inflammatory process, e.g., in acne. IL-8 has been shown to be induced in SZ95 sebocytes by *Propionibacterium acnes*, IL-1α, lipopolysaccharides, as well as calcitonin gene-related peptide, and to be inhibited by linoleic acid.[1–3] Interestingly, α-MSH at 10^{-9} M was found to partially abrogate IL-1β-induced IL-8 secretion in SZ95 sebocytes. By

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[3]Seltmann H, Rudawski IM, Holland KT, Orfanos CE, Zouboulis ChC: Propionibacterium acnes does not influence the interleukin-1a/interleukin-8 cascade in immortalized human sebocytes *in vitro*. *J Invest Dermatol* 114:816, 2000 (abstr.)

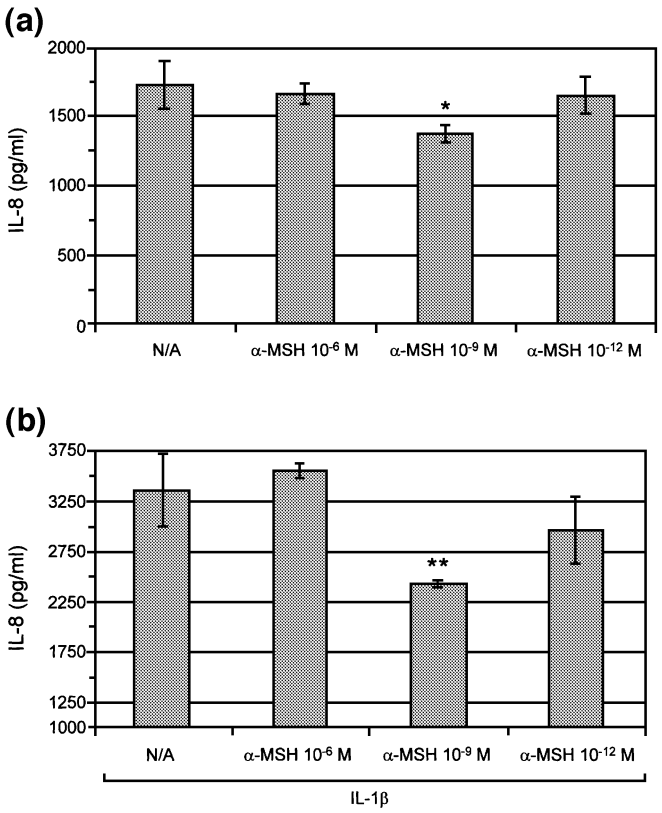

Figure 4. α-MSH modulates IL-8 secretion by SZ95 sebocytes. Cells were treated with α-MSH at different doses as indicated or were left untreated (*a*). In a second set of experiments α-MSH at different concentrations was coincubated with IL-1β (50 ng per ml) (*b*). The amount of IL-8 in the culture supernatants after incubation for 48 h was determined by ELISA. Data are means from three individual experiments ± SD. *p-value 0.06; **p-value 0.02; N/A=untreated cells.

modulating IL-8 secretion, α-MSH could act as a modulator of inflammatory responses of the pilosebaceous unit. Our findings on the modulatory activity of α-MSH on IL-8 release by IL-1β-stimulated SZ95 sebocytes are in accordance with our recent studies showing that IL-8 secretion by human dermal fibroblasts can be modulated by α-MSH (Böhm *et al*, 1999c). As it has been shown that the immunomodulatory activity of α-MSH can partly be abrogated by pharmacologic blockade with butoxamine, a β_2-adrenergic receptor antagonist (Ichiyama *et al*, 1999), however, it remains to be determined whether other receptors than MC-1R are involved in eliciting the biologic responses by α-MSH in human sebocytes.

In summary, we have shown here that sebocytes *in vitro* and *in situ* express MC-1R and that α-MSH can partially abrogate the inductive effects of IL-1β on IL-8 secretion by SZ95 sebocytes. Detection of the MC-1R in SZ95 sebocytes as presented in this paper provides the base for ongoing studies in our laboratory to clarify whether α-MSH can affect lipogenesis in human sebocytes.

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