Expression of CD1d in human scalp skin and hair follicles: hair cycle related alterations

M A Adley, H A Assaf, M Hussein

Background: CD1d belongs to a family of antigen presenting molecules that are structurally and distantly related to the classic major histocompatibility complex class I (MHC I) proteins. However, unlike MHC I molecules, which bind protein antigens, CD1d binds to lipid and glycolipid antigens. CD1d is expressed by cells of lymphoid and myeloid origin, and by cells outside of the lymphoid and myeloid lineages, such as human keratinocytes of psoriatic skin.

Aims: To investigate whether CD1d is also expressed in sun exposed skin and in the immunoprivileged anagen hair follicle.

Materials/Methods: CD1d immunoreactivity was studied in human scalp skin and hair follicles of healthy women in situ by immunofluorescent and light microscopic immunohistology. Skin biopsies were obtained from normal human scalp containing mainly anagen VI hair follicles from women (age, 53–57 years) undergoing elective plastic surgery.

Results: CD1d showed strong immunostaining in human scalp skin epidermis, pilosebaceous units, and eccrine glands. In the epidermis, CD1d was strongly expressed by basal and granular keratinocytes. In hair follicles, CD1d was expressed in the epithelial compartment and showed hair cycle related alterations, with an increase in the anagen and a reduction in the catagen and telogen phases.

Conclusions: These results suggest that CD1d plays a role in human scalp skin immunology and protection against lipid antigen rich infectious microbes. They also raise the question of whether keratinocytes of the immunoprivileged anagen hair follicle can present lipid antigens to natural killer T cells. These data could help provide new strategies for the manipulation of hair related disorders.

CD1d is a member of the CD1 family of transmembrane glycoproteins, which form a third lineage of antigen presenting molecules distantly related to the classic major histocompatibility complex (MHC) molecules of the immune system. However, unlike the first and second lineages of antigen presenting molecules (the MHC class I and class II molecules), CD1 family molecules have evolved to bind lipids and glycolipids. CD1 family molecules are closely related to MHC class Ia and Ib proteins by sequence homology, domain organisation (x1, x2, x3, and β2 microglobulin), and association with β2 microglobulin, rather than to class II molecules. In contrast to MHC class I molecules, which are polymorphic, CD1d molecules are not polymorphic and are encoded by linked genes outside the MHC complex; the gene for CD1d is located on chromosome 1 in humans.

The CD1 family is divided into two groups by sequence homology: group I, which consists of the CD1a, CD1b, and CD1c isoforms; and group II, which includes CD1d. Only the group II CD1d isoforms are preserved in humans, mice, rats, rabbits, and monkeys. Sequence similarity is substantially higher for the same isotype from different species than for different isoforms within the same species, suggesting that each group of CD1 molecules could have a different function.

“CD1d plays a crucial role in several immunoregulatory functions within the human and mammalian body.”

CD1d is essential for the development and activation of a subset of T cells known as natural killer T (NK-T) cells, which are characterised by the expression of receptors used by NK cells and invariant Vα2-Jα3 T cell receptors, such as Vα24Jα2Q in humans and Vα14Jα281 in mice. NK-T cells recognise self or non-self glycolipids presented by the CD1d molecule and respond by secretion of cytokines, most notably interferon γ and interleukin 4. The synthetic glycolipid molecule α galactosylceramide stimulates human and mouse NK-T cells in a CD1d restricted manner. Therefore, via the production of cytokines secreted by NK-T cells, CD1d plays a crucial role in several immunoregulatory functions within the human and mammalian body, including protection against autoimmune diseases, microbial infection, and cancer. In mice, it was shown that CD1d promotes ultraviolet induced carcinogenesis by inhibiting apoptosis and thereby preventing the elimination of potentially malignant keratinocytes and fibroblasts.

Recently, CD1d expression and NK-T cells were demonstrated in the epidermis of acute and chronic psoriatic plaques. The mammalian hair follicle undergoes lifelong transformations from a resting stage (telogen) to a growth stage, characterised by rapid cell proliferation and keratinocyte differentiation with production of pigmented hair fibre (anagen), and finally to an apoptosis induced and stress associated involution stage (catagen), which leads again into telogen. The hair follicle is an immunoprivileged organ, mainly characterised by MHC class I negativity and an immunosuppressive cytokine milieu. Recent studies showed that human hair follicle keratinocytes of sun protected skin express CD1d. Whether CD1d is expressed in human scalp hair follicles and whether its expression undergoes hair cycle related changes is still unclear. Therefore, our current study aims to explore the expression of CD1d in human scalp skin and hair follicles at different cycle stages.

Abbreviations: IRS, inner root sheath; MHC, major histocompatibility complex; NK, natural killer; ORS, outer root sheath; TBS, Tris buffered saline; TSA, tyramide signal amplification
CD1d in human scalp skin and hair follicles

MATERIALS AND METHODS

Skin samples
Skin biopsies from healthy human scalp containing mainly anagen VI hair follicles were obtained after informed consent from women (age, 53–57 years) undergoing elective plastic (cosmetic) surgery. After surgery, samples were maintained in Williams E medium (Biochrom KG Seromed, Berlin, Germany) for transportation at 4°C for up to 24 hours. Skin specimens used for cryosections were frozen abruptly in liquid nitrogen and stored at −80°C until use. Before immunostaining, samples were embedded and processed for longitudinal cryosections (8 μm). Sections were dried, fixed in cold acetone (−20°C), and stored at −20°C until used for immunohistochemistry.

Immunohistochemistry
Cryosections of normal human scalp skin were immunostained using mouse monoclonal IgG1 antihuman CD1d (BioSource International, Camarillo, California, USA). Two labelling techniques were performed to visualise antigen–antibody complexes; avidin–biotin complex labelling (Vector Laboratories, Burlingame, California, USA) and the highly sensitive immunofluorescent tyramide signal amplification (TSA) labelling method (PerkinElmer Life Science, Boston, Massachusetts, USA). For the avidin–biotin complex labelling method, cryosections were washed in Tris buffered saline (TBS; 0.05M, pH 7.6) and preincubated with avidin–biotin blocking solution (Vector Laboratories), followed by incubation with protein blocking agent (Immunotech, Krefeld, Germany) to prevent non-specific binding. Sections were then incubated with the primary antibody diluted in TBS (1/200) for 30 minutes at room temperature. Thereafter, sections were incubated with streptavidin horseradish peroxidase (1/50 in blocking buffer) for 30 minutes at room temperature. Finally, tetramethylrhodamine isothiocyanate–diamidino-2-phenylindole and mounting in levamisole (Dako Corporation, Carpenteria, California, USA). The TSA signals were visualised under a fluorescence microscope (Carl Zeiss, Jena, Germany). Finally, sections were counterstained with Meyer’s haemalum, covered with Kaiser glycerol (Dako, Glostrup, Denmark), and stored at 4°C for microscopic examination and analysis.

For the TSA labelling technique, cryosections were washed in Tris/acid/Tween buffer (pH 7.5), followed by washing in 3% hydrogen peroxide (H₂O₂). Sections were then incubated with a lower concentration of the primary antibody diluted in Tris/acid blocking buffer (pH 7.2; 1/1000 dilution) overnight at 4°C. Next, sections were washed in Tris/acid/Tween and incubated with Cy3 conjugated F(ab)2 fragments of goat antimouse IgG secondary antibody (Jackson Immunoresearch Laboratories, West Grove, Pennsylvania, USA) diluted in blocking buffer (1/200) for 30 minutes at room temperature. Thereafter, sections were incubated with streptavidin horseradish peroxidase (1/50 in blocking buffer) for 30 minutes at room temperature. Finally, tetramethylrhodamine isothiocyanate–tyramide amplification reagent was added (1/50 dilution in amplification diluent provided with the kit) for 30 minutes at room temperature, followed by counterstaining with 4’,6’-diamidino-2-phenylindole and mounting in levamisole (Dako Corporation, Carpenteria, California, USA). The TSA signals were visualised under a fluorescence microscope (Carl Zeiss, Jena, Germany).

Positive controls
The walls of the blood vessels served as positive controls (fig 1F).

Negative controls
Additional sections, running in parallel but with omission of the primary antibody, served as negative controls (fig 2J).

RESULTS
The positive and negative controls showed positive and negative reactivity, respectively, validating our immunostaining results.

CD1d is prominently expressed in the epidermis, some dermal elements, hypodermis, the eccrine sweat glands, and sebaceous glands.

In the epidermis, immunoreactivity for CD1d was detected prominently in the keratinocytes of the basal layer and three
to four layers of the stratum granulosum directly beneath the stratum corneum (fig 1A,C; table 1). CD1d immunopositivity was also seen in the stratum spinosum, but with weaker intensity than in the basal and granular layers (fig 1A,C). In the dermis, weak CD1d immunoreactivity was seen in some dendritic cells of the papillary dermis adjacent to the epidermal basal layer (fig 1A,C) and in other dermal cell types around hair follicles (fig 2F,H) and blood vessels (fig 1F). Prominent CD1d expression was found in blood vessel walls and endothelial elements (fig 1F). In the hypodermis, CD1d immunostaining was intense in adipose tissue cells surrounding the anagen hair follicle bulb (fig 2G). In the eccrine sweat glands, strong CD1d immunostaining was found in all cells, with expression on the cell membrane and throughout the cytoplasm, and staining was stronger in the sweat ducts (fig 1E). In the sebaceous glands, strong CD1d immunostaining was detected in the peripheral sebocytes and moderate staining was seen in the central sebocytes (fig 1B,D).

CD1d is expressed in human scalp hair follicles and shows hair cycle dependent alterations

In anagen VI hair follicles (fig 2A–G; table 2), CD1d immunoreactivity was prominently detected in the outer root sheath (ORS), in the inner root sheath (IRS), and in the differentiating corticocytes. Weak, if any, CD1d expression was found in the hair matrix cells and the dermal papilla (fig 2D,G,K). In the ORS, CD1d was found in almost all follicle regions, including the proximal hair bulb region, the central region, and the distal region, with different staining

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BV, blood vessels; DS, dermal stroma; HF, hair follicle; SB, stratum basale; SBG, sebaceous gland; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum; SWG, sweat gland; +, weak expression; ++, strong expression; ++++, very strong expression.
intensities. In the hair bulb region, CD1d immunoreactivity was strong and confined to the outermost keratinocytes of the ORS (fig 2D,G). In the lower portion of the central region (lower suprabulbar), CD1d immunopositivity was prominent in the outer basal layer and single keratinocytes of the inner layers (fig 2C,F). In the upper portion of the central region (upper suprabulbar), CD1d expression in the ORS became stronger and increased dramatically, extending to include almost all the layers of keratinocytes (fig 2B,F). In the distal region of the follicle, CD1d immunostaining in the ORS was seen in the outer basal and innermost layers of keratinocytes (fig 2A,E,H). In the infundibulum, CD1d expression was found in the outer basal and innermost layers, and in single intermediate keratinocytes, matching its expression in the epidermis (figs 1B,D, 2K). In the IRS, CD1d immunopositivity was prominent in the central region (fig 2B,C,E,F) of the hair follicle. Positivity was seen in both the outer telocytes’ layer and inner Huxley’s layer of the IRS. The newly differentiated corteocytes in the hair bulb showed strong CD1d immunostaining (fig 2D,G), whereas scattered single corteocytes in the proximal hair shaft showed weak CD1d staining (fig 2C,F).

Weak CD1d immunoreactivity was seen in single keratinocytes in the outer basal layer of the ORS in catagen stage hair follicles compared with anagen VI hair follicles. However, intermediate expression was found in the lowest involuted part of the hair follicle known as the epithelial strand (fig 2I). In telogen stage hair follicles, weak CD1d immunoreactivity was confined to single keratinocytes of the ORS (not shown).

**DISCUSSION**

CD1d plays a crucial role in mammalian skin biology as an antigen presenting molecule that binds lipid and glycolipid antigens. Our current study revealed that CD1d is expressed by epidermal keratinocytes of normal human scalp skin and this is supported by Bonish et al., who reported the expression of CD1d by keratinocytes of normal human skin from regions of the body other than the scalp. They found a high expression rate in the superficial keratinocytes close to the lipid rich stratum corneum. We also found intense CD1d expression in other epidermal layers including basal keratinocytes. This implies the involvement of basal and suprabasal keratinocytes in binding glycolipid antigens to activate NK-T cells to secrete interferon γ, and that these cells play a role in the immunity of human scalp skin. CD1d has also been shown to play a regulatory role in inflammatory skin diseases. CD1d deficient MRL-lpr/lpr mice have more frequent and more severe skin disease than their wild-type littermates, with increased local infiltration by mast cells, lymphocytes, and dendritic cells, including Langerhans cells. NK-T cells, which are activated by CD1d stimulation, were shown to mediate cutaneous inflammatory reactions to fly larvae, suggesting a role for CD1d in protection against fly larvae infestation. There is strong evidence that CD1d is a crucial regulator of ultraviolet induced carcinogenesis, by inhibiting apoptosis and thereby preventing the elimination of potentially malignant keratinocytes and fibroblasts. In mice, CD1d was also found to mediate contact sensitivity by activating B1 cells to produce IgM, which activates complement to promote T cell passage into the tissues. In psoriasis, a severe autoimmune skin disease, CD1d is overexpressed by keratinocytes, suggesting that it plays a role in regulating and mediating the pathogenesis of cutaneous autoimmune diseases.

"The most intriguing finding of our current study is the strong expression of CD1d by the pilosebaceous unit, which is the site of the common multifactorial skin disease of young adults, acne." Our current study revealed that CD1d is prominently expressed in the epithelial compartment of anagen VI hair follicles, mainly in IRS and IRS keratinocytes, melanocytes, and some corteocytes. This could mean that CD1d plays an important role in supporting the anagen phase by inhibiting apoptosis, and is thus involved in regulating the human hair cycle. Several studies have shown that keratinocyte proliferation and differentiation are induced by lipids. N-Acylated forms of sphingolipids, such as ceramides, have been shown to promote keratinocyte differentiation, and sphingosine and sphingosylphosphorylcholine promote keratinocyte proliferation. The high amounts of CD1d produced during anagen might bind lipid molecules and induce keratinocyte proliferation and differentiation, thus supporting anagen. This view is supported by the finding that CD1d deficient mice have severe skin disorders, manifested as hair loss and scab formation. Alternatively, as an antigen presenting molecule, CD1d binds available glycolipid antigens and activates NK-T cells, so that it may play a role in immunity and protection of the hair follicle. However, this raises the question of whether the proximal anagen hair follicle can mount an immune response; it is thought to be an immune privileged organ because its compartments do not express the classic MHC class I antigen presenting molecules and it is characterised by an immunosuppressive cytokine milieu. Previous studies have shown that human and murine proximal hair follicles are negative for peptide antigen presenting cells but the role of lipid and glycolipid antigen presenting cells in these pilosebaceous units is unclear. Although our current study revealed strong expression of CD1d in the proximal anagen hair follicle, the coexistence of NK-T cells within anagen hair follicle compartments was not investigated. This should be investigated in future studies. However, the presence of a few NK-T cells around the anagen hair follicle may support our results and indicate a role for anagen hair follicle keratinocytes in lipid antigen presentation to NK-T cells (R Paus, personal communication, 2002). Our present study also revealed strong expression of CD1d in the eccrine sweat gland, suggesting a role for CD1d in the normal functioning of the sweat gland and/or that sweat gland cells play a role in the human scalp skin immune system, by presenting lipid and glycolipid molecules to NK-T cells. Lampert et al. reported the expression of MHC class II antigen presenting molecules in the lining cells of the sweat gland, more evidence of a role for the sweat gland in the immune system of the skin; however, these authors also suggested that MHC class II molecules might have a role in the development and the normal function of adult sweat gland cells. Our present results are supported by those of Bonish et al., who showed the expression of CD1d in sweat glands of skin areas other than the scalp.

The most intriguing finding of our current study is the strong expression of CD1d by the pilosebaceous unit, which is the site of the common multifactorial skin disease of young adults. A recent study has shown that CD1d is overexpressed by the pilosebaceous unit, and that these cells play a role in the human scalp skin immune system, by presenting lipid and glycolipid molecules to NK-T cells. This finding suggests a role for CD1d in immunity and protection of the hair follicle.
Take home messages

- The expression pattern of CD1d in human scalp skin suggests that it plays an important role in the immunology of this tissue and in the protection against lipid antigen rich infectious microbes.
- These results also suggest that keratinocytes of the immunoprivileged anagen hair follicle can present lipid antigens to natural killer T cells.
- These data could help provide new strategies for the manipulation of hair related disorders, such as alopecia.

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Case 2. Multicystic tumour with thickened septa.

Case 2. Typical oncocytic cells with eosinophilic cytoplasm covering the tumour septa.

Rare, and is often misdiagnosed as renal carcinoma with a cystic component. Here, we report two consecutive cases of multicellular cystic renal oncocytoma. A 67 year old man presented with a sudden pain in his left back. He had no relevant medical history. Physical examination showed a microscopic haematuria. Ultrasonography, computed tomography (CT) scan, and magnetic resonance imaging (MRI) uncovered a large heterogeneous tumour in the left kidney. The tumour measured 7 cm in its greatest diameter, and was largely cystic with multilocular septa. Enhancement of the septa and the nodular central component showed up well on MRI (fig 1). A radical nephrectomy was performed. After a follow up of 26 months, the patient is well without recurrence or metastasis. A 47 year old woman without relevant antecedents presented with diffuse abdominal pain. A CT scan and MRI studies were performed and showed a small 3.5 cm multicellular cystic tumour in the upper pole of the left kidney. Multiple thickened septa were more easily seen on the MRI than the CT scan. Septa and wall were well enhanced on contrast imaging. A partial nephrectomy was performed, and eight months later the patient is well.

On gross examination, both tumours were predominantly cystic, multilocular, with thickened septa, and with no central scar. Microscopically, septa were covered by cuboidal or columnar eosinophilic cells (fig 2, 3). The cytoplasm was abundant and granular. The nuclei were rounded with one or several nucleoli. No severe atypia or necrosis was noted. No hobnail cells were found. Mitotic activity was low (less than one mitotic figure/10 high power fields). A small area of classic solid tubulocystic oncocytoma was identified in each case. In the first case, tumour cysts focally invaded the perirenal adipose tissue. No vascular invasion was seen. On immunohistochemistry, tumour cells were diffusely positive for epithelial membrane antigen and pancytokeratin and focally positive for cytokeratin 7. Staining for cytokeratin 20, high molecular weight cytokeratin, and CD10 was negative. Oncocytoma is a renal adenoma that makes up 3–5% of all renal tumours. Classically, oncocytoma is a solid mass that develops in the renal parenchyma with a central fibrous scar. Oncocytoma with prominent macroscopic cystic features is very unusual and should be distinguished from other renal cystic tumours, such as multicellular clear cell carcinoma or cystic nephroma. Preoperatively, in our cases, no distinction was possible by imaging studies; in particular, no central scar was seen. Therefore, these tumours were classified as suspected cystic tumours, Bosniak’s class 3.

Only microscopic examination can identify the characteristic oncocytic cells covering the tumour septa. However, a minor component of usual oncocytoma is also often seen. In the first case, focal invasion of the perirenal adipose tissue was present. This unusual feature is seen in less than 10% of oncocytomas in large series, and has no effect on the prognosis. Oncocytomas may present limited central microcystic degeneration but a prominent multicystic presentation is very unusual. Only isolated cases have been reported in the English literature. The diagnosis cannot be made radiologically. In the literature, one case resembled a haemorrhagic cyst on imaging. In a recent series of 28 cases of atypical renal cystic tumours evaluated by imaging guided biopsy, one case of cystic oncocytoma was diagnosed. The prognosis of this variant is excellent; metastasis has not been reported. The clinical course appears to be very similar to that of usual oncocytoma and these tumours may be managed by partial nephrectomy.

References

CORRECTION
Adley MA, Assaf HA, Hussein M. Expression of CD1d in human scalp skin and hair follicles: hair cycle related alterations. J Clin Pathol 2005; 58: 1279–82. The surname of the first author was spelt incorrectly. The correct name is Adly MA.