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Langerhans cells from human oral epithelium are more effective at stimulating allogeneic T cells *in vitro* than Langerhans cells from skin

B. HASSÉUS*, M. JONTELL*, G. BERGENHOLTZ* & U. I. DAHLGREN*† *Departments of Endodontology/Oral Diagnosis/Oral Medicine and †Rheumatology and Inflammatory Research, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden

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SUMMARY

This report is focused on the functional capacity of Langerhans cells (LC) in the epithelium of skin and oral mucosa, which both meet different antigenic challenges. The capacity of LC from human oral and skin epithelium to provide co-stimulatory signals to T cells *in vitro* was compared. LC in a crude suspension of oral epithelial cells had a significantly enhanced T cell co-stimulatory capacity compared to skin epithelial cells. This applied both to cultures with concanavalin A (con-A)-stimulated syngeneic T cells and to a mixed epithelial cell lymphocyte reaction involving allogeneic T cells. The co-stimulatory capacity of oral and skin epithelial cells was reduced by >70% if monoclonal antibodies against HLA-DR, -DP and -DQ were added to the cultures with allogeneic T cells, indicating the involvement of HLA class II expressing LC. Immunohistochemistry revealed that 6% of the epithelial cells were CD1a + LC in sections from both oral and skin epithelium. Interleukin (IL)-8 production was higher in cultures of oral epithelial cells and con-A stimulated T cells than in corresponding cultures with skin epithelial cells as accessory cells. The results suggest that LC in human oral epithelium are more efficient at stimulating T cells than those of skin.

Keywords co-stimulation dendritic cells human MHC T lymphocytes

INTRODUCTION

The oral mucosa and intestine are exposed to large amounts of antigens emanating from food, bacteria, viruses, fungi and their by-products. In skin, not harbouring such a diverse microbiota, the quantity of antigenic exposure assumable is less. This difference in antigenic load may demand different immune responses.

Langerhans cells (LC) are a subpopulation of the bone marrow-derived dendritic cells (DC). They are antigen-presenting cells (APC), capable of internalizing and processing antigens [1]. Because they reside in epithelium of skin and mucosal membranes they may be the primary target cell for antigens entering oral mucosa and skin. Human LC express CD1a, human leucocyte antigen (HLA) -DR, -DP, -DQ, CD80, CD83, CD86, CD40, Langerin/CD207 and Birbeck's granulae [2–6]. After antigen uptake they migrate to regional lymph nodes where peptides, in the context of major histocompatibility complex (MHC) class II molecules, are presented to T cells with appropriate T cell receptors. This first signal to the T cell, together with a second signal, delivered in part by the interaction between CD80 and CD86 molecules on the LC and CD28 and CTLA4 (CD152) ligands on the T cell, results in expansion, but also regulation of T cell clones [7,8]. During the migration from the epithelium LC mature functionally with increased expression of MHC class II molecules, CD80, CD86 and adhesion molecules but decreased expression of CD1a and Birbeck's granulae [9–11]. Freshly isolated LC from skin are inefficient as APC, while DC found in draining lymph nodes are highly immunostimulatory [10]. Freshly isolated epidermal LC are also poor stimulators of allogeneic T cells in a mixed epithelial cell lymphocyte reaction (MELR) in comparison with cultured epidermal LC [12,13]. Recent knowledge on DC maturation has been gained from DC generated from human CD34-positive progenitor cells [3,14]. Expression and up-regulation of CD83 enhance allogenic T cell response, thus indicating maturation of dendritic cells [15]. Cytokines, especially granulocyte-macrophage colony stimulating factor (GM-CSF) and tumour necrosis factor (TNF), are essential in LC/DC maturation [14,16,17].

Keratinocytes and LC produce interleukin (IL)-8, which are chemotactic for T cells and neutrophils [18,19]. LC carry receptors for this chemokine [20]. Proinflammatory cytokines such as IL-1β and TNF induce IL-8 secretion [21]. Thus LC both produce and are influenced by these substances.

Studies on DC/LC derived from skin, oral mucosa, airways, peripheral blood and gut show differences in maturation relating

Correspondence: Dr Bengt Hasséus, Department of Endodontology/Oral Diagnosis, Faculty of Odontology, The Sahlgrenska Academy at Göteborg University, Box 450, SE 405 30 Göteborg, Sweden.
E-mail: Bengt.Hasseus@odontologi.gu.se

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to the origin of the cells, manifested as a variation in the capacity to induce a T cell response [12,22–25]. To mount an appropriate response to antigen at various body compartments it is most likely that DC/LC are adapted to the local environment. We have reported earlier that in rodents, freshly isolated LC from oral epithelium have potent T cell co-stimulatory capacity compared to skin LC [25]. The present study was undertaken in order to determine whether there is a similar functional difference between human oral and skin LC.

MATERIALS AND METHODS

Subjects

Healthy volunteers (six men, age range 29–46 years, mean 41 years, median 44 years) without medical histories of allergies, chronic diseases or ongoing medication were asked to participate in the experiments.

Biopsies

Biopsy areas were washed with 0·1% chlorhexidine solution (Ipex Medical AB, Helsingborg, Sweden). Punch biopsies (5 mm and 3 mm in diameter) of oral mucosa (bucca) and skin (ventral side of lower leg) were obtained under local anaesthesia (prilocaine hydrochloride 30 mg/ml, felypressin 0·54 μg/ml; Citanest Octopressin®, Astra, Södertälje, Sweden). Biopsy specimens with 5 mm diameter were transferred immediately to 0·9% saline for use in cell cultures, while 3 mm specimens were transferred to Histocon® (Histolab, Bethlehem Trading Ltd, Göteborg, Sweden), and snap-frozen in embedding medium (OCT Compound, Miles, IN, USA) for use in immunohistochemical analysis. Venous blood was drawn from each volunteer. The Ethical Committee at Göteborg University approved the study.

Cell suspensions

T cells. Peripheral blood mononuclear cells (PBMC) were isolated on Lymphoprep® from heparinized venous blood (20 ml) according to the manufacturer's instructions (Nycomed, Pharma AS, Oslo, Norway). Cells were then seeded in culture flasks (Nunc AS, Roskilde, Denmark) and incubated at 37°C for 45 min in Dulbecco's modification of Eagle's medium (DMEM) containing 10% heat-inactivated fetal calf serum (FCS), 1% L-glutamine, gentamycin (25 mg/l), penicillin (100 U/ml) and streptomycin (100 μg/ml; DMEM + +). Non-adherent cells were recovered and residual class II molecule-expressing cells were removed by the following procedures: (i) incubation of recovered cells (5×10^6) with mouse antihuman HLA-DR, -DP, -DQ monoclonal antibody (100 μg/ml; clone Tü39, Pharmingen, San Diego, CA, USA) in 4°C for 30 min; (ii) repeated washes in cold medium to remove free antibodies; (iii) incubation for 60 min at 37°C under gentle stirring with immunomagnetic beads coated with a sheep antimouse IgG according to the manufacturer's instructions (Dynabeads® M-280, Dynal AS, Oslo, Norway); (iv) separation of HLA-DR, -DP and -DQ molecule-expressing cells with a magnet. The viability of the recovered cells was estimated by trypan blue exclusion and consistently exceeded 95%.

Oral and skin epithelial cells. Dissected oral mucosal and skin specimens were put into 0·2% chlorhexidine solution (Hibitane Dental, ICI-Pharma, Stockholm, Sweden) for 10 s and rinsed in phosphate buffered saline (PBS) for 10 s. Specimens were then floated on Dispase type II (Boehringer-Mannheim, GmBH, Mannheim, Germany) diluted to a concentration of 1·2 U/ml in DMEM + + for 2 h. After incubation, epithelia were separated easily from the underlying connective tissue, placed with the subepithelial side up in Petri dishes, covered with 0·5% trypsin (Trypsine, Life Technologies, Paisley, UK) and incubated further at 37°C for 30 min in 5% CO_2 atmosphere. Immediately after this incubation procedure, tissue sheets were covered with DMEM + + supplemented with 20% FCS to inactivate the trypsin. To release oral epithelial and epidermal cells, sheets were first transferred to a vial containing fresh DMEM + + supplemented with 20% FCS and then subjected to pipetting. After sedimentation a supernatant containing released cells was harvested. The cell suspensions were washed twice in DMEM + + and cells were counted. Cell viability was assessed by trypan blue exclusion.

Immunohistochemistry

Immunohistochemistry to determine the number of CD1a- and CD83 molecule-expressing cells in oral and skin epithelium was performed on frozen sections (4 μm) prepared and air-dried for 15 min. The sections were then fixed in acetone for 10 min at 4°C and air-dried for 20 min. All incubations were followed by extensive rinsing in Tris-HCL buffer (pH 7·6) containing 0·9% NaCl (TBS) for at least 15 min. Endogenous peroxidase activity was inhibited by incubating the sections in 0·3% H_2O_2 in TBS for 5 min. After this the sections were incubated with 4% bovine serum albumin (BSA) in TBS for 30 min at room temperature. Incubation with a mouse anti-CD1a monoclonal antibody (NA1/34; Serotec Ltd, Oxon, UK) diluted in 4% BSA in TBS was performed at 4°C overnight. Sections were then incubated with biotinylated $F(ab)_2$ IgG rabbit antimouse antibodies (Dako AS, Glostrup, Denmark) diluted in 4% BSA in TBS for 30 min. Incubation with the secondary antibody was followed by incubation with avidin–biotin–peroxidase complex (Dakopatts AS) for 30 min in room temperature.

Incubation with a mouse anti-CD83 monoclonal antibody (HB15e; Serotec Ltd) diluted in 4% BSA in TBS was performed for 30 min at room temperature. This was followed by incubation for 30 min at room temperature with Dako EnVision™ (peroxidase, antimouse, Dakopatts AS).

Sections were then developed for 10 min in 3-amino-9-ethyl-carbaxol (10 mg) dissolved in dimethyl-sulphoxide (6 ml) in sodium acetate (50 ml, 0·02 M, pH 5·5) and 4 μl H_2O_2 (30%) and counterstained with Mayer's haematoxylin. The monoclonal antibody and secondary antibody were diluted in 4% BSA in TBS. Negative controls were incubated with 4% BSA in TBS instead of monoclonal antibody (MoAb). Quantitative analysis was conducted on 1–2 consecutive sections of each biopsy. Two or three high-power fields (HPF × 200) in the periphery and the middle of the sections were selected for cell counting. In a light microscope with a chilled colour CCD camera, computerized images of the HPF were obtained. The sections were then analysed with the NIH Image Software (National Institute of Health, Bethesda, USA). Within the epithelium all nucleated positively and negatively stained cells were counted. Results were expressed as the mean percentage of positively stained cells (CD1a) or number of positive cells/mm^2 (CD83).

Interleukin-8 (IL-8) enzyme-linked immunosorbent assay (ELISA)

The presence of IL-8 in supernatants from cell cultures, derived from two subjects, was examined by ELISA. Briefly, 96-well

plates (Immunoplate, Maxisorp, Nunc, Denmark) were coated with goat antihuman IL-8 antibody (10 μg/ml; clone AB-208-NA, R&D Systems, UK) diluted in carbonate buffer (pH 9·0) overnight at 4°C. This was followed by repeated washing in PBS and the plates were blocked with 2% BSA for 2 h. Recombinant IL-8 (32 ng/ml–0·5 ng/ml; R&D systems, UK) was used as a standard. The standard and duplicate samples, in serial dilutions, were incubated for 3 h at room temperature. This was followed by overnight incubation at 4°C with a rabbit antihuman IL-8 antibody (1 μg/ml; clone P-801, Endogen, Boston, MA, USA) followed by incubation for 2 h with an alkaline phosphatase conjugated goat antirabbit IgG antibody (1 : 1000; clone D487, Dakopatts, Copenhagen, Denmark). Captured IL-8 was visualized with the substrate P-nitrophenyl phosphate (Sigma, Stockholm, Sweden). Absorbance was measured at 405 nm with an ELISA counter (Spectra MAX, Molecular Devices Corp., CA, USA).

Functional assays

T cells ($2·5 \times 10^5$ cells/well) were incubated with 2·5 μg/ml concanavalin A (con A; Pharmacia LKB Biotechnology AB, Uppsala, Sweden) together with syngeneic oral or skin epithelial cells (1×10^4 cells/well). In some cultures mouse anti-HLA-DR, -DP, -DQ MoAbs were added (25 μg/ml; clone Tü39; Pharmingen). As controls, T cells and epithelial cells were incubated separately. All incubations were performed in duplicate or triplicate at 37°C for 72 h in 5% CO_2 atmosphere in 96-well U-bottomed culture plates (Nunc AS) containing 0·2 ml DMEM + + 0. Following 48 h of incubation, 5 μCi/ml of [^{3}H]-thymidine (methyl-[^{3}H]-thymidine; 24 Ci/nMol, Amersham, Buckinghamshire, UK) was added to each well. After 72 h the cells were harvested onto glass fibre filters in a Skatron harvester (Skatron, Flow Laboratories, Oslo, Norway) and counted in a liquid scintillator.

Supernatants from cell cultures from two subjects, as described above, were harvested after 72 h. Culture plates were centrifuged at 200 ***g*** for 10 min and 150 μl aliquots of supernatants were collected and frozen in –70°C for analysis of IL-8 production.

Allogeneic T cells ($2·5 \times 10^5$ cells/well) from one subject served as responder cells in a mixed epithelial cell lymphocyte reaction (MELR). Oral or skin epithelial cells (1×10^4 cells/well) from another subject served as stimulator cells. Some cultures received a mouse antihuman HLA-DR, -DP, -DQ monoclonal antibody (25 μg/ml; Tü39, Pharmingen). As controls, T cells and epithelial cells were incubated separately. All incubations were performed in duplicate or triplicate at 37°C for 72 h in 5% CO_2 atmosphere in 96-well U-bottomed culture plates (Nunc AS) containing 0·2 ml DMEM + + and following 72 h of incubation, 5 μCi/ml of [^{3}H]-thymidine (methyl-[^{3}H]-thymidine; 24 Ci/nMol, Amersham, Buckinghamshire, UK) was added to each well. After 96 h the cells were harvested onto glass-fibre filters in a Skatron harvester (Skatron) and counted in a liquid scintillator.

In a mixed lymphocyte reaction, peripheral blood mononuclear cells (total $2·5 \times 10^5$ cells/well) from two subjects were mixed in equal numbers in triplicate cultures at 37°C in 5% CO_2 atmosphere in 96-well U-bottomed culture plates (Nunc AS) containing 0·2 ml DMEM + + 0. Following 24, 48, 72 and 96 h of incubation, 5 μCi/ml of [^{3}H]-thymidine (methyl-[^{3}H]-thymidine; 24 Ci/nMol, Amersham) was added to each well and cells were harvested 24 h later and counted as described above. In some cultures mouse anti-HLA-DR, -DP, -DQ MoAbs were added (25 μg/ml; Tü39; Pharmingen). Following 72 h of incubation, the cells were pulsed with 5 μCi/ml of [^{3}H]-thymidine harvested and counted as described above. Control cultures received an irrelevant MoAb of the same isotype (25 μg/ml; mouse IgG2α,κ; Sigma, St Louis, MO, USA), when cell yield permitted.

Statistical analysis

Statistical analyses of differences between groups were performed using the Wilcoxon signed-rank test. A *P*-value <0·05 was considered as a significant difference.

RESULTS

CD1a molecule-expressing Langerhans cells and CD83 molecule-expressing cells in oral and skin epithelium

CD1a-positive cells in oral and skin epithelium were located preferentially in the suprabasal portion of the epithelium and showed CD1a-positive cells with dendritic morphology. Enumeration of positive and negative cells revealed a mean frequency of 5·8% (range: 1·0–10·5) CD1a-positive LC in oral epithelium and 5·7% (range: 3·8–8·0) CD1a-positive LC in skin epithelium (Table 1).

CD83-positive cells in oral and skin epithelium were located preferentially in the suprabasal portion of the epithelium. In oral epithelium some CD83-positive cells showed the characteristic dendritic morphology of LC, while dendritic appearance was less pronounced in skin epithelium. Enumeration of the number of positive cells revealed a mean number of 40 positive cells/mm^2 (range: 3–120) in oral epithelium and seven positive cells/mm^2 (range: 0–15) in skin epithelium (Table 2).

Table 1. CD1a-positive Langerhans cells (LC) in biopsies from oral and skin epithelium

Subject no.	Frequency of LC in oral epithelium (%)	Frequency of LC in skin epithelium (%)
1	Not done	Not done
2	6·6 ± 3·1	8·0 ± 3·2
3	10·5 ± 1·4	3·8 ± 2·0
4	Not done	Not done
5	5·0 ± 3·2	6·3 ± 0·4
6	1·0 ± 0·1	4·6 ± 3·0

Frequency of CD1a-positive Langerhans cells in sections from subjects. Data are expressed as the percentage (%) of positive cells ± standard deviation.

Table 2. CD83-positive cells in biopsies from oral and skin epithelium

Subject no.	CD83-positive cells in oral epithelium (no. of cells/mm^2)	CD83-positive cells in skin epithelium (no. of cells/mm^2)
1	18 ± 2	14 ± 0·4
2	3 ± 1	0
3	120 ± 8	0
4	Not done	Not done
5	20 ± 3	15 ± 1
6	Not done	Not done

CD83-positive cells in sections from subjects. Data are expressed as the number of positive cells/mm^2 ± standard deviation.

Cell yield
Oral biopsies yielded a mean of $2{\cdot}6 \times 10^5$ cells (range: $1–3{\cdot}8 \times 10^5$ cells), while skin biopsies gave a mean of $4{\cdot}7 \times 10^5$ cells (range: $1–6{\cdot}1 \times 10^5$). Cell viability exceeded 95%. Dead cells were large cells with keratinocyte morphology and without nucleus.

Co-stimulatory capacity of oral and skin LC incubated with con-A-stimulated T cells
Incubation of oral and skin epithelial cell suspensions containing LC, with syngeneic T cells in the presence of con-A, resulted in a mean T cell proliferation response 1·5 times higher in cultures with oral epithelial cells than in cultures with skin epithelial cells ($P < 0{\cdot}05$; Table 3). In all cultures containing oral epithelial cells and con-A-stimulated T cells proliferation was higher than in cultures containing skin epithelial cells and T cells, although two subjects (nos 3 and 5) showed little difference (Table 3). Con-A stimulation of peripheral blood mononuclear cells showed substantial interindividual variation (Table 3). Cultures of oral and skin epithelial cells without T cells or con-A never gave counts per million (cpm) values above 500, confirming insignificant proliferation of epithelial cells alone.

Capacity of oral and skin LC to stimulate allogeneic T cells
Oral and skin epithelial cells were incubated with allogeneic T cells. The oral epithelial cells induced 1·3 times higher mean response in the allogeneic T cells than did the skin epithelial cells ($P < 0{\cdot}05$; Table 4). T cells incubated with syngeneic oral and skin epithelial cells did not show any significant difference in proliferative response (Table 4, $P > 0{\cdot}05$). In all subjects oral epithelial cells induced higher allogeneic T cell responses than did skin epithelial cells, although a considerable interindividual variation was registered (Table 4). Also, three subjects (nos 3, 5 and 6) showed little difference between oral and skin epithelial cells in allogeneic T cells response, but in all three subjects the allogeneic T cells response induced by oral epithelial cells was higher than when skin epithelial cells were used for stimulation (Table 4). One subject (no. 2) had a marked increase in autoproliferation of syngeneic and allogeneic T cells (Table 4), which may be caused by incomplete purification of T cells. However, any remaining accessory cells in the T cell suspension would have affected the oral and skin epithelial cell cultures equally.

The mean T cell response in the five MELR assays was reduced by >70% when anti-HLA-DR, -DP, -DQ monoclonal antibodies were added to the cultures of both oral and skin epithelial cells and allogeneic T cells (Table 4). Cultures of oral and skin epithelial cells without T cells or con-A never gave cpm values above 500, confirming insignificant proliferation of epithelial cells alone. In mixed lymphocyte reactions, the anti-HLA -DR, -DP, -DQ monoclonal antibodies reduced the proliferation of peripheral blood mononuclear cells by >50% compared to cultures with isotype-matched monoclonal antibodies present (data not shown).

IL-8 production in cultures of con-A stimulated T cells and oral or skin epithelial cells
Oral or skin epithelial cells were cultured with con-A-stimulated syngeneic T cells and supernatants were collected after 72 h. In the cultures containing oral epithelial cells there was a higher concentration of IL-8 than in cultures containing skin epithelial cells (Fig. 1). Also, without con-A stimulation cultures of oral epithelial cells and T cells produced IL-8 (Fig. 1). In cultures of skin epithelial cells or T cells alone IL-8 production was diminutive (Fig. 1). Cell yields did not allow assay of oral epithelial cells separately.

DISCUSSION

The present study indicates clearly that human oral epithelial cells provided T cells with more efficient co-stimulation than cells from skin epithelium in a MELR or during con-A stimulation. The co-stimulatory capacity in the MELR was dependent on HLA-DR, -DP and -DQ-expressing cells.

These findings are in line with a previous study in which we report that rat oral epithelial cells cultured with allogeneic T cells or syngeneic con-A-stimulated T cells are superior co-stimulatory cells compared to skin epithelial cells [25]. The allogeneic T cell stimulating capacity of epithelial cells was reliant on MHC class II molecule-expressing cells, as depletion of such cells resulted in abolished T cell proliferation [25]. In healthy human epidermis LC are the only cells expressing HLA-DR, -DP -DQ, and even if keratinocytes during pathological conditions and in culture can express class II molecules they are not able to deliver co-stimulatory help to T cells in a MELR [26–28]. Thus, the T cell stimulation is conditional of LC**.**

Topographically, LC are the first professional APC that may encounter a certain antigen [2]. The two-step signalling process,

Table 3. Co-stimulatory activity of human oral and skin epithelial cells, including Langerhans cells, incubated with syngeneic T cells and con-A

Subject no.	Oral epithelial cells + T cells + con-A	Skin epithelial cells + T cells + con-A	Oral epithelial cells + T cells	Skin epithelial cells + T cells	T cells + con A	T cells	Peripheral blood mononuclear cells + con-A	Peripheral blood mononuclear cells
1	13573 ± 84	6011 ± 2738	330[a]	127[a]	2204 ± 363	63[a]	19991 ± 3087	10734 ± 532
2	36950 ± 9537	29716 ± 19082	1060 ± 89	853 ± 306	13518 ± 1285	482 ± 42	91534 ± 8701	2938 ± 528
3	8828 ± 2520	6109 ± 1579	473 ± 101	499 ± 343	1243 ± 613	339 ± 41	26043 ± 1096	3323 ± 467
4	41748 ± 10816	21113 ± 11080	640 ± 297	725 ± 391	1856 ± 567	780 ± 373	110014 ± 25251	Not done
5	17295 ± 3967	16741 ± 3099	597 ± 106	474 ± 159	547 ± 41	476 ± 70	13501 ± 3144	Not done
		$P < 0{\cdot}05$		Not significant				

Accessory cell capacity of fresh oral and skin epithelial cells (1×10^4 cells/well) in con-A stimulation of syngeneic T cells ($2{\cdot}5 \times 10^5$ cells/well) and con-A-stimulated peripheral blood mononuclear cells ($2{\cdot}5 \times 10^5$ cells/well). The data are expressed as mean cpm ± standard deviation of triplicate culture wells (except as noted) in five experiments on five subjects; [a]cpm from one well.

Table 4. Co-stimulatory activity of human oral and skin epithelial cells incubated with allogeneic or syngeneic T cells in the mixed epithelial cell lymphocyte reaction

Subject no.	Oral epithelial cells + allogeneic T cells	Skin epithelial cells + allogeneic T cells	Oral epithelial cells + syngeneic T cells	Skin epithelial cells + syngeneic T cells	Oral epithelial cells + allogeneic T cells + antibodies against HLA DR, DP, DQ	Skin epithelial cells + allogeneic T cells + antibodies against HLA DR, DP, DQ	Allogeneic T cells	Syngeneic T cells
2	58169 ± 5626	44055 ± 2149	29516 ± 343	22229 ± 1955	4576 ± 1116	2390 ± 9	16532 ± 1245	26114 ± 556
3	10682 ± 3692	9273 ± 1595	1158 ± 158	3340 ± 2437	1905 ± 642	2126 ± 570	443 ± 28	234 ± 50
4	5627 ± 2025	2248 ± 1211	1841 ± 1704	426 ± 107	2146 ± 1324	1293 ± 249	1214 ± 72	262[a]
5	2846 ± 906	2450 ± 491	697 ± 202	369 ± 168	1119[a]	632 ± 44	740 ± 309	418 ± 21
6	9546 ± 330	8456 ± 492	2545 ± 190	2547 ± 328	3899 ± 693	2496 ± 222	443 ± 28	300 ± 45
		$P < 0.05$		Not significant				

Accessory cell capacity of fresh oral and skin epithelial cells (1×10^4 cells/well) in stimulation of allogeneic T cells (2.5×10^5 cells/well). The data are expressed as mean cpm ± standard deviation of triplicate culture wells in five experiments on five subjects except those noted; [a]cpm from one well.

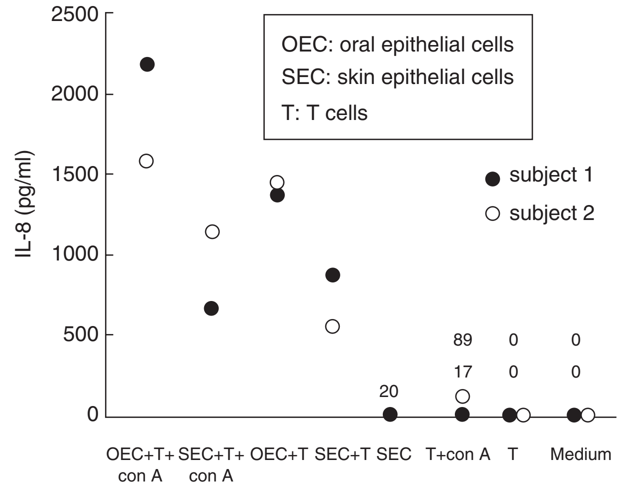

Fig. 1. IL-8 production, measured by ELISA, in supernatants from cultures of oral and skin epithelial cells (1×10^4 cells/well) and T cells (2.5×10^5 cells/well) with or without con-A present following 4 days of incubation. Data are from two subjects, cultures of skin epithelial cells only from one subject.

necessary to induce a T cell response, starts with presentation of peptides by professional APC to a T cell with appropriate specificity, followed by a second signal, delivered in part by CD80 and CD86 present on the APC [29]. Difference in signal strength and temporal sequence between CD80 and CD86 give either co-stimulation or suppression of the T cell response or induction of regulatory T cell [8,30]. Thus, LC are part of the intricate system for regulation of the immune responses both to foreign and self antigens.

It is conceivable that keratinocytes produce soluble factors necessary for LC maturation, as *in vitro* culturing of purified LC or generation of DC from progenitor cells demand the addition of extrinsic factors [12]. Key cytokines in LC maturation are GM-CSF together with TNF, which can be used to generate DC/LC from CD34-positive progenitor cells [3]. Keratinocytes can produce GM-CSF and TNF [18]. Thus, a suspension of epithelial cells containing both LC and keratinocytes constitute a good environment for LC. The experimental design in this study, using whole suspensions of epithelial cells in cultures, ensures keratinocyte-derived factors vital for LC viability.

Analysis of human epidermal cells with flow cytometry has shown that LC constitute 2–5% of the epithelial cells [31]. Our findings of approximately 6% CD1a-expressing cells with dendritic morphology in sections of oral and skin epithelium is close to that figure. The interindividual range in frequency of CD1a-expressing cells is high and most subjects have a slightly higher frequency of CD1a-expressing cells in skin than in oral epithelium. Thus, the superior co-stimulatory capacity of oral epithelial cells is not due to higher numbers of LC in oral epithelium compared to skin epithelium.

In healthy human epidermis LC are the only cells expressing MHC class II molecules [26,27]. However, during pathological conditions and in culture keratinocytes can be stimulated to express class II molecules [27,32,33]. Keratinocytes can also express CD80 and CD86, which has been shown in epithelium of patients with atopic dermatitis [34]. IFN-γ treatment of keratinocytes does not seem to induce CD80 and CD86 expression [35].

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However, even if keratinocytes do express CD80 and CD86 they fail to stimulate T cells in MELR [28]. Consequently, it is reasonable to assume that the cells responsible for the T cell proliferation in our assays were the HLA-DR, -DP, -DQ and CD80-, CD86-expressing LC. Cell yield did not permit concomitant culture of irrelevant isotype matched monoclonal antibodies in the MELR, but the anti-HLA DR, -DP, -DQ monoclonal antibodies inhibited cell proliferation in MELR and mixed lymphocyte reactions [36]. The incomplete blocking of T cell proliferation by the anticlass II antibodies could be explained by CD8-positive T cell proliferation.

In the experiments with both syngeneic and allogeneic T cells as responder cells and oral or skin epithelial cells as stimulator cells there was a considerable interindividual difference in allogeneic T cell proliferation. However, there was always the same relation in the T cell response between cultures with oral and skin epithelial cells, i.e. oral epithelial cells induced higher T cell proliferation in each individual.

The oral cavity, being the first part of the gastrointestinal tract, is constantly exposed to antigens from microbes and food. This antigenic influx may cause an up-regulation of the maturation stage of LC. LC isolated from skin epithelium show low co-stimulatory potential *in vitro,* while culturing them for 2–5 days before adding T cells increase the co-stimulatory capacity [10,37]. In contrast, fresh oral LC have high co-stimulatory potential *in vitro*, without prior culturing [25]. Similarly, Liu *et al*. have shown that fresh DC derived from intestinal epithelium have a functional capacity resembling mature DC [23]. The intestine, just as the oral cavity, is loaded with microbes and food antigens. It could be expected that this would bestow oral LC with an enhanced capacity to induce a T cell response. Zhou *et al*. report that *in vitro*-generated CD1a$^+$ CD83$^+$ cells are potent stimulatory cells in allogeneic mixed leucocyte reactions [15]. In the present study CD83-positive cells were found in higher numbers in oral epithelium than in skin epithelium, thus supporting the finding that oral DC have higher T cell stimulating capacity than skin DC. The difference in activity between oral and skin DC may be a result of difference in maturation stimuli, e.g. bacterial products. If this is the case we do not know, but a recent study showed that bacterial stimuli had a greater effect on oral than on skin keratinocytes [38]. This might also be the case for oral and skin DC.

Several studies emphasize the importance of proinflammatory cytokines, such as IL-8, in local inflammatory responses to exogenous antigens [39–43]. IL-8 is a potent chemoattractant and activator of inflammatory response [44], which is produced by epithelial cells provoked by bacteria and other stimuli [39,41]. In this study IL-8 was assessed in cultures of oral and skin epithelial cells and con-A-stimulated T cells in two subjects. The cultures with oral epithelial cells had a higher IL-8 concentration than cultures with the skin epithelial cells. The higher concentration of IL-8 in the oral epithelial cell suspension might reflect the presence of various danger signals in the oral mucosa which are not present in the skin. It may also be that the increased immunogenicity of the oral DC is caused by the increased production of IL-8. We will try investigate this possibility by neutralizing IL-8 with monoclonal antibodies. Several investigations have shown that production of IL-8 by epithelial cells is dependent on both the type of bacterial stimulation and time of provocation [21,41,43].

In conclusion, we show that fresh oral LC have a T cell co-stimulating capacity superior to that of cultured skin LC. The results suggest that fresh oral LC have a phenotype, which resemble mature DC. It is likely that local environmental factors in the oral cavity are responsible for this. It can also be speculated that the clinical behaviour of immunomediated skin and mucosal diseases is influenced by this fact.

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