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Flow cytometric analysis of cytokine receptors on human Langerhans' cells. Changes observed after short-term culture

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SUMMARY

It is well established that granulocyte–macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-1 and tumour necrosis factor-α (TNF-α) are involved in Langerhans' cell (LC) development and dendritic cell traffic. However, little is known about the pattern of cytokine receptors on human LC and their modulation during different stages of maturation. The expression of cytokine receptors was studied by flow cytometry on both freshly isolated LC (fLC) and 72-hr cultured LC (cLC). Epidermal cell suspensions enriched in LC were obtained after skin trypsinization and Ficoll–Hypaque gradient. LC were identified by their CD1a positivity. Although the majority of fLC were positive for the α chain of GM-CSF receptor (GM-CSFR), the β chain of GM-CSFR was detected only on 15% of CD1a$^+$ cells. fLC were also positive for IL-1 receptor (IL-1R) type 1, IL-1R type 2, 75 000 molecular weight TNF receptor (TNFR) and interferon-γ receptor (IFN-γR). IL-6R and its transducing signal gp130 were present in a subset of fLC. Granulocyte colony-stimulating factor receptor (G-CSFR), macrophage colony-stimulating factor receptor (M-CSFR), the α and β chain of IL-2R, IL-4R, IL-7R, IL-8R and 55 000 molecular weight TNFR were not detected on fLC. After culture, LC up-regulated the expression of both the α and β chains of GM-CSFR, IL-1R type 2, α and β chains of IL-2R, IL-6R and gp130. In contrast, IL-1R type 1 and 75 000 molecular weight TNFR were down-modulated and the expression of IFN-γR was not affected by culture. These results suggest that LC undergo changes in the cytokine receptor repertory during *in vitro* maturation.

INTRODUCTION

Langerhans' cells (LC) represent immature elements of the dendritic cell (DC) family. During their *in vivo* maturation intraepidermal LC emigrate, via afferent lymphatics, from the epithelium to the T-cell areas of draining lymph nodes where they become interdigitating DC.[1,2] It is also known that freshly isolated LC (fLC), after short-term (2–3 days) culture in bulk epidermal cell suspensions, mature to cells with morphology, phenotype and T-cell stimulatory capacity similar to lymphoid DC.[3,4] During this process, cultured LC (cLC) lose their ability to uptake native antigens and acquire the capacity to cluster and to activate efficiently T cells.[5] Different cytokines are involved in LC maturation and trafficking. Granulocyte–macrophage colony-stimulating factor (GM-CSF) and interleukin-1α (IL-1α) mediate the *in vitro* maturation and differentiation of LC.[6,7] Also under culture conditions, the simple addition of tumour necrosis factor-α (TNF-α) maintains LC viability without inducing DC maturation.[8] Intracutaneous injection of IL-1 induces on epidermal LC a significant up-regulation of major histocompatibility complex (MHC) class II molecules, an important increase in their immunostimulatory properties and a decrease of the intraepithelial DC number. These changes resemble those observed during the course of contact sensitivity.[9,10] It was demonstrated that TNF-α provides an important signal for LC migration from skin.[11,12] On the contrary, other experimental models have suggested that TNF-α might prevent LC trafficking from epidermis to draining lymph nodes.[13] TNF-α increases the expression on LC of α6 integrin and intercellular cell adhesion molecule-1 (ICAM-1).[14,15] It is well documented the primary role of GM-CSF and TNF-α in the *in vitro* differentiation of human, mouse and rat DC from haematopoietic precursors.[16,17] GM-CSF might also play some role during the process of recruitment

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Abbreviations: cLC, cultured Langerhans' cells; DC, dendritic cells; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; fLC, freshly isolated Langerhans' cells; FSC, forward scatter; G-CSFR, granulocyte colony-stimulating factor receptor; GM-CSF, granulocyte–macrophage colony-stimulating factor; GM-CSFR, granulocyte–macrophage colony-stimulating factor receptor; ICAM-1, intercellular cell adhesion molecule 1; IL, interleukin; IL-R, interleukin receptor, IFN-γR, interferon-γ receptor; LC, Langerhans' cells; LFA-3, lymphocyte function associated antigen-3; mAb, monoclonal antibody; M-CSFR, macrophage colony-stimulating factor receptor; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; PMA, phorbol myristate acetate; R-PE, R-phycoerythrin; SSC, side scatter; TNF-α, tumour necrosis factor-α; TNFR, tumour necrosis factor receptor.

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and differentiation of LC in peripheral tissues.[18,19] It is still uncertain whether such responses may represent a direct effect of different ligands on specific cytokines receptors expressed on the LC cell membrane. In this study we analysed the expression and modulation of the cytokine receptors at different stages of LC maturation after short-term *in vitro* culture.

MATERIALS AND METHODS

Monoclonal antibodies (mAb)

A panel of murine mAb from the Cytokine Receptor Panel of the Fifth International Workshop on Human Leucocyte Differentiation Antigens (Boston, 1993) was used to study the cytokine receptor phenotype of fLC and cLC. The mAb 8G6 and 3D7 directed against the α and β chains of GM-CSF receptor (GM-CSFR) respectively, were kindly provided by Dr Angel Lopez (Institute of Medical and Veterinary Science, Adelaide, Australia). The information regarding all these antibodies is detailed in Table 1.

Preparation and culture of epidermal cell suspensions

Epidermal cell suspensions were obtained from normal human

Table 1. List of monoclonal antibodies

Specificity	CD	Clone	Susceptibility of epitope to trypsinization*	Isotype
Cytokine receptors				
IL-1R; Type-1	w121a	hIL-1R1-M1	+/−	IgG1
IL-1R; Type 2	w121b	hIL-1R2-M22	+/−	IgG2b
IL-2R; 55 000 mol. wt.	25	anti-Tac	+	?
(α chain)		IL2 R-M1	+	?
IL-2R; 75 000 mol. wt.	122	2R-B	+/−	IgG1
(β chain)		Mik-b1	+/−	IgG2a
IL-4R (α chain)	w124	hIL-4R-m57	+/−	IgG1
IL-6R	126	B-F19	+/−	IgG1
		B-E23	+	IgG1
		B-R6	+	IgG1
IL-6R-gp130	w130	AM64	+/−	IgG
IL-7R (α chain)	w127	hIL-7R-M20	+	IgG1
IL-8R	w128	B-G20	+	IgM
		B-F25	NT	IgM
GM-CSFR	w116	hGM-CSFR-M1	NS	IgG1
(α chain)		8G6	+	IgG1
GM-CSFR, IL-3R and IL-5R common β chain	—	3D7	+/−	IgG1
G-CSFR	—	hGCSFR-M1	+/−	IgG1
M-CSFR (CSF-1R)	115	7-7A3-17	+	IgG
IFN-γR	w119	GIR-208	NS	IgG1
TNFR; 55 000 mol. wt.	120a	htr-9	+	IgM
		MR1-1	+/−	IgG1
		MR1-2	+/−	IgG1
TNFR; 75 000 mol. wt.	120b	hTNFR-M1	+/−	IgG2b
		MR2-1	+/−	?
		utr-1	+/−	IgM
Controls				
Class I like	1a	OKT6 (FITC)	+/−	IgG1
Class I (MHC)	—	W6/32	+/−	IgG2a
HLA-DR	—	L243	NS	IgG2a
Fcγ-RII	32	IV.3	NS	IgG2b
gp39 ligand	40	G28.5	+/−	IgG1
ICAM-1	54	F10.2	+	IgG1
LFA-3	58	BRIC 5	+/−	IgG2a
B7.1	80	BB1	+/−	IgM
B7.2	86	BU63	+/−	IgG1

* NS, not susceptible; +/−, fluorescence mean channel (FMC) up to 25% lower than control cells not trypsinized; +, FMC 25–50% lower than control cells; NT, not tested. Those mAb from the Cytokine Receptor Panel (Vth International Workshop on Human Leucocyte Differentiation Antigens) that showed a reduction in their FMC higher than 50% were not included in the present study and are not listed.

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skin of women undergoing corrective plastic surgery. Skin samples were attached to a cork plate, split-cut with a dermatome (1 mm thickness), cut into small square pieces and then incubated for 20 min at 37° with 0·20% trypsin (Gibco, Grand Island, NY) in buffer (0·68% NaCl, 0·04% KCl, 0·1% glucose, 0·22% $NaHCO_2$, pH 7·2–7·4). The enzyme was stopped by washing the skin samples with RPMI-1640 supplemented with 20% fetal calf serum (FCS) at 4°. The epidermal cells were detached from the dermis by gently scraping with a scalpel and the cell suspension was obtained by blowing in and out for 5 min with a Pasteur pipette. After filtration through sterile steel mesh (pore diameter: 35 μm), cells were washed three times in RPMI-1640 at 4° supplemented with 20% FCS. Cells were counted and the viability was assessed with ethidium bromide and orange acridine. The suspension contained 0·5–1·5% LC, as judged by CD1a staining, and viability was more than 90%. LC enrichment was achieved by Ficoll–Hypaque gradient with a density of 1·077 g/cm^3. After 15 min of centrifugation at 300 *g* the cells of the interface were collected and washed twice in RPMI-1640 with 20% FCS. After this procedure, LC were enriched to 6–9% CD1a$^+$ cells.

For epidermal cells short-term cultures, 40–80 $\times 10^6$ epidermal bulk cells from each sample were cultured in RPMI-1640 supplemented with 15% heat-inactivated AB human serum, 20 mmol/l HEPES buffer, 2 mmol/l L-glutamine, 200 U/ml penicillin and 20 μg/ml gentamicin, in a CO_2 atmosphere of 5%, at 37°. After 72 hr of culture, the non-adherent fraction was harvested and subjected to a Ficoll–Hypaque density (1·077 g/cm^3) gradient, to eliminate debris, dead cells and most keratinocytes. Viability exceeded 80%, and the percentage of CD1a$^+$ cells ranged from 8 to 10%. Each experiment was done at least three times with epidermal cells obtained from different donors.

Trypsinization of cell suspensions

To analyse the susceptibility to trypsin of epitopes from different clusters of differentiation (CD), peripheral blood mononuclear cells (PBMC) and different cell lines were incubated in RPMI-1640 containing 0·20% trypsin, for 25 min at 37°. Control cells were identically treated, but without the enzyme. After labelling by indirect immunofluorescence, the mean intensity of fluorescence of both enzyme-treated and control cells was compared by flow cytometry.

Double immunostaining and analysis by flow cytometry

Epidermal cells suspensions enriched in LC were incubated with pooled human AB serum (1/10) 30 min, to block Fc receptors, and then with the primary mAb (Table 1) at optimal concentrations (1 : 50, mAb concentration : 1 μg/10^6 cells) for another 60 min. As the second step, cells were labelled with R-phycoerythrin (R-PE)-conjugated goat anti-mouse immunoglobulins (Dako, Denmark) for 30 min. Cells were then incubated with normal mouse serum (1/10) for 15 min, in order to block any residual free goat anti-mouse immunoglobulin binding sites. In the final step cells were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-CD1a (Ortho, USA) for 45 min. All incubations and washings were done at 4° with RPMI-1640 containing 5% FCS and 0·1% NaN_3. Suspensions of enriched epidermal cells labelled successively with irrelevant isotype-matched primary mAb, R-PE-conjugated goat anti-mouse immunoglobulins, normal mouse serum, and FITC-conjugated irrelevant IgG1 were used as negative controls. Fluorescence intensity of 5×10^4 fresh or cultured epidermal cells was analysed by a FACStar plus (Becton Dickinson, Mountain View, CA). Dead cells were excluded by gating with propidium iodide.

RESULTS

Cell surface expression of cytokine receptors on fLC

Double immunostaining of epidermal cell suspensions enriched in fLC by density gradient were analysed by flow cytometry immediately after labelling. The fLC were identified by their CD1a positivity. The localization of fLC in a forward scatter (FSC) versus side scatter (SSC) dot plot was accomplished by gating of the CD1a$^+$ cell population. Compared with CD1a$^-$ cells, most fLC were small to medium in size with low cytoplasmic granularity. By gating the CD1a$^+$ cells on a FSC versus SSC dot plot, the percentage of fLC was enriched electronically from 6–9% to a mean of 23% (16–32%).

Preparation of fLC-enriched suspensions required that thin fragments of skin were exposed to trypsin. Proteolytic treatment of this type might be expected to trim off trypsin-sensitive cell surface epitopes. In the first series of experiments the influence of 0·20% trypsin (in our hands the lowest trypsin concentration that disrupts the epidermis after 20 min of incubation at 37°) on the determinants recognized by the mAb used in this study was investigated. PBMC of normal individuals, phytohaemagglutinin-stimulated PBMC, Epstein–Barr virus-transformed B cells and the cell lines HL60, U937 and CIR (all of them used as positive controls) were pretreated with 0·20% trypsin for 20 min at 37°, immunostained and subjected to flow cytometry analysis. The sensitivity of different epitopes to trypsinization was assessed comparing the fluorescence mean intensity of trypsinized cells versus non-treated control cells. Only those mAb from the Cytokine Receptor Panel of the Fifth International Workshop on Human Leucocyte Differentiation Antigens that were not affected or showed up to 50% reduction in the fluorescence mean intensity were selected for the present study and are listed in Table 1.

The majority of fLC (more than 80% of CD1a$^+$ cells) expressed the GM-CSF receptor (GM-CSFR) α chain with the two mAb tested (Fig. 1). On the other hand, the GM-CSFR β chain was expressed on a small subset of CD1a$^+$ cells (15%). None of the cells obtained from fresh epidermal cells suspensions showed staining with anti-granulocyte colony-stimulating factor receptor (G-CSFR) or anti-macrophage colony-stimulating factor receptor (M-CSFR) mAb. Both CD1a$^+$ and CD1a$^-$ epidermal cells (most keratinocytes) were weakly positive for the anti-IL-1 receptor (IL-1R) type 1 mAb. Unlike keratinocytes, fLC (78% of CD1a$^+$ cells) were also positive for the presence of IL-1R type 2. Consistent results of three different experiments showed that only one mAb (B-F19) of the three anti-IL-6R mAb was positive on a subpopulation of 30% of CD1a$^+$ cells. The signal transducer chain of the IL-6R, gp130, was present both in a subset of fLC (27% of CD1a$^+$ cells) and a subpopulation of keratinocytes. The α and β chains of IL-2R, as well as the cytokine-binding chains of IL-4R, IL-7R and IL-8R were not detected in any cell from the fresh epidermal cell

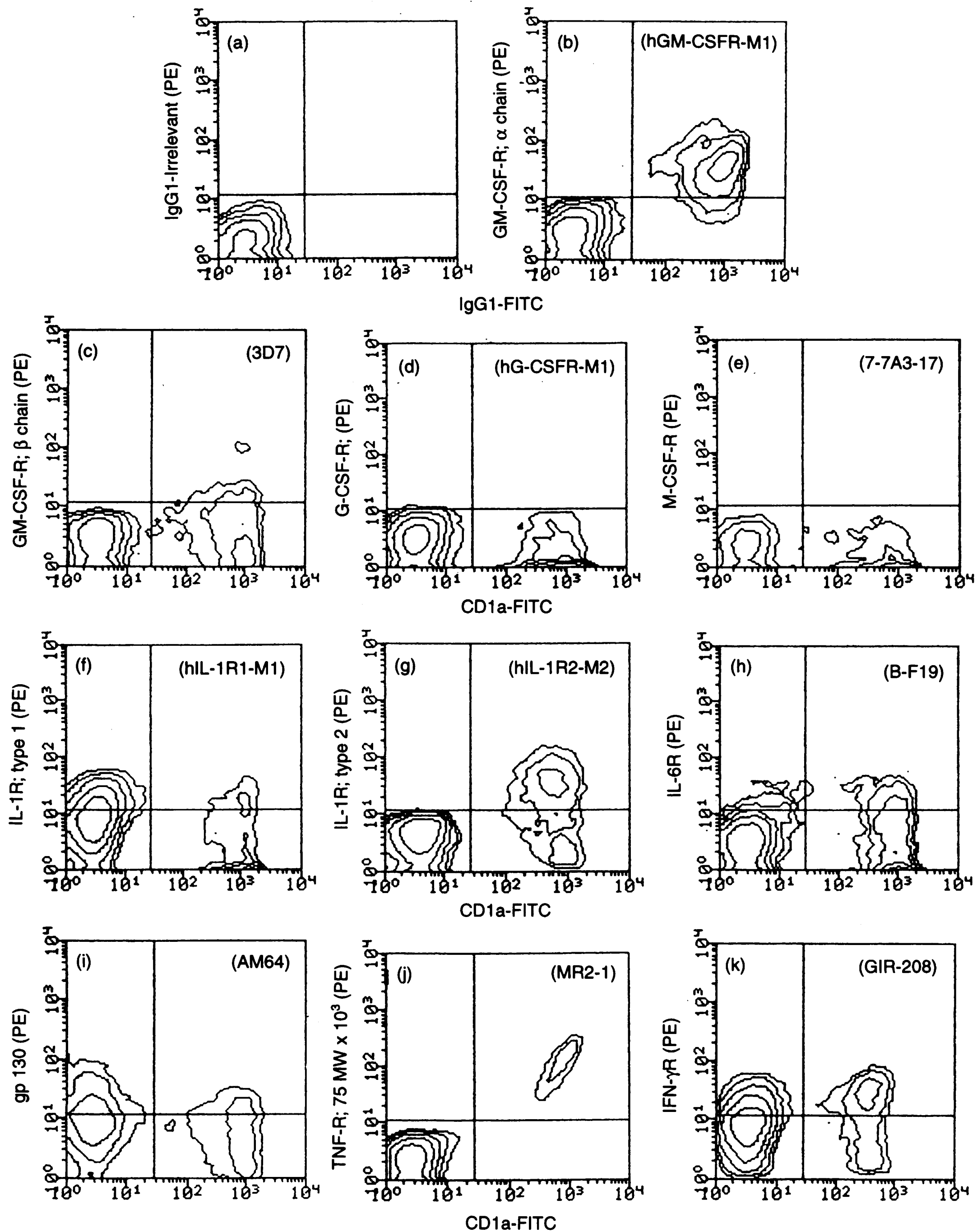

Figure 1. Expression of cytokine receptors by human epidermal LC. Epidermal cell suspensions were obtained from skin and stained for simultaneous expression of cytokine receptors (vertical axis) and CD1a (horizontal axis) and analysed by flow cytometry. (a) Vertical and horizontal markers were set to include >98% of negative cells in the left lower quadrant when isotype control IgG1 was used instead of primary mAb. Similar results were obtained with isotype controls IgG2a, IgG2b, and IgM (not shown). (b–k) fLC express α and β chain GM-CSFR, upper quadrant of the contour plot IL-1R type 1 and type 2, IL-6R, IL-6R-gp130, 75 000 molecular weight TNFR and IFN-γR. G-CSFR and M-CSFR were not detected on CD1a$^+$ cells. The name of the mAb used in each experiment is in the right upper quadrant of the contour plot.

suspension. All fLC expressed the 75 000 molecular weight TNF receptor (TNFR) (negative on keratinocytes). On the contrary, while the 55 000 molecular weight TNFR showed a weak positivity on keratinocytes, it was completely absent on CD1a$^+$ cells. Keratinocytes and a subset of fLC (67% of CD1a$^+$ cells) expressed similar levels of interferon-γ receptor (IFN-γR).

To exclude the binding to cytokine receptors on intra-epidermal T cells, control immunostaining with anti-CD3 mAb was included in all cases (data not shown).

Cytokine receptors expression on cLC

The expression of cytokine receptors on LC was analysed after 72-hr culture in bulk epidermal cell suspension. As expected, cLC up-regulated MHC class I and II molecules and showed a strong expression of the adhesion molecules ICAM-1 (CD54) and lymphocyte function-associated antigen 3 (LFA-3, CD58) (Fig. 2). Although still detectable, CD1a and CD32 showed a decreased expression. The costimulatory signals B7.1 (CD80) and B7.2 (CD86) as well as the CD40 molecule, all of them negative or expressed weakly by a low number of fLC, were expressed by 100% of cLC.

The phenotype of cytokine receptors on LC after 72 hr of culture demonstrated a different pattern of expression (Table 2 and Fig. 3). The cLC up-regulated both the α and the β chains of GM-CSFR and increased the expression of IL-1R type 2, the cytokine-binding chain of IL-6R and gp130. The weak positivity observed for IL-1R type 1 on fLC was completely down-modulated upon culture. Other cytokine receptors, that were not found initially on fLC, such as α and β chains of IL-2R, were detected on cLC. The 75 000 molecular weight TNFR was significantly down-modulated after culture and the 55 000 molecular weight TNFR remained absent. The level of IFN-γR expression did not show a significant change on cLC membrane compared to fLC. IL-4R, IL-7R and IL-8R remained undetectable after 72 hr of culture.

DISCUSSION

In the present study we demonstrated by flow cytometry the expression of receptors for GM-CSF, IL-1, IL-2, IL-6, TNF-α and IFN-γ on human LC. We also confirmed previous reports demonstrating the presence of the α chain of IL-2R (CD25) and 75 000 molecular weight TNFR on human DC.[3,20–22]

The absence of detectable levels of some receptors on isolated LC raises the question whether fLC, the *in vitro* analogue of epidermal LC, would have expressed any of the not-detected cytokine receptors if isolated without enzymatic digestion. This problem can be partially answered by the results obtained by means of immunostaining of LC in epidermal sheets. Previous studies have demonstrated that those mAb that were negative on fLC by flow cytometry were also not detectable on epidermal sheets or cryostat tissue sections (ref. 22 and Larregina *et al.*, unpublished observation). Lack of specific staining on LC in epidermal sheets or in tissue sections indicates that this expression if present, must be extremely low.

LC lack detectable levels of receptors for G-CSF and M-CSF, but express GM-CSFR. These data, in concordance with a study performed in murine DC, give further support for the concept that human DC represent a highly specialized subset of myeloid cells with a distinctive pattern of cell differentiation with respect to monocytes/macrophage or granulocytic cells.[23]

The GM-CSFR consists of a unique α subunit and a β

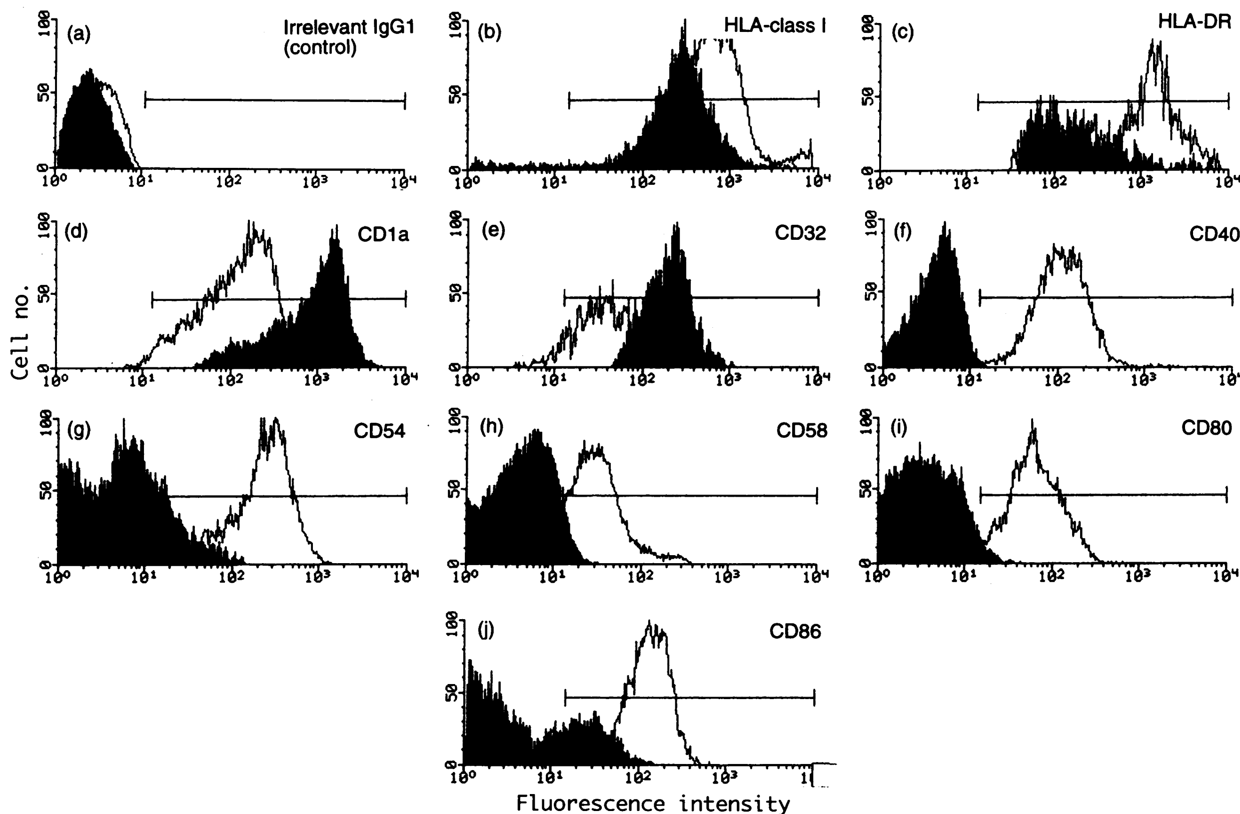

Figure 2. Maturation of fLC upon short-term culture monitored by flow cytometry. fLC were cultured for 3 days in an epidermal cell suspension and then processed for double staining using mAb anti-HLA-Class I, HLA-DR, CD1a, CD32, CD40, CD54, CD58, CD80 and CD86 revealed by PE-conjugated anti-mouse immunoglobulin followed by FITC-conjugated anti-CD1a. The fLC are represented by shaded areas and cLC by open areas. Graphs illustrate only the population of LC gated by their positivity for CD1a. (a) Range bars were set to include < 1% of positive cells when isotype control IgG1 mAb was used instead of primary mAb. Similar results were obtained when IgG2a, IgG2b and IgM isotype controls were used (data not shown). (b–j) After culture LC up-regulated HLA-Class I, HLA-DR, CD40, CD54, CD58, CD80 and CD86. The expression of CD1a and CD32 decreased on cLC.

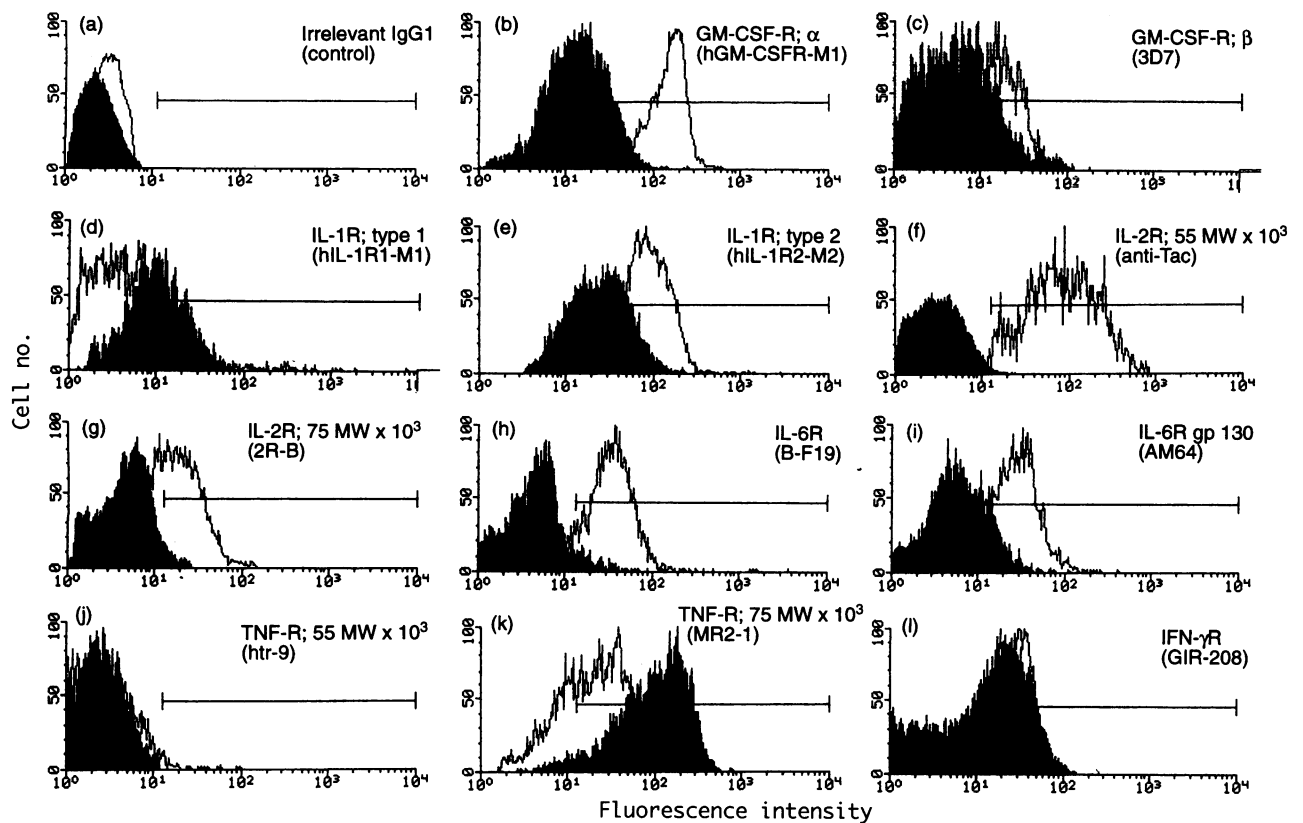

Figure 3. Changes in the expression of cytokine receptors on LC upon short-term culture monitored by flow cytometry. fLC were cultured for 3 days in an epidermal cell suspension and then processed for double staining using primary mAb specific for cytokine receptors, revealed by PE-conjugated anti-mouse immunoglobulins followed by FITC-conjugated anti-CD1a. Histograms illustrate only the population of LC gated by their positivity for CD1a. fLC are represented by shaded areas and cLC by empty areas. (a) Range bars were set to include < 1% of positive cells when isotype control IgG1 mAb was used instead of primary mAb. Similar results were obtained when IgG2a, IgG2b and IgM isotype controls were used (data not shown). After culture LC up-regulated the expression of α and β chain GM-CSFR (b, c), IL-1R type 2 (e), IL-6R (h), and IL-6R-gp130 (i). cLC expressed *de novo* 55 000 molecular weight IL2-R (f) and 75 000 molecular weight IL-2R (g) and down-regulated IL-1R type 1 (d) and 75 000 molecular weight TNFR (k). 55 000 molecular weight TNFR remained negative along culture (j) and IFN-γR did not show change (l).

subunit. The α chain binds the GM-CSF with low affinity. The β chain (shared with IL-3R and IL-5R) is the signal transducing subunit and together with the α subunit constitutes the high-affinity receptor.[24–26] The subcutaneous injection of GM-CSF does not induce significant changes in intraepidermal LC, whereas under similar conditions IL-1β triggers intraepidermal LC maturation. Based on these observations, it has recently been postulated that IL-1 might up-regulate the β subunit of GM-CSFR.[9,10,23] Similar results were demonstrated on the human haematopoietic cell line TF-1.[27] TNF-α might also contribute to such changes in as much as it can up-regulate GM-CSFR in DC precursors and in acute myeloid leukemia cells.[28,29] The up-regulation of the β subunit on the surface of epidermal LC would increase the affinity of GM-CSFR for its ligand and might trigger the signal for DC maturation. The data presented here seem to confirm such speculation. We observed that although most fLC expressed the α subunit only a small subset of them showed detectable levels of β chain on the cell surface. After culture the β subunit of GM-CSFR was up-regulated and detected in more than 50% of cLC. It is known that keratinocytes are a great source of IL-1α and TNF-α and LC expressed mRNA for IL-1β.[30] These cytokines are released to the medium and might be the factors that modulate in a paracrine or autocrine fashion the GM-CSFR β subunit in cLC.

Two types of IL-1R have been characterized, IL-1R type 1 and IL-1R type 2.[31] Functional experiments, wherein both types of IL-1R were blocked by using either anti-IL-1R type 1 or anti-IL-1R type 2 mAb, demonstrated that the biological effect of IL-1 is mediated through the IL-1R type 1. Moreover, it was recently suggested that IL-1R type 2 not only has no signalling function but also acts as a molecular trap for IL-1. Thus, both membrane and soluble forms of IL-1R type 2 might play a negative control on IL-1 function.[32] It was demonstrated here that human LC show negligible levels of IL-1R type 1 and a predominant expression of IL-1R type 2. The same pattern was observed in other myeloid cells such as monocytes, polymorphonuclear leucocytes, and myelomonocytic cell lines. Schuler *et al.* found that after 24 hr of culture, murine LC express 1200 IL-1R type 1/cell.[23] Lower values were detected in mouse spleen cells or in the afferent lymph of sheep.[23,33] The low number of IL-1R type 1 per DC is very close to the sensitivity threshold of flow cytometry. Some proteolytic damage during trypsinization could also explain the low fluorescence mean intensity values detected in the present study. Interestingly, this study demonstrated that human LC

Table 2. Expression of cytokine receptors on fLC and cLC*

Cytokine receptors	fLC	cLC
IL-1R; Type 1	+ (20%)	↓ (5%) (#)
IL-1R; Type 2	+ + (78%)	↑ (95%)
IL-2R; 55 000 mol. wt. (α chain)	–	↑ (97%)
IL-2R; 75 000 mol. wt. (β chain)	–	↑ (75%)
IL-4R (α chain)	–	–
IL-6R	+ (30%)	↑ (90%)
IL-6R-gp130	+ (27%)	↑ (80%)
IL-7R (α chain)	–	–
IL-8R	–	–
GM-CSFR (α chain)	+ + (80%)	↑ (100%)
GM-CSFR (βc chain)	+ (15%)	↑ (27%)
G-CSFR	–	–
M-CSFR	–	–
IFN-γR	+ + (67%)	= (87%)
TNFR; 55 000 mol. wt.	–	–
TNFR; 75 000 mol. wt.	+ + + (98%)	↓ (75%)

* +, weak staining (log FMC between 10^0–10^1); + +, moderate staining (log FMC between 10^1 and 10^2); and + + +, strong staining (log FMC between 10^2 and 10^3). In round brackets the mean percentage of LC positive for each marker (only CD1a$^+$ cells were included).

(#) ↓, Down-regulation; ↑, up-regulation; =, no shift of FMC.

express IL-1R type 2, which was not found in the DC of mice or sheep.[23,33] Species differences in IL-1R tissue distribution or distinct methodological approaches could account for such discrepancy. The biological implication of this finding on the maturation of human LC remains to be established.

Although no biological effects of IL-2 have been described on LC, several authors reported the up-regulation of the IL-2R α chain (CD25) after short-term culture of both LC and lymph-borne DC in mice, rats and humans.[3,34,35] Our findings demonstrated that cLC express both α and β subunits of the IL-2 high-affinity receptor complex. Although the IL-2β chain is considered the major signalling molecule, a third component of IL-2R termed γ chain is critical for receptor-mediated signal transduction.[36] Unfortunately, mAb that recognize the human IL-2R γ chain are not yet available. Further studies are needed to demonstrate whether IL-2R have some biological function on cLC.

There are two distinct TNFR, TNFR type 1 (molecular weight 55 000) and TNFR type 2 (molecular weight 75 000), each encoded by separate genes.[37–39] Both receptors share significant homologies in their extracellular sequences, with repeat cysteine-rich extracellular domains, which define a larger family of cellular and viral proteins that includes the nerve growth factor receptor, Fas/APO-1 antigen (CD95), CD40, CD27 and CD30 among others.[39,40] Sequence homology between human and murine 55 000 molecular weight TNFR is most conserved in the extracellular segment, whereas the 75 000 molecular weight TNFR is most preserved in the intracellular domain.[40] The weak homology of the extracellular segment explains the species specificity of the 75 000 molecular weight TNFR for its ligand. In this regard, several authors reported that in mice, only murine TNF was able to maintain LC viability during culture as well as to induce LC to emigrate from the epidermis towards the draining lymph node.[8,12] In the present study we confirmed by flow cytometry the expression of the 75 000 molecular weight TNFR and the absence of the 55 000 molecular weight TNFR in human fLC and cLC. Ryffel *et al.* by means of immunohistochemistry on frozen sections demonstrated the same pattern of TNFR expression in intraepidermal LC and interdigitating reticulum cells of secondary lymphoid tissues in humans.[21] Although the 55 000 molecular weight TNFR seems to be more prevalent in epithelial cells and the 75 000 molecular weight TNFR in cells of myeloid and lymphoid origin, the distribution of both receptors is quite ubiquitous.[41,42] As a matter of fact, within the myeloid lineage mature granulocytes express both species of TNFR to about equal extent whereas peripheral blood monocytes express the TNFR of 75 000 molecular weight. The presence of only this TNFR in both monocytes and DC is in agreement with the close ontogenic relationship between these two cell types.[21,43] The cLC can be considered to be activated DC that express on their cell surface all the molecules necessary for antigen presentation for both naive and memory T cells [i.e. high levels of MHC class I and class II molecules, the adhesion molecules ICAM-1 (CD54) and LFA-3 (CD58), and the costimulatory signals B7.1 (CD80) and B7.2 (CD86)]. The cLC down-modulate the 75 000 molecular weight TNFR as part of their maturation process during culture. In agreement with this observation, it is important to notice that effector T lymphocytes activated by phorbol myristate acetate (PMA) or anti-CD3, granulocytes activated by *N*-formyl-Met-Leu-Phe or PMA, and macrophages activated by IFN-γ also down-modulate their TNF-binding capacity.[42,44,45] The proteolytic cleavage (shedding) of TNFR seems to be the mechanism by which activated cells reduce their TNF-binding capacity.[42,44,46] Although the way by which cLC modulate the expression of 75 000 molecular weight TNFR is unknown, down-modulation of cell surface receptors might be one of the mechanisms by which LC might control the deleterious effects resulting from high levels of TNF-α released by activated T cells to DC microenvironment during antigen presentation.

In summary, the pattern of cytokine receptors and its modulation upon culture were analysed on LC by means of a broad panel of mAb and flow cytometry. Although, further studies are required to determine whether the receptors detected in this study are biologically active, the results presented here suggest important changes in the repertoire of cytokine receptors during LC differentiation.

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