Cultured Human Langerhans Cells Resemble Lymphoid Dendritic Cells in Phenotype and Function

Nikolaus Romani, Ph.D., Angela Lenz, Herta Glassel, Ph.D., Hella Stössel, Ursula Stanzl, Otto Majdic, M.D., Peter Fritsch, M.D., and Gerold Schuler, M.D.

Departments of Dermatology and Internal Medicine (HG), University of Innsbruck, Innsbruck, and Institute of Immunology (OM), University of Vienna, Vienna, Austria

Freshly isolated murine epidermal Langerhans cells (LC) are weak stimulators of resting T cells. Upon culture their phenotype changes, their stimulatory activity increases significantly, and they come to resemble lymphoid dendritic cells. Resident murine LC, therefore, might represent a reservoir of immature dendritic cells. We have now used enzyme cytochemistry, a panel of some 80 monoclonal antibodies, and immunofluorescence microscopy or two-color flow cytometry, as well as transmission electron microscopy, to analyse the phenotype and morphology of human LC before and after 2 - 4 d of bulk epidermal cell culture. In addition, LC were enriched from bulk epidermal cell cultures, and their stimulatory capacity was tested in the allogeneic mixed leukocyte reaction and the oxidative mitogenesis assay. Cultured human LC resembled human lymphoid dendritic cells in morphology, phenotype, and function. Specifically, LC became non-adherent upon culture and developed sheet-like processes (so-called "veils"), decreased their surface ATP/ADP'ase activity, and lost nonspecific esterase activity. As in the mouse, surface expression of MHC class I and II antigens increased significantly, and FcII receptors were significantly reduced. Markers that are expressed by dendritic cells (like CD40) appeared on LC following culture. Cultured human LC were potent T-cell stimulators. Our findings support the view that resident human LC, like murine LC, represent immature precursors of lymphoid dendritic cells in skin-draining lymph nodes. *J Invest Dermatol 93:600–609, 1989*

The relationship of Langerhans cells (LC) [1] to dendritic cells (DC) [2,3] and macrophages [4] has been unclear. DC have a common group of features in all species analyzed so far [3], such as dendritic shape including characteristic veils, lack of Fc receptors, abundant class I and II products, absence of many macrophage and lymphocyte traits, and, most importantly, potent stimulatory function for 1°immune responses. DC were first identified in cell supensions prepared from lymphoid organs, but evidence is now accumulating that DC are more widely distributed (veiled cells in the lymph, peripheral blood DC, and interstitial DC). Resident (The term "resident LC" is defined here as LC in situ as well as freshly isolated LC.) epidermal LC, though class II positive and dendritic in shape, show some clear-cut differences from DC, as they express markers found on macrophages and are rather weak in stimulating a 1° immune response [1,2,5]. We have observed that murine LC upon culture undergo significant changes in phenotype and function and come to resemble lymphoid DC [6]. The properties that are considered macrophage-like (such as presence of F4/80 antigen, Fc receptor, membrane ATP'ase, nonspecific esterase) decreased significantly or were lost. The cells developed cytoplasmic veils, increased class I and II products, and increased their stimulatory capacity 10 - 30-fold [6,7]. This maturation of LC was mediated by the combined action of GM-CSF and IL-1 [8,9]. These findings led to the concept that LC in situ might represent a reservoir of immunologically immature DC, which can be mobilized in response to exogenous cytokines released locally in response to epidermal injury, deposition of contact allergens, etc.

We have now performed a detailed analysis of the phenotype of resident human LC (rLC) and of LC after 2-4 d of bulk epidermal cell (EC) culture (rLC). We have also tested the stimulatory capacity of cLC for resting T cells. Our data suggest that human LC undergo a similar process of maturation as observed in the murine system.

MATERIALS AND METHODS

Processing of Skin Specimens Normal human, female breast skin was obtained from plastic surgery and processed immediately. To prepare cryosections small pieces were excised, embedded in Tissue-Tek II OCT (Miles Laboratory, Inc., Naperville, IL), rapidly frozen in melting isopentane, and stored in liquid nitrogen until use. Four-micrometer cryosections were cut vertically, mounted on albuminized slides, dried, fixed in acetone (10 min at 20°C), and

Manuscript received March 9, 1989; accepted for publication June 19, 1989.

This work was supported by a grant from the Austrian Science Foundation (project No. P6170M) and the Austrian Ministry of Science.

Reprint requests to: Nikolaus Romani, Ph. D., Department of Dermatology, University of Innsbruck, A-6020 Innsbruck, Austria.

Part of this work was presented at the ADF (Arbeitsgemeinschaft für Dermatologische Forschung) meetings in Vienna, 1985, and in Cologne, 1988

Abbreviations:
ATCC: American Type Culture Collection
cLC: cultured LC
DC: dendritic cell(s)
EC: epidermal cell(s)
FcR: Fc-Receptor
FCS: fetal calf serum
FITC: fluorescein isothiocyanate
LC: epidermal Langerhans cell(s)
MLR: mixed leukocyte reaction
MoAb: monoclonal antibody
PBMC: peripheral blood mononuclear cells
rLC: resident LC
TRITC: rhodamin isothiocyanate

0022-202X/89/$03.50

stained. From the remainder of the skin specimen split-thickness skin was removed by using a dermatome and processed to prepare epidermal sheets and EC suspensions. Epidermal sheets were peeled from small pieces of split-thickness skin following treatment with ammonium-thiocyanate [10], rinsed in phosphate-buffered-saline, fixed in acetone (10 min at 20°), and stained. To obtain EC suspensions split-thickness skin was exposed for 16 h at 4°C to 0.25% trypsin in phosphate-buffered saline (Flow Laboratories, Irvine, Scotland). The epidermis was then peeled off and disaggregated by teasing with glass rods and by vigorous pipetting in cold MEM (1x Minimum Essential Medium Eagle, modified, Cat. No. 12-102-54; Flow Laboratories) with 10% FCS (Seromed, Biochrom KG, Berlin, FRG). The resulting EC suspension was then filtered through nylon mesh (59 μm mesh size) to remove cell clumps and finally washed three times in culture medium (we used MEM with 10% FCS, 1 mM L-glutamine, and 50 μg/ml gentamycin sulfate added). The EC suspensions (viability 74-93%, 1.2 ± 0.5% LC as identified by staining for CD1a antigens) were then processed for immunolabeling immediately or following culture.

Enrichment of LC From Cultured EC For bulk epidermal cell culture 25 - 30 × 10^6 EC were plated in 20 ml culture medium per 75 cm^2 culture flask (Falcon, Lincoln Park, NJ, Cat.No. 3024). After 2-4 d the nonadherent fraction, which then contained most LC (as identified by staining for HLA-DR or CD1a antigens), was harvested and floated on dense albumin as described for murine EC cultures [6]. The interface (viability >80%) contained most of the viable LC (enriched to 10%-70%). The recovery of LC ranged from 3% to 40% of the starting population. We did not systematically analyze the reasons for this variability, but our impression was that a low plating efficiency of the keratinocytes paralleled low recovery and enrichment of cLC. If the LC recovery was less than 8% of the starting population the respective samples were not included in this study.

Immunolabeling Techniques We used a large panel of mouse monoclonal antibodies (MoAb) as purified reagents or as hybridoma culture supernatants to analyze the phenotype of human LC (Tables I and II). We are thankful to the many colleagues who generously provided us with the antibodies needed for this study (Tables I and II). All antibody dilutions and washes were in phosphate-buffered saline containing 1% bovine serum albumin. All antibodies were tested at saturating or supersaturating levels. Positive control staining was obtained by the use of an appropriate positive tissue or cell population (human skin, lymphoid tissues, peripheral blood mononuclear cells, and subfractions thereof). Negative controls to rule out Fc-receptor-mediated binding and/or unspecific adsorption of antibodies consisted of replacement of the first MoAb with unrelated or unreactive MoAb of the same isotype, and with phosphate-buffered saline. For the detection of antigens of rLC we relied upon the examination of sheets for two reasons. First, staining of sheets detects membrane as well as intracellular antigens, and is thus most sensitive. Second, it circumvents exposure to trypsin, and thus allows the visualization of trypsin-sensitive antigens, which might escape detection in freshly prepared EC suspensions.

To detect the binding of MoAb to cryosections we used a streptavidin-biotin-peroxidase complex technique (as prescribed by the manufacturer; Amersham Int., Amersham, UK). For double labeling we used 3-amino-9-ethylcarbazol instead of 3,3′ diaminobenzidine as a substrate for peroxidase to visualize the binding of the first antibody. Staining with alkaline phosphatase anti-alkaline phosphatase complex as prescribed by the manufacturer (Dianova, Hamburg, FRG; Cat.No. M800) was then used to identify CD1a on LC.

For staining of sheets we used the following staining sequence: 1. MoAb for 16 h at 4°C with constant shaking, then 2. biotinylated species-specific sheep $F(ab')_2$ anti-mouse Ig (Amersham), followed by 3. streptavidin FITC (Amersham) for 90 min., 37°C each. For strongly expressed antigens we sometimes used only a two-step technique: a. MoAb, then b. fluorescein or rhodamin isothiocyanate

Table I. The Phenotype of Resident Epidermal Langerhans Cells[a]

CD Classification, Specificity	Monoclonal Antibodies	Reactivity with LC (Sheet, Suspension)	Source reference[b]
MHC antigens and leukocyte common antigen			
HLA-DR	DR	+	Becton Dickinson
HLA-DP	DP	+	Becton Dickinson
HLA-DQ	Leu10	+	Becton Dickinson
	8C4	−	Steinman [42]
HLA-DR + DQ	9.3F10	+	Steinman [42]
HLA-DQ?	RFD1	+ (subset only)	Poulter [20]
Invariant chain cytoplasmic	VIC-Y1	+	Knapp [43]
HLA-A,B	W6/32	+	ATCC
CD45	HLe	+	Becton Dickinson
	T29/33	+	Hybritech
Myeloid cell markers			
CD14	VIM13	− (a rare LC+)	Knapp [44]
	FMC-17, FMC-32	−	Zola [22]
	3C10	−	Steinman [22]
	63D3	−	ATCC
CD15	LeuM1	− (a rare LC+) + (after neuraminidase pretreatment)	Becton Dickinson
	VIMD5, VIM10	−	Knapp [23,44,45]
CDw65	VIM2, VIM8	−	Knapp [23,46,47]
CD68	EBM11	−	Dakopatts [23]
Macrophages/monocytes	VIM5, VIMD2	−	Knapp [23,44]
	2.4	−	Hogg [22]
WS group[c] 08	MAX.1	−	Emmrich [23]
WS group[c] 11D	MAX.2	−	Emmrich [23]
Unique	MAX.3	−	Emmrich [23]
Monocyte/platelet	OKM5	− (a rare LC+)	Ortho
FDC[d], subset of monocytes	Ki-M4	−	Behring

[a] All CD numbers higher than 45 have been assigned in the latest Leukocyte Differentiation Antigen Workshop [23].
[b] Commercial sources: ATCC, American Type Culture Collection (Rockville, MD); BD, Becton Dickinson (Mountain View, CA); Dakopatts (Roskilde, Denmark); Ortho (Raritan, NJ); Behringwerke (Marburg/Lahn, FRG); Boehringer (formerly Hybritech) (Indianapolis, IN).
[c] WS group: Fourth Leukocyte Differentiation Antigen Workshop [23].
[d] FDC: follicular dendritic cells.

Table II. The Phenotype of Resident Epidermal Langerhans Cells[a]

CD Classification Specificity	Monoclonal Antibodies	Reactivity with LC (Sheet, Suspension)	Source reference[b]
B-cell markers			
CD10	VIL-A1	−	Knapp [48]
CD19	Leu12	−	Becton Dickinson
CD20	Leu16	−	Becton Dickinson
CD22	Leu14	−	Becton Dickinson
CD24	OKB2	−	Ortho
	BA-1	−	Hybritech
	VIBC5	−	Knapp [49]
	VIBE3	−	Knapp [35]
CD40	G-28-5	− (subset of LC+)	Ledbetter [22,23]
T cell markers			
CD1a	OKT6	+	Ortho
CD1b	NUT2	−	Berti [22,23]
CD1c	M241, L161	+ (varying%)	Berti [22,23]
CD3	OKT3, Leu4	−	Ortho, BD
CD4	OKT4	−[c]	Ortho
	Leu3a + b	+ (varying%)	Becton Dickinson
CD5	Leu1	−	Becton Dickinson
CD7	Leu9	−	Becton Dickinson
CD8	OKT8, Leu2a, IOT8	−	Ortho, BD, IT
TCR alpha/beta	WT31	−	Becton Dickinson
TCR gamma/delta	delta-TCS1	−	Rittershaus [51]
	TCR-delta1	−	Rittershaus [52]
Fc-receptors			
CD64, FcRI	32.2	−	Fanger [23,53,54]
	10.1[d]	−	Hogg [22, 55, 56]
CDw32, FcRII	IV.3	+ (weak on most LC[e])	Fanger [23, 53, 54]
	CIKM5	+ (weak on most LC[e])	Pilkington [22,23]
CD16, FcRIII	Leulla	−	Becton Dickinson
	3G8	−	Steinman [58]
Complement receptors and adhesion molecules			
CD35, CR1	3F11, 5D11	−	Schulz [59]
CD21, CR2	1C8, 2G7, RFD5	−	Schulz [60]
CD11a, LFA-1, gp180/95	Ts1/22	− (<15% faintly +)[f]	Springer [22,61]
CD11b, CR3, gp155/95	Leu15	− (<15% faintly +)[f]	Becton Dickinson
	OKM1	−	Ortho
	VIM12	−	Knapp [44]
	M522	−	Schulz [62]
CD11c, gp150/95	LeuM5	− (variable% faintly +)[g]	Becton Dickinson
	3.9	− (variable% faintly +)[g]	Hogg [22,56]
	Ki-M1	−	Behring [22]
CD18	IB4	+	Wright [22,63]
	Ts1/18	+ (subset only)	Springer [22,61]
CD54, ICAM-1[h]	RR1.1	−	Rothlein [64]
	7F7	−	Stauder [23]
Activation markers			
CD25	CD25-3G10,CD25-4E3	−	Knapp [23]
Activated B and T cells	LA45	−	Knapp [66]

[a] All CD numbers higher than 45 have been assigned in the latest Leukocyte Differentiation Antigen Workshop [23].
[b] Commercial sources: BD, Becton Dickinson (Mountain View, CA); Ortho (Raritan, NJ); Behringwerke (Marburg/Lahn, FRG); Boehringer (formerly Hybritech) (Indianapolis, IN); IT, Immunotech (Marseille, France).
[c] OKT4 reactivity of resident LC has been described [50].
[d] Not definitively assigned to CD64.
[e] By flow cytometry.
[f] In sheet preparations.
[g] in some individuals, low levels of CD11c expression on resident LC have been reported [57].
[h] ICAM: intercellular adhesion molecule.

(FITC or TRITC) conjugated species-specific sheep $F(ab')_2$ anti-mouse IgG + IgM (Grub Antibodies, Scandic, Vienna, Austria). In most experiments sheets were further processed for staining of CD1a (or class II antigens) to identify LC and were then incubated in mouse Ig (50μg/ml, 30 min., 20°C) to block any residual free sheep anti-mouse immunoglobulin (Ig) binding sites and finally exposed to TRITC or FITC labeled OKT6 (the hybridoma was obtained from the American Type Culture Collection [ATCC], Rockville, MD; MoAb was purified and conjugated to the fluorochromes by standard techniques), or FITC anti-HLA-DR (Becton Dickinson, Mountain View, CA). After staining, sheets were mounted in phosphate buffered saline/glycerol/azide, dermal side up, and viewed with a Leitz Ortholux II fluorescence microscope.

Fresh EC were stained in suspension. Cultured LC were enriched (see above) and stained either in suspension or following attachment (on ice) to poly-L-lysine coated multiwell-slides (8-well multi-test slide, Flow, UK). In either case the same staining sequence was used as for sheets, except that the cells were exposed to the reagents on ice for 30 min per step, and streptavidin phycoerythrin (Amersham) was used instead of streptavidin FITC for flow cytometry analysis. After staining, the cells were either analyzed immediately or fixed in 1% paraformaldehyde in phosphate buffered saline and evaluated within 24 h by fluorescence microscopy and/or two-color flow cytometry (using a FACStar, Becton Dickinson, Mountain View, CA; $1 - 2 \times 10^4$ cells were analyzed per sample). Weak staining may be due to nonspecific binding to EC as shown by Cooper et al [11].

Therefore, if need be, dead cells were identified by staining unfixed samples with propidium iodide [11] and excluded by appropriate software gating.

Cytochemistry Staining for membrane ATP'ase, ADP'ase, and nonspecific esterase was performed by standard techniques [12-14] on sheets, fresh EC suspensions, and enriched cLC that were cytospun.

Electron Microscopy Fractions of enriched cLC were fixed for 1 h at 20°C in precooled half-strength Karnovsky's paraformaldehyde-glutaraldehyde fixative as described [6]. Cells were then washed, pelleted in 20% bovine serum albumin in phosphate buffered saline, fixed for another 30 min, and then processed for transmission electron microscopy by routine methods.

T-cell Proliferative Assays To test the stimulatory capacity of LC for unprimed T cells we used the primary mixed leukocyte reaction (MLR) [15] and the polyclonal response of periodate-modified T cells ("oxidative mitogenesis") [16]. Besides cLC, peripheral blood mononuclear cells (PBMC) were used as stimulators. PBMC were prepared as described [17]. Graded doses of irradiated (3000 rad, ^{137}Cs) stimulator cells were added to 1.5×10^5 allogeneic T cells in microwells (Flat Bottom Microtest III plate, Falcon) in medium with 10% human AB serum and 1 μg/ml indomethacin was added. For the allogeneic MLR, T cells were unmodified and [^{3}H] Thymidine uptake (4 μCi/ml) was measured at 96-114 h. For the oxidative mitogenesis assay, the T cells were periodate-modified as described [18] and [^{3}H] Thymidine uptake was measured at 48-64 h. To prepare T cells, the nonadherent fraction of PBMC was separated into low- and high-density fractions by a continuous Percoll gradient as described [17]. High-density fractions were put on nylon wool columns, and the nonadherent cells were used as T cells.

RESULTS

Resident Human Langerhans Cells

Antigenic Profile: Epidermal sheets rather than cryostat sections proved to be much more sensitive for the demonstration of antibody binding to rLC, as we already observed in the mouse [19]. To analyze rLC in suspension we stained unseparated fresh EC because enrichment of LC from fresh EC suspensions by any of the published negative selection methods inevitably leads to the loss of LC subsets.

Reactivity of MoAb to MHC Antigens and Leukocyte Common Antigen with Resident LC: Resident LC as identified by CD1a staining uni-

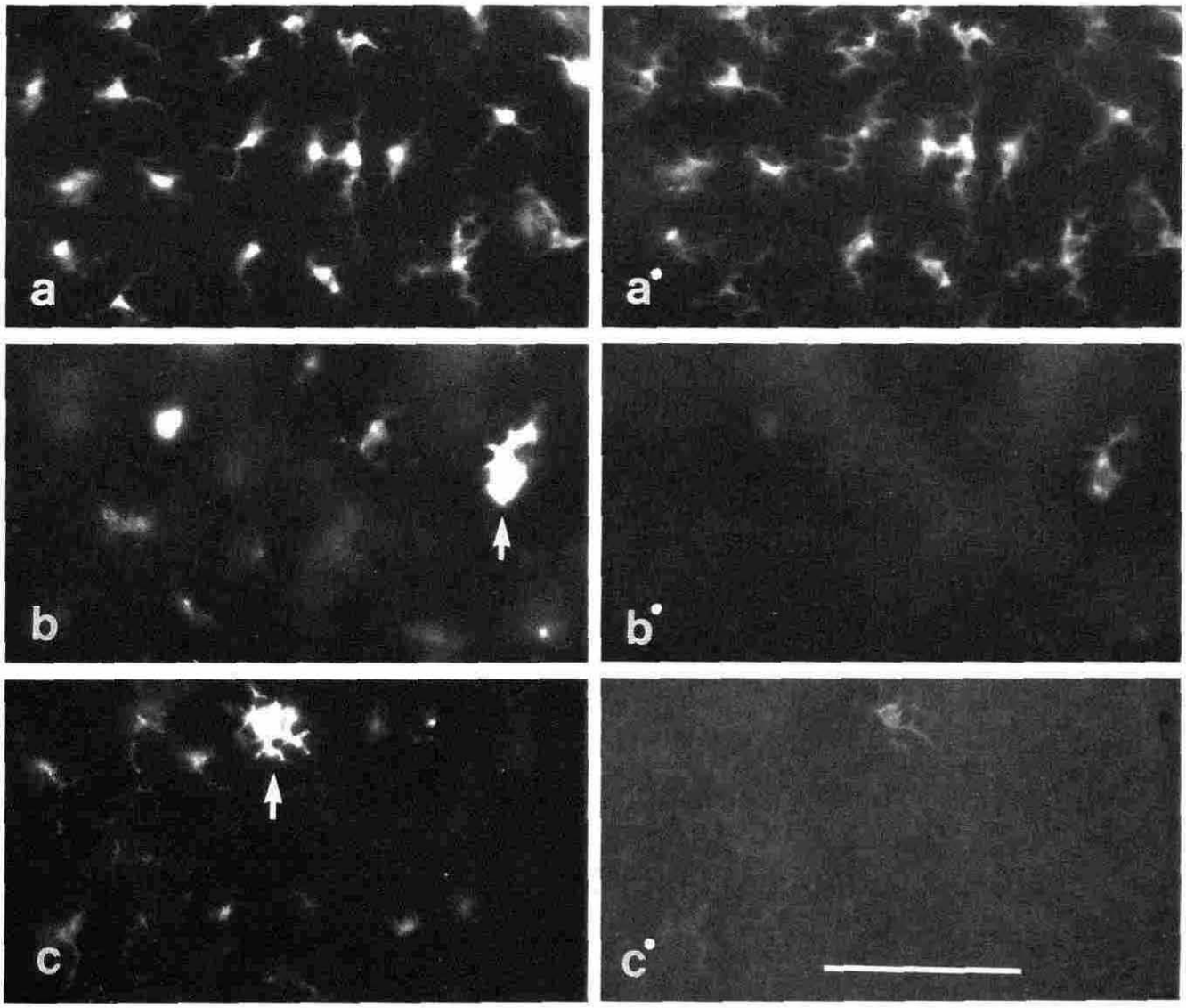

Figure 1. Double immunofluorescence of epidermal sheets. HLA-DR (*a*) and CD1 (a°) antigens overlap. Those LC that express HLA-DR more abundantly and are located in a more basal position (*arrows* in *b,c*) are stained by the MoAb RFD1 (b°) and G-28-5 (anti - CD40) (c°). *Bar:* 100 μm, Magnification: × 290

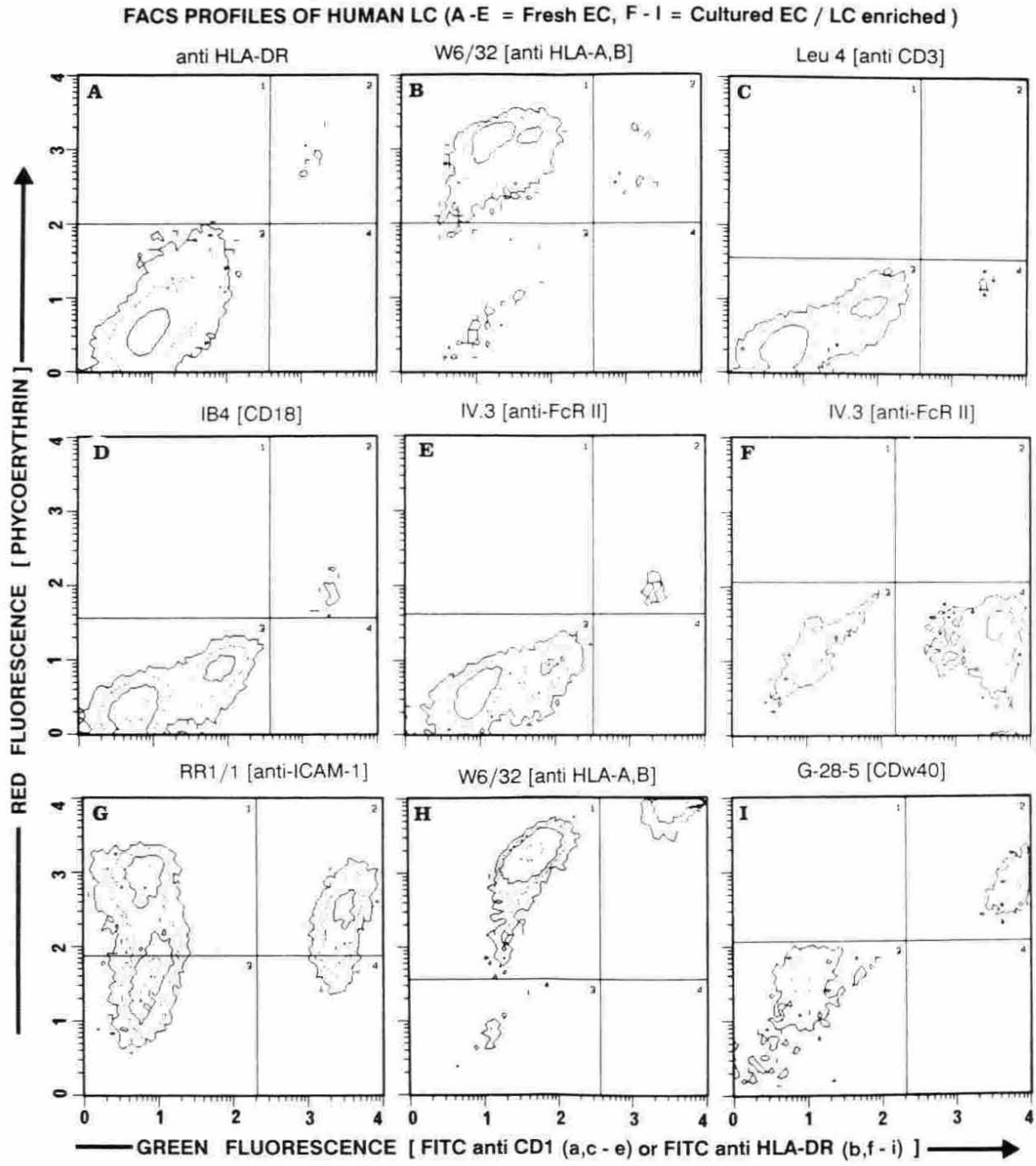

Figure 2. Examples of two-color flow cytometry analysis of freshly isolated (*A-E*) human EC and cultured EC (enriched for LC) (*F-I*). EC were double stained with the MoAb listed above each two-color plot (specificities of the MoAb are given in brackets, see also Tables I and II) and with FITC anti - CD1a mAb (*A, C-E*) or FITC anti - HLA-DR mAb (*B, F-I*) to identify LC. Green (FITC) and red (phycoerythrin) fluorescence are plotted as logarithmically increasing intensities on the *X* and *Y axis*, respectively. Staining with several isotype matched control MoAb (not shown) was used to determine background staining, which is indicated by the *horizontal* and *vertical bars*. It is demonstrated that freshly isolated LC express HLA-DR (*A*), CD1a antigens (*A*), HLA-A,B (*B*), CD18 (*D*), and FcRII (*E*), but lack CD3 (*C*). Note that FcRII is no longer detectable on cultured human LC (*F*); surface MHC class I and II antigens increased severalfold (*B,H*). CD40 is absent from the cell surface of virtually all rLC (Fig 1*c*) but is expressed on the surface of all LC following culture (*I*). Also, ICAM-1 emerged on most cultured LC (*G*). The staining of HLA-DR negative cells (keratinocytes), but not of HLA-DR positive ones (LC), was likely non-specific, as propidium iodide staining identified the former as non-viable.

formly expressed CD45 (leukocyte common antigen), class I MHC antigens, and class II MHC antigens, including antibodies specific for HLA-DP, DQ, and DR antigens as well as the invariant chain (Figs. 1 and 2; Table I). Consistently, a small fraction of LC (5%-10%) stained more brightly with anti-HLA-DR MoAb in both EC suspensions and epidermal sheets. The vast majority of these LC were located in a basal position within the epidermis. The MoAb RFD1, which recognizes a class II antigen/epitope strongly expressed by DC [20], stained a variable percentage of LC very faintly in sheets. The small subset of HLA-DR-rich LC was stained more intensely however (Fig 1*b*).

Reactivity of MoAb to Myeloid Antigens with Resident LC: None of the antibodies tested uniformly reacted with rLC. The MoAb VIM13,

Table III. Phenotype of Resident Versus Cultured Human Epidermal LC

Property	Resident LC	Cultured LC
Morphology		
cytoplasmic veils	−	+
Birbeck granules	+	↓
Cytochemistry		
Membrane ATPase	+	↓
Nonspecific esterase	+	−
Antigenic profile		
MHC class I	+	↑
MHC class II	+	↑
HLA-DQ? (mAb's RFD1, 8C4)	− (subset +)	+
CD40	− (subset +)	+
CD1a	+	↓
CDw32 (FcRII)	+	−
CD24 (VIBE3 mAb)	−	+
CD25 (IL-2 Receptor)	−	+
LA45 antigen	−	+ (very weak)
CD54 (ICAM-1)	−	+ (75%)

LeuM1, and OKM5 stained an occasional LC weakly (Table I). When EC suspensions were pretreated with neuraminidase, however, most LC bound LeuM1 MoAb like interdigitating cells in the lymph node [21].

Reactivity of MoAb to Lymphocyte Antigens with Resident LC: Resident LC lacked all the well-defined B lymphocyte antigens we tested. Staining of EC (in suspension or cytospun) with a polyclonal Ab to human Ig (FITC goat anti-human Ig, Ortho, Raritan, NJ) failed to reveal immunoglobulin on the surface or in the cytoplasm of rLC. A varying percentage (up to 40%) of LC stained faintly with MoAb G-28-5 (CD40) in epidermal sheets, whereas in suspension only an occasional LC was stained. The small subset of LC, which expressed class II antigens more abundantly and was usually located in a more basal position, was stained more clearly (Fig. 1*c*).

With two exceptions, all MoAb to T lymphocyte antigens we tested failed to react with rLC. As it is well known, all rLC strongly expressed CD1a antigens (Figs 1*a* and 2), whereas CD1b was absent from LC, and a varying percentage of rLC reacted with MoAb to CD1c [22,23]. MoAb Leu 3a+b (anti-CD4) gave variable weak labeling of rLC, whereas the other CD4 antibody used, OKT4, was consistently negative in our hands.

Reactivity of MoAb to Defined Receptors, Adhesion Molecules, and Activation Antigens with Resident LC: We were able to demonstrate the presence of Fc-receptors (FcR) type II (CDw32) on rLC by using either MoAb IV.3 or CIKM5 and flow cytometry (Fig.2E). In contrast, MoAb to FcRI (CD64) and FcRIII (CD16) did not react with rLC.

Most of the antibodies against complement receptors and adhesion molecules we tested failed to bind to rLC. Only CD18 was expressed by most rLC on their surface as shown by two-color flow cytometry (Fig 2*D*) using MoAb IB4 or MoAb Ts1/18 (Ts1/18 detected fewer rLC than IB4). MoAb to CD11a, CD11b, and CD11c stained a subset of rLC very faintly in some, but not all, patients. We did not find CD21, CD35, or CD54 (ICAM-1) in suspension or in specimens which had not been exposed to trypsin (sheets, cryosections). None of the antibodies to activation markers tested, i.e., MoAb to IL-2 receptors and to the LA45 antigen [66], reacted with rLC (Table II).

Cytochemical Studies: Resident human LC, like murine LC, expressed membrane ATP' and ADP'ase as well as nonspecific esterase (not shown).

Cultured Human Langerhans Cells

Antigenic Profile: Most antigens were expressed at constant levels during culture (Table III). A few significant changes occurred, however. Most importantly, as in the mouse, MHC class I and II antigen expression increased several fold after 2-4 d of culture (Fig 2*H*). All cLC were uniformly stained by MoAb RFD1 and 8C4, which recognize class II antigens / epitopes strongly expressed by DC [20, 42]. CD1a antigen decreased somewhat but was still readily detectable. Sometimes we also observed a slight increase in the expression of CD18 (not shown). In contrast, ICAM-1 molecules consistently emerged on the surface of about two-thirds of the cLC (Fig 2*G*). CD40 appeared on the cell surface of most LC following culture (Fig 2*I*). CDw32 antigen (FcRII), however, was no longer detectable on cLC (Fig 2*F*). Three antigens that were not found initially on rLC but appeared upon culture included interleukin-2 receptor (CD25), as described previously [24], the lymphocyte activation-associated LA45 antigen [66], and a particular CD24 epitope recognized by MoAb VIBE3 (Table III).

Cytochemistry: Membrane ATP / ADP'ase activity of human LC significantly decreased upon culture but did not disappear completely. Nonspecific esterase became negative (Table III).

Morphology: Cultured human LC, like murine cLC, had developed numerous processes including thin sheet-like ones, the so-called "veils" (Figs 3 and 4*a*). The cLC were rich in cytoplasm; the nuclei were irregular in shape with a rim of heterochromatin and small nucleoli. The cytoplasm contained many mitochondria, few free polysomes and ribosomes, and little rough endoplasmic reticulum. The Golgi region was well developed with many electron-lucent, non-coated vesicles. The cLC contained a variable number of 0.2–

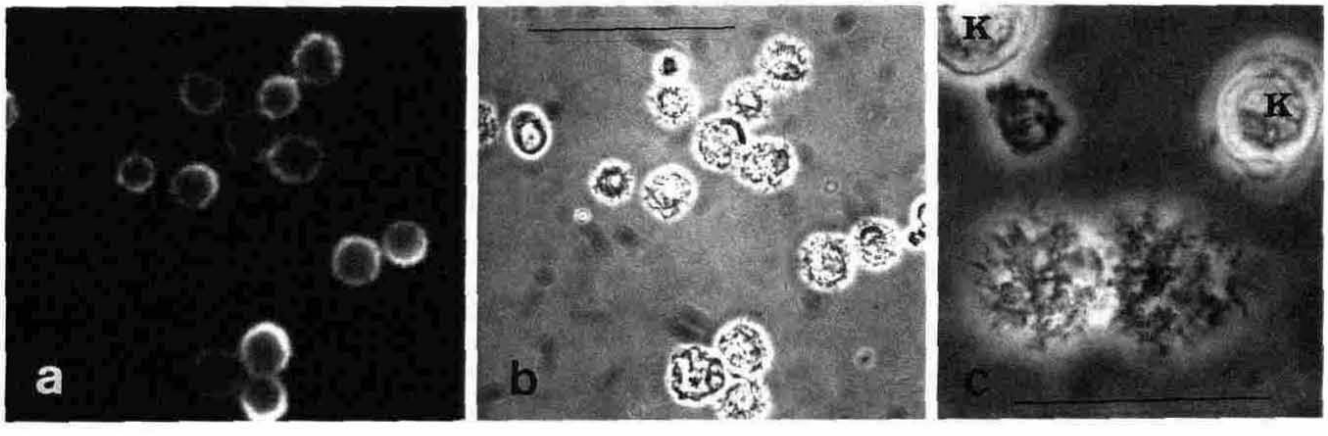

Figure 3. Light microscopic appearance of cultured (3 d) human LC (enriched fraction). Immunofluorescence staining for HLA-DQ antigens identifies LC (*a*), which display a hairy or spiny cell surface when viewed in phase contrast (*b*). When the cells are viewed in a viable state with an inverted phase contrast microscope it becomes apparent that most LC exhibit sheet-like processes or veils (*c*). *K*: contaminating keratinocytes. *a,b*: Bar, 100 μm, Magnification: × 275; *c*: *Bar*, 50 μm, Magnification: × 675.

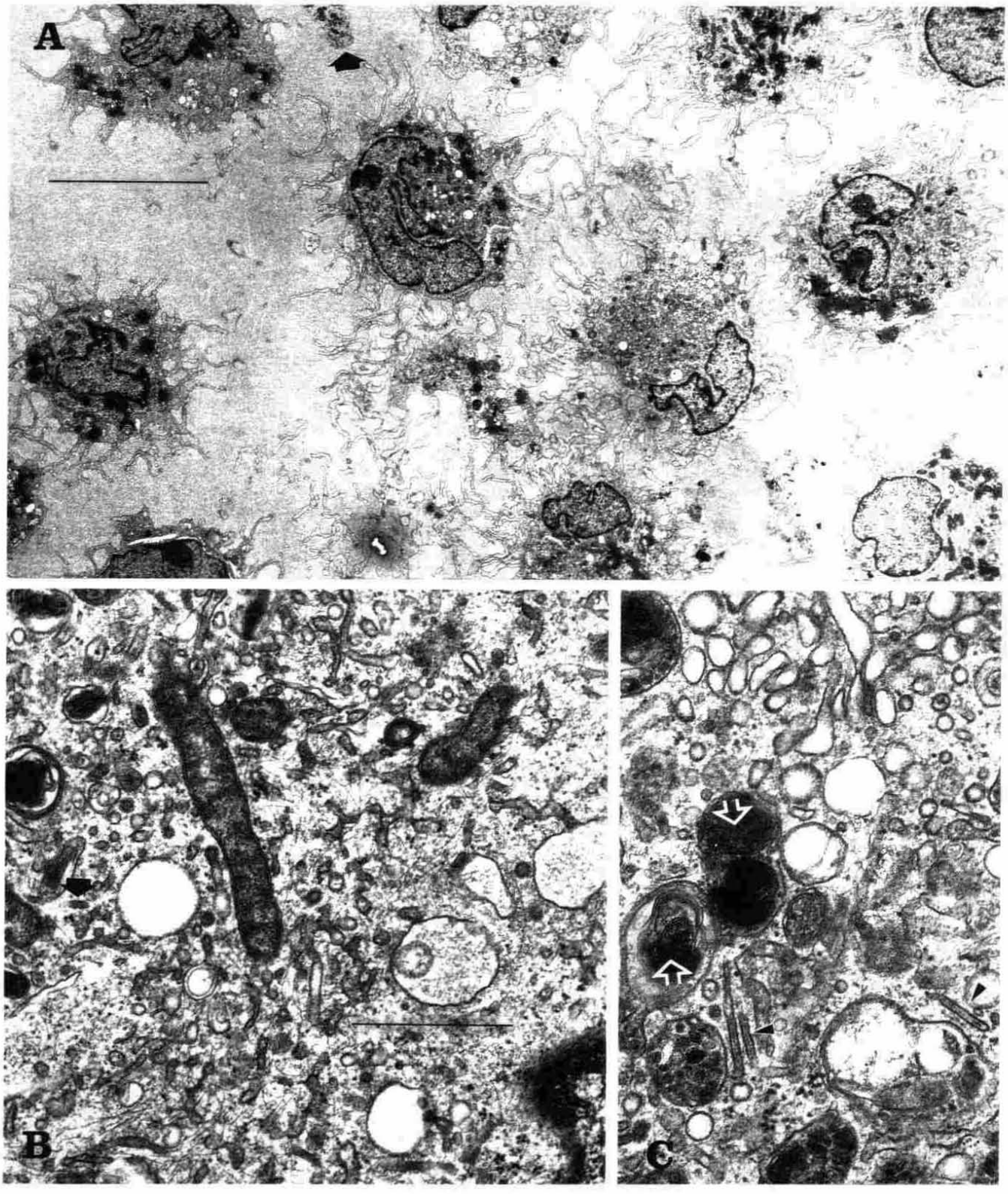

Figure 4. Ultrastructural appearance of cultured (3 d) human LC. Low-power view illustrates the thin sheet-like cytoplasmic processes extending from the surface of the LC. Note that LC are significantly enriched as indicated by the presence of only one profile of a contaminating keratinocyte (*arrow*). *Bar:* 10 μm, Magnification: × 2.755. *B,C*: High-power view of cultured human LC. The LC on the left (*B*) illustrates the typical appearance of the cytoplasm with many mitochondria and smooth-surfaced tubular profiles, an occasional LC granule (Birbeck granule) (*arrow*), and a few larger electron-lucent vacuoles. The LC on the right (*C*) is representative of the subset that contains a variable number of secondary lysosomes (phagolysosomes or autophagic vacuoles) (*open arrows*) in its cytoplasm. Note LC granules (*arrowheads*). *Bar:* 1 μm, Magnification: × 27.550.

0.6 μm vacuoles as well as many smooth tubular profiles similar in appearance to those seen in interdigitating cells in lymphoid areas and in synovial fluid DC [25] (Fig 4*b*). A variable percentage of the cLC contained Birbeck granules mainly in the area of the Golgi region, although less than in rLC. Lysosomes were scarce, but a variable percentage of the cLC contained phagolysosomes and/or autophagic vacuoles. Lipid droplets were observed in some cLC (Fig 4).

Stimulatory Activity of Cultured LC: Cultured LC were compared to PBMC for stimulatory activity in the allogeneic MLR and the polyclonal mitogenesis of periodate-modified T cells (oxidative mitogenesis assay). Cultured LC proved to be potent accessory cells for these primary T-cell responses (Fig 5). Their stimulatory activity was comparable to that of human blood DC [18].

DISCUSSION

It is an accepted, although not finally proven, view that lymphoid dendritic cells/interdigitating cells in skin-draining lymph nodes are in part derived from epidermal LC that have taken up antigen and migrated via afferent lymph to lymph nodes to stimulate T cells [1,2,5]. Differences in phenotype between epidermal LC in situ and lymphoid DC argued against such a relationship, but this no longer holds because we have shown that murine LC in vitro differentiate into cells that very closely resemble DC in phenotype and function [6]. This work now demonstrates that human LC, when put in culture, change their phenotype to one comparable with murine cLC and are potent stimulators of resting T cells.

The basic knowledge of the antigenic profile of rLC came from several studies that used one or a few MoAb for analysis [1]. An extensive study of the phenotype of resident human LC was contributed by Katz and co-workers when searching for a human analogue of the murine Thy1+ dendritic epidermal cell [11]. We have now added new information by testing additional MoAb as well as comparing resident with cultured LC. All these studies clearly show that rLC express the leukocyte common antigen (CD45) and MHC class I and II antigens but lack lineage specific antigens, including those found on myeloid cells and B and T lymphocytes. The absence of myeloid antigens from human LC is in contrast to the expression of the myeloid / macrophage antigen F4/80 on murine LC in situ [19], which is lost during culture [6]. There is evidence, however, that putative precursors of human epidermal LC bear myeloid antigens upon entry but lose them upon residency in the epidermis [29], and that such markers may be induced in disease states [30-32]. Just like murine rLC [6,19], human rLC express FcRII (CDw32) (Fig 2*E*).

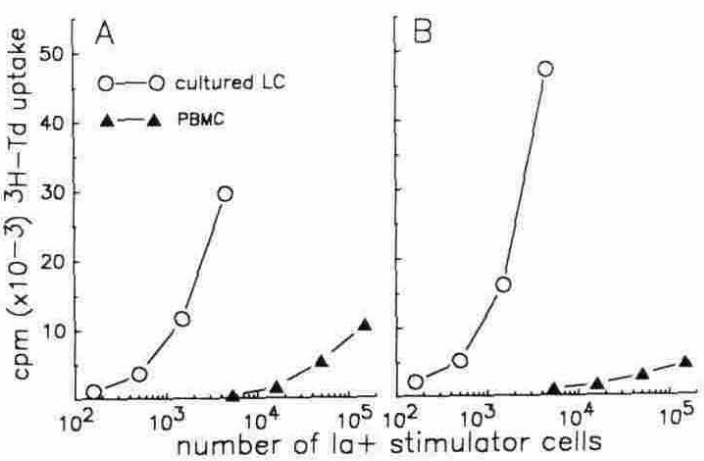

Figure 5. Cultured human LC but not PBMC are potent accessory cells in the allogeneic MLR (*A*) and the oxidative mitogenesis assay (*B*). Background (T cells only) was < 1000 cpm. For details see text.

Regarding T-cell markers, it is noteworthy that Groh et al have recently reported that resident human LC might bear CD3 and T-cell receptor gamma/delta [26,27]. We, like Cooper and co-workers [11] did not find CD3 antigen on rLC (Fig 2*C*), however, and we were also unable to demonstrate binding of two MoAb specific for the T-cell receptor gamma/delta using either sheets, fresh EC suspensions, or cLC. It might be of interest in this context that CD3 antigen is definitely absent from murine LC [28, and our unpublished results], and that in the course of the pilot experiments for this study a faint granular cell surface staining on a subset of LC was occasionally noted with some isotype control antibodies when sensitive staining procedures were used. This was possibly due to FcR-mediated binding of MoAb or passive adsorption of antigens.

During 2-4 d of bulk EC culture human LC changed (Table III). They became nonadherent and had a low buoyant density. Their membrane ATP / ADP'ase activity decreased significantly but did not disappear completely as in the mouse [6]. Nonspecific esterase, however, was no longer detectable. As has been observed in the mouse [6,33,34], surface class I and II MHC antigens increased severalfold (Fig 2*H*), whereas FcRII (CDw32) were no longer detectable (Fig 2*F*). A lymphocyte activation antigen, LA45, and (as described in Ref 24) IL-2 receptors (CD25) appeared (Table III). It is of interest that cLC were strongly and uniformly stained on their surface by the following three MoAb, which also react with DC: MoAb RFD1 (which recognizes a MHC class II antigen / epitope strongly expressed on DC) [20], MoAb VIBE3 [which recognizes a particular CD24 epitope [35] and stains blood DC (our unpublished observations)], and MoAb G-28-5 (anti-CD40) (which stains interdigitating cells in the lymph node [22] as well as isolated tonsil dendritic cells [36]). The finding that a small subset of rLC, which is located in a more basal position and strongly expresses class II molecules, was stained with the MoAb RFD1 and G-28-5 might indicate that the respective LC are in a process of maturation.

Cultured human LC proved potent stimulators of resting T cells as tested in the allogeneic MLR and the oxidative mitogenesis assay. As the capacity to bind to T cells by an antigen-independent mechanism seems critical for the unique capacity of DC and cLC [2,5,7] to stimulate resting T cells, one might have expected that human LC would significantly upregulate any of the defined adhesion molecules. Indeed, ICAM-1 appeared on cLC. This molecule is, however, most likely not responsible for antigen-independent clustering of LC with T cells, as this phenomenon in the mouse cannot be blocked by anti-LFA-1MoAb [65]. This may indicate that novel membrane molecules, yet to be identified, are involved. Overall, cultured human LC resemble lymphoid dendritic cells [17,25,36] in phenotype and function. This supports the view that lymphoid DC in skin-draining lymph nodes might indeed be derived from skin LC, even though the phenotype of cLC and lymph node DC in situ is not identical. It is conceivable, however, that the final phenotype is not acquired until LC have settled in the lymph node. In concordance with this assumption is the observation that, apart from phenotypic differences between members of the DC family, even in a given subpopulation of DC the antigenic profile is influenced by the microenvironment (e.g., LC in the tonsil epithelium [22], like interdigitating cells in the lymph node, strongly express CD11c, while LC in the skin and tonsil DC [36] virtually lack this antigen).

The morphology of cultured human LC was also consistent with their being in vitro equivalents of DC [25,36,38]. As compared to murine LC two differences were observed. First, Birbeck granules decreased significantly in number but did not completely disappear from most human LC upon culture. In this context it is interesting that the subpopulation of resident human LC, which, like cLC, strongly expresses class II antigens on a given ultrathin section, displays only few or no Birbeck granules [39]. Second, in contrast to what we observed in the mouse, a considerable portion of cultured human LC contained secondary lysosomes. These differences presumably reflect the fact that resident human LC possess more Birbeck granules as well as lysosomes from the very beginning. Therefore, even if a comparable percentual reduction of both organelles would occur during culture, human LC would less likely score

negative on a given ultrathin section. One has also to consider that both organelles are dynamic. In the mouse it has recently been observed that following hapten application the number of Birbeck granules in situ increases rapidly [40]. Also a portion of the murine DC isolated from the lymph node 24 h following hapten application exhibit Birbeck granules as well as a high lysosomal content [41] unlike what would be expected from the published ultrastructure of DC [25]. In addition, we observed recently that freshly isolated LC in vitro quite surprisingly are capable of ingesting even large particles under appropriate conditions (unpublished observations).

We are now investigating whether human LC significantly increase their stimulatory function on a per cell basis and functionally mature in vitro in response to certain cytokines as in the mouse [6,8,9]. These studies were not feasible until we recently learned how to enrich human LC from freshly prepared EC suspensions.

We thank the many colleagues who provided us with valuable MoAb (Tables I to III), Dr. J. Varga and Dr. E. Kämpgen for carefully reading the manuscript. We are very grateful to Dr. H. Anderl and his co-workers at the Department of Plastic Surgery, University of Innsbruck, who provided us with skin specimens and thus made this study possible.

REFERENCES

1. Wolff K, Stingl G: The Langerhans Cell. J Invest Dermatol 80:17s-21s, 1983
2. Steinman RM, Inaba K, Schuler G, Witmer M:Stimulation of the immune response: contributions of dendritic cells. In: Steinman RM, North RJ (eds.). Mechanisms of Host Resistance to Infectious Agents, Tumors, and Allografts. Rockefeller University Press, New York, 1986, pp 71-97
3. Steinman RM, Inaba K: Antigen presentation by dendritic cells. In: Pernis B, Silverstein SC, Vogel HJ (eds.). Processing and Presentation of Antigens. Academic Press, New York 1988, pp 167-177
4. Cohn ZA: The macrophage - versatile element of inflammation. The Harvey Lecture Series 77:63-80, 1983
5. Schuler G, Inaba K, Witmer M, Steinman R: The immunologic properties of epidermal Langerhans cells. In: Pernis B, Silverstein S, Vogel HJ (eds.). Processing and Presentation of Antigens. Academic Press, New York, 1988, pp 179-184
6. Schuler G, Steinman RM: Murine epidermal Langerhans cells mature into potent immunostimulatory dendritic cells in vitro. J Exp Med 161:526-546, 1985
7. Inaba K, Schuler G, Witmer MD, Valinsky J, Atassi B, Steinman RM: Immunologic properties of purified epidermal Langerhans cells. Distinct requirements for stimulation of unprimed and sensitized T lymphocytes. J Exp Med 164:605-613, 1986
8. Witmer-Pack MD, Olivier W, Valinsky J, Schuler G, Steinman RM: Granulocyte/macrophage colony-stimulating factor is essential for the viability and function of cultured murine epidermal Langerhans cells. J Exp Med 166:1484-1498, 1987
9. Heufler C, Koch F, Schuler G: Granulocyte-macrophage colony-stimulating factor and interleukin-1 mediate the maturation of murine epidermal Langerhans cells into potent immunostimulatory dendritic cells. J Exp Med 167:700-705, 1988
10. Juhlin L, Shelley WB: New staining techniques for the Langerhans cell. Acta Derm Venereol 57:289-296, 1977
11. Cooper KD, Breathnach SM, Caughman SW, Palini AG, Waxdal MJ, Katz SI: Fluorescence microscopic and flow cytometric analysis of bone marrow-derived cells in human epidermis: a search for the human analogue of the murine dendritic Thy-1+ epidermal cell. J Invest Dermatol 85:546-552, 1985
12. Wolff K, Winkelmann RK: Quantitative studies on the Langerhans cell population of guinea pig epidermis. J Invest Dermatol 48:504-513, 1967
13. Chaker MB, Tharp MD, Bergstresser PR: Rodent epidermal Langerhans cells demonstrate greater histochemical specificity for ADP than for ATP and AMP. J Invest Dermatol 82:496-500, 1984
14. Li CY, Lam KW, Lam LT: Esterases in human leukocytes. J Histochem Cytochem 21:1-12, 1973
15. Steinman RM, Inaba K: Stimulation of the primary mixed leukocyte reaction. CRC Crit Rev Immunol 5:331-348, 1985
16. Klinkert WEF, LaBadie JH, O'Brien JP, Beyer CF, Bowers WE: Rat dendritic cells function as accessory cells and control the production of a soluble factor required for mitogenic responses of T cells. Proc Natl Acad Sci USA 77:5414-5418, 1980
17. Young JW, Steinman RM: Accessory cell requirements for the mixed leukocyte reaction and polyclonal mitogens, as studied with a new technique for enriching blood dendritic cells. Cell Immunol 111:167-182, 1988
18. Van Voorhis WC, Valinsky J, Hoffman E, Luban J, Hair LS, Steinman RM: Relative efficacy of human monocytes and dendritic cells as accessory cells for T cell replication. J Exp Med 158:174-191, 1983
19. Romani N, Stingl G, Tschachler E, Witmer MD, Steinman RM, Shevach EM, Schuler G: The Thy-1-bearing cell of murine epidermis. A distinctive leukocyte perhaps related to natural killer cells. J Exp Med 161:1368-1383, 1985
20. Poulter LW, Campbell DA, Munro C, Janossy G: Discrimination of human macrophages and dendritic cells by means of monoclonal antibodies. Scand J Immunol 24:351-357, 1986
21. Hsu S-M, Yang K, Jaffe ES: Phenotypic expression of Hodgkin's and Reed-Sternberg cells in Hodgkin's disease. Amer J Pathol 118:209-217, 1985
22. McMichael AJ (ed.): Leucocyte Typing III: White Cell Differentiation Antigens. Oxford University Press, Oxford, 1988, p 1050
23. Knapp W (ed.): Leukocyte Typing IV: White Cell Differentiation Antigens. Oxford University Press, Oxford, in press.
24. Groh V, Tschachler E, Romani N, Wolff K, Stingl G: Tac expression by cultured human Langerhans cells (abstr). J Invest Dermatol 87:142, 1986
25. Steinman RM, Schuler G, Romani N, Kaplan G:Dendritic Cells. In: Zucker-Franklin D, Greaves MF, Grossi CE, Marmont AM (eds.). Atlas of Blood Cells - Function and Pathology, Vol. 1. E.E edi-ermes and Lea and Febiger, Philadelphia, 1988, pp 361-377
26. Groh V, Yokozeki H, Yokoyama WM, Foster CA, Koning F, Brenner MB, Stingl G: T cell receptors gamma/delta on dendritic cells of the human epidermis (abstr). J Invest Dermatol 90:565, 1988
27. Groh V, Yokozeki H, Yokoyama WM, Koning F, Foster CA, Stingl G: The human epidermis harbors CD1+/CD3+/TCR gamma+/HLA-DR+ dendritic cells (abstr). J Invest Dermatol 90:241, 1988
28. Stingl G, Koning F, Yamada H, Yokoyama WM, Tschachler E, Bluestone JA, Steiner G, Samelson LE, Lew AM, Coligan JE, Shevach EM: Thy-1+ dendritic epidermal cells express T3 antigen and the T-cell receptor gamma chain. Proc Natl Acad Sci USA 84:4586-4591, 1987
29. Murphy GF, Messadi D, Fonferko E, Hancock WW: Phenotypic transformation of macrophages to Langerhans cells in the skin. Amer J Pathol 123:401-406, 1986
30. Wood GS, Turner RR, Shiurba RA, Eng L, Warnke RA: Human dendritic cells and macrophages - In situ immunophenotypic definition of subsets that exhibit specific morphologic and microenvironmental characteristics. Amer J Pathol 119:73-82, 1985
31. Groh V, Gadner H, Radaszkiewicz T, Rappersberger K, Konrad K, Wolff K, Stingl G: The phenotypic spectrum of histiocytosis X cells. J Invest Dermatol 90:441-447, 1988
32. Franklin WA, Mason DY, Pulford K, Falini B, Bliss E, Gatter KC, Stein H, Clarke LC, McGee JO'D: Immunohistological analysis of human mononuclear phagocytes and dendritic cells by using monoclonal antibodies. Lab Invest 54:322-335, 1986
33. Witmer-Pack MD, Valinsky J, Olivier W, Steinman RM: Quantitation of surface antigens on cultured murine epidermal Langerhans cells: rapid and selective increase in the level of surface MHC products. J Invest Dermatol 90:387-394, 1988
34. Shimada S, Caughman SW, Sharrow SO, Stephany D, Katz SI: Enhanced antigen-presenting capacity of cultured Langerhans' cells is associated with markedly increased expression of Ia antigen. J Immunol 139:2551-2556, 1987
35. Sugita K, Majdic O, Stockinger H, Holter W, Köller U, Peschel Ch, Knapp W: Use of a cocktail of monoclonal antibodies and human complement in selective killing of acute lymphocytic leukemia cells. Int J Cancer 37:351-357, 1986
36. Hart DNJ, McKenzie JL: Isolation and characterization of human tonsil dendritic cells. J Exp Med 168:157-170, 1988

37. Tew JG, Thorbecke GJ, Steinman RM: Dendritic cells in the immune response: Characteristics and recommended nomenclature (A report from the Reticulo-endothelial Society Committee on Nomenclature). J Reticuloendothel Soc 31:371-380, 1982

38. Hoefsmit ECM, Duijvestijn AM, Kamperdijk EWA: Relation between Langerhans cells, veiled cells, and interdigitating cells. Immunobiology 161:255-265, 1982

39. Dezutter-Dambuyant C, Cordier G, Schmitt D, Faure M, Laquoi C, Thivolet J: Quantitative evaluation of two distinct cell populations expressing HLA-DR antigens in normal human epidermis. Br J Dermatol 111:1-11, 1984

40. Kolde G, Knop J: Different cellular reaction patterns of epidermal Langerhans cells after application of contact sensitizing, toxic, and tolerogenic compounds. A comparative ultrastructural and morphometric time-course analysis. J Invest Dermatol 89:19-23, 1987

41. Macatonia SE, Knight SC, Edwards AJ, Griffiths S, Fryer P: Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. J Exp Med 166:1654-1667, 1987

42. Zvaifler NJ, Steinman RM, Kaplan G, Lau LL, Rivelis M: Identification of immunostimulatory dendritic cells in the synovial effusions of patients with rheumatoid arthritis. J Clin Invest 76:789-800, 1985

43. Quaranta V, Majdic O, Stingl G, Liszka H, Hönigsmann H, Knapp W: A human Ia cytoplasmic determinant located on multiple forms of invariant chain (γ, γ_2, γ_3). J Immunol 132:1900-1905, 1984

44. Knapp W, Majdic O, Stockinger H, Bettelheim P, Liszka K, Köller U, Peschel Ch: Monoclonal antibodies to human myelomonocyte differentiation antigens in the diagnosis of acute myeloid leukemia. Med Oncol Tumor Pharmacother 4:257-262, 1984

45. Majdic O, Liszka K, Lutz D, Knapp W: Myeloid differentiation antigen defined by a monoclonal antibody. Blood 58:1127-1133, 1981

46. Macher BA, Buehler J, Scudder P, Knapp W, Feizi T: A novel carbohydrate differentiation antigen on fucogangliosides of human myeloid cells recognized by monoclonal antibody VIM-2. J Biol Chem 263:10186-10191, 1988

47. Majdic O, Bettelheim P, Stockinger H, Aberer W, Liszka K, Lutz D, Knapp W: M2, a novel myelomonocytic cell surface antigen and its distribution on leukemic cells. Int J Cancer 33:617-623, 1984

48. Knapp W, Majdic O, Bettelheim P, Liszka K: VIL-A1, a monoclonal antibody reactive with acute lymphatic leukemia cells. Leuk Res 6:137-147, 1982

49. Knapp W, Majdic O, Bettelheim P, Liszka K, Aberer W, Stingl G: Typing of leukemic cells with monoclonal antibodies. Ann N Y Acad Sci 420:251-260, 1983

50. Groh V, Tani M, Harrer A, Wolff K, Stingl G: Leu-3/T4 expression on epidermal Langerhans cells in normal and diseased skin. J Invest Dermatol 86:115-120, 1986

51. Wu Y-J, Tian W-T, Snider RM, Rittershaus C, Rogers P, LaManna L, Ip SH: Signal transduction of gamma/delta T cell antigen receptor with a novel mitogenic anti-delta antibody. J Immunol 141:1476-1479, 1988

52. Band H, Hochstenbach F, McLean J, Hata S, Krangel MS, Brenner MB: Immunochemical proof that a novel rearranging gene encodes the T cell receptor delta subunit. Science 238:682-684, 1987

53. Anderson CL, Guyre PM, Whitin JC, Ryan DH, Looney RJ, Fanger MW: Monoclonal antibodies to Fc receptors for IgG on human mononuclear phagocytes. J Biol Chem 261:12856-12864, 1986

54. Hogg N: The structure and function of Fc receptors. Immunol Today 9:185-187, 1988

55. Anderson CL, Looney RL: Human leukocyte Fc receptors. Immunol Today 7:264-265, 1986

56. Dougherty GJ, Selvendran Y, Murdoch S, Palmer DG, Hogg N: The human mononuclear phagocyte high-affinity Fc receptor, FcRI, defined by a monoclonal antibody, 10.1.Eur J Immunol 17:1453-1459, 1987

57. Staquet MJ Dezutter-Dambuyant C, Schmitt D, Thivolet J:Adhesion-mediating molecules of human Langerhans cells (abstr). J Invest Dermatol 92:522, 1989

58. Fleit HB, Wright SD, Unkeless JC: Human neutrophil Fc gamma receptor distribution and structure. Proc Natl Acad Sci USA 79:3275-3279, 1982

59. Schulz TF, Helmberg A, Hosp M, Petzer A, Dierich MP: Enzyme-linked immunoassay to monitor the purification of, and to screen for monoclonal antibodies against C3b receptor. Complement 2:219-229, 1985

60. Petzer A, Schulz TF, Stauder R, Eigentler A, Myones BL, Dierich MP: Structural and functional analysis of CR2/EBV receptor by means of monoclonal antibodies and limited tryptic digestion. Immunology 63:47-53, 1988

61. Sanchez-Madrid F, Krensky AM, Ware CF, Robbins E, Strominger JL, Burakoff SJ, Springer TA: Three distinct antigens associated with human T lymphocyte-mediated cytolysis: LFA-1, LFA-2 and LFA-3. Proc Natl Acad Sci USA 79:7489-7493, 1982

62. Schulz TF, Scharfenberger H, Lambris JD, Rieber P, Riethmüller G, Dierich MP: Antigenic relationship between the alpha-chain of C3, a leucocyte-surface antigen involved in the activation of phagocytic cells, and a 50,000 MW B-cell antigen. Immunology 54:791-800, 1985

63. Wright SD, Rao PE, Van Voorhis WC, Craigmyle LS, Iida K, Talle MA, Westberg EF, Goldstein G, Silverstein SC: Identification of the C3bi receptor of human monocytes and macrophages by using monoclonal antibodies. Proc Natl Acad Sci USA 80:5699-5703, 1983

64. Rothlein R, Dustin ML, Marlin SD, Springer TA: A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. J Immunol 137:1270-1274, 1986

65. Inaba K, Steinman RM: Monoclonal antibodies to LFA-1 and to CD4 inhibit the mixed leukocyte reaction after the antigen-dependent clustering of dendritic cells and T lymphocytes. J Exp Med 165:1403-1412, 1987

66. Majdic O, Schnabl E, Stockinger H, Gadd S, Maurer D, Radaszkiewics T, Gaugitsch H, Lindley I, Hofer E, Knapp W: LA45, an activation-induced human lymphocyte antigen with strong homology to MHC class I molecules. In: Knapp W (ed.). Leukocyte Typing IV: White Cell Differentiation Antigens. Oxford University Press, Oxford, in press