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Cultured human Langerhans' cells are superior to fresh cells at presenting native HIV-1 protein antigens to specific CD4$^+$ T-cell lines

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SUMMARY

Cultured Langerhans' cells (CLC) exhibit enhanced antigen-presenting function compared to freshly isolated LC (FLC), but they are commonly believed to be inefficient at processing intact proteins. In this study, FLC and CLC from normal, human immunodeficiency virus (HIV) seronegative volunteers were compared for their ability to present the HIV-1 envelope glycoprotein gp120 or reverse transcriptase (p66) antigens to autologous, specific CD4$^+$ T cell lines. Epidermal cell suspensions enriched for LC were prepared from suction blister roofs. FLC stimulated T cells at lower antigen concentrations compared to unfractionated peripheral blood mononuclear cells (PBMC). CLC were more potent on a per cell basis than FLC, PBMC or adherent monocytes at presenting native gp120, native p66 or immunogenic peptides. CLC were also more efficient than FLC or PBMC in terms of the amount of antigen required for T-cell activation. Chloroquine and leupeptin inhibited presentation of intact p66, but not of an immunodominant peptide, by FLC or CLC, thus indicating that both cells utilize antigen-processing mechanisms that are based on intracellular acidification and protease activity. Incubation of CLC with monoclonal antibodies against HLA-DR, CD11b, CD18, CD50, CD54, CD58 or CD80, but not anti-major histocompatibility complex class I (MHC-I), inhibited antigen-specific T-cell proliferation to varying degrees. We conclude that human CLC retain the ability to process and present protein antigens potently to CD4$^+$ T cells. Thus, CLC have the capacity to participate actively in the generation and maintenance of T-helper cell immunity to viral antigens during HIV-1 infection.

INTRODUCTION

Dendritic cells (DC) are a population of widely distributed leucocytes with potent antigen-presenting function that play a dominant role in T-cell priming both *in vitro* and *in vivo*.[1–4] In keeping with data obtained from mice, work from our laboratory has shown that human peripheral blood DC are more efficient than B cells or adherent monocytes in presenting the human immunodeficiency virus-1 (HIV-1) envelope glycoprotein gp120 to specific CD4$^+$ T-cell lines. Moreover, DC were required for the *in vitro* generation of gp120-specific T-cell lines from unprimed volunteers.[5] Langerhans' cells (LC), i.e. the DC of epidermis, are the critical antigen-presenting cells (APC) for the induction of T-cell immunity against antigens that are present in or penetrate through stratified squamous epithelia.[6,7] LC from HIV-infected subjects contain HIV-1 DNA and mRNA,[8,9] and can release HIV virions,[10] indicating that LC are productively infected with HIV-1 *in vivo*. This raises the possibility that HIV-1 antigens are processed by LC as exogenous proteins and gain access to the major histocompatibility complex (MHC) class II pathway for presentation to CD4$^+$ T-helper lymphocytes.

LC undergo profound changes in both immunophenotype and functional properties during short-term culture. Compared to freshly isolated LC (FLC), cultured LC (CLC) exhibit increased expression of membrane MHC and costimulatory molecules. In parallel, CLC become more potent stimulators of allogeneic T cells and they acquire the ability to prime hapten-specific naive T cells.[11–14] Although several works have shown that both CLC and cultured DC maintain substantial processing capacity,[2,15,16] other detailed studies have documented that both cell types become rather inefficient at processing and presenting native protein antigens.[17,18] Because of difficulties in obtaining autologous LC, few studies have compared human FLC with CLC for the capacity to process and present intact protein antigens. Cohen & Katz[19] have shown that human CLC maintain the capacity to process recall protein antigen but are

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Abbreviations: APC, antigen-presenting cells; CLC, cultured LC; DC, dendritic cells; FLC, freshly isolated LC; LC, Langerhans' cells.

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not superior to FLC in presenting them to peripheral blood lymphocytes,[19] and Teunissen *et al.*[11] have confirmed that CLC can effectively present *Candida albicans* antigen.

In this study, we examined the ability of FLC and CLC to present native HIV-1 protein antigens and immunogenic peptides to specific $CD4^+$ T-cell lines generated from HIV seronegative individuals.

MATERIALS AND METHODS

Culture media

RPMI-1640 supplemented with heat-inactivated 10% fetal calf serum, 25 mM HEPES, 0·1 mM non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate (all Biochrom KG, Berlin, Germany), 50 μM 2-mercaptoethanol (Merck, Darmstadt, Germany), 100 U/ml penicillin and 100 μg/ml streptomycin (Biochrom) (complete medium) was used to culture epidermal cells and peripheral blood mononuclear cells (PBMC). RPMI-1640 containing 2 mM L-glutamine, 50 μM 2-mercaptoethanol and 5% autologous plasma was used to generate T-cell lines and in T-cell proliferation assays.

Antigens

Glycoprotein 120 and p66 were of the HIV BRU strain produced in CHO cells and were purchased from Microgenesys (Meriden, CT). The gp120 was heat denatured to abolish CD4 binding capacity.[20] A panel of 15-mer peptides with a six amino acid overlap and encompassing the entire p66 molecule was synthesized, as described elsewhere.[21] The numbering is derived from the Los Alamos Database 1990.[22] Peptides 29 (LAENREILKEPVHGV, 302–316, C4), 30 (EPVHGVYY-DPSKDLI, 311–325, D4) and 43 (LEKEPIVGAETFYD, 428–442, E6) were shown to be immunodominant *in vitro* for donor 1.

Monoclonal antibodies (mAb)

The following mouse mAb to human antigens were used at saturating concentrations in the antigen-presentation assay: anti-MHC class I (W6/32, IgG2a, 10 μg/ml; Dako, Copenhagen, Denmark); anti-HLA-DR (L243, IgG1, 10 μg/ml; Becton Dickinson, San Jose, CA); anti-CD54 [intracellular adhesion molecule-1 (ICAM-1)] (84H10, IgG1, 10 μg/ml; Immunotech, Marseille, France); anti-CD50 (ICAM-3) (BRIC79, IgG2a, 3 μg/ml; kindly provided by Dr F. Spring of the International Blood Group References Laboratory, Bristol, UK), anti-CD58 (LFA-3) (AICD58, IgG2a, 10 μg/ml; Immunotech); anti-CD18 (BL5, IgG1, 10 μg/ml; Immunotech); and anti-CD11b (Bear1, IgG1, 10 μg/ml; Immunotech); anti-CD80 (B7–1) (L304, IgG1, 10 μg/ml; Becton Dickinson; and B7-24, IgG2a, 10 μg/ml, kindly provided by Dr M. de Boer of Innogenetics, Ghent, Belgium).

Generation of T-cell lines

T-cell lines specific for gp120 or p66 were generated from two healthy HIV-1 seronegative individuals, as described in detail elsewhere.[23] Briefly, 2×10^6 PBMC were cultured with an equal number of antigen-pulsed (5 μg/ml; 4 hr) and γ-irradiated autologous PBMC as APC for 5 days. Thirty U/ml of human recombinant interleukin-2 (hrIL-2; kindly provided by Hoffman-La Roche, Basel, Switzerland) was then added, and after 10 days 2×10^5 blasts were stimulated with antigen-pulsed, irradiated autologous APC. Human recombinant-IL-2 was added 3 days later, and this cycle was repeated at least four times to establish antigen-responsive T-cell lines. In the APC assays reported below, T cells were used 15 days after restimulation.

Preparation of APC

Epidermal cell suspensions were prepared from suction blisters raised over flexor forearm skin. Briefly, blister tops (epidermal sheets) were trypsinized (0·25% for 60 min at 37°), disaggregated by repeated aspiration and expulsion through a sterile pipette, and then added to 0·05% DNAse (ICN Biomedicals, Costa Mesa, CA). Epidermal cell suspensions were enriched for LC by density gradient centrifugation using Ficoll–Paque (density 1·077 g/ml; Pharmacia, Uppsala, Sweden) and used either freshly isolated or after 48 hr culture in complete medium. In some experiments, 200 ng/ml human recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF); a gift of Dr G. Corbetta, Sandoz, Italy) was added to the culture medium to improve LC survival. Both freshly procured and cultured epidermal cell preparations contained 5–10% LC, as assessed by flow cytometry or fluorescence microscopy with anti-CD1a (OKT6, IgG1, 10 μg/ml; Ortho Diagnostic System, Raritan, NJ) (data not shown). The actual number of LC was determined in each experiment. LC-enriched epidermal cells were not irradiated before functional studies, as they do not proliferate significantly. PBMC were prepared from heparinized peripheral venous blood by density gradient centrifugation over Ficoll–Paque (1·077; Pharmacia). In some experiments, PBMC were cultured in complete medium and, after 2 hr, non-adherent cells were removed by pipetting and the adherent monocytes detached with EDTA and by scraping with a rubber policeman. Flow cytometry analysis of PBMC and adherent monocytes showed the presence of 10% and > 90% bright $CD14^+$ cells, respectively (data not shown). B-lymphoblastoid cell lines (EBV-B cells) were established from PBMC by previously published methods.[24]

T-cell proliferation assay

T-cell proliferation was evaluated by culturing 2×10^4 specific T cells in U-bottomed 96-well plates (Nunc, Roskilde, Denmark) with APC (γ-irradiated unfractionated PBMC, γ-irradiated adherent monocytes, γ-irradiated EBV-B cells or LC-enriched epidermal cells) incubated with antigen at the concentrations reported below. Duplicate cultures were pulsed with 0·5 μCi [^{3}H]thymidine (specific activity 5 Ci/mmol; Amersham Int., Amersham, UK) after 72 hr and harvested 8 hr later. This was found to be the optimal time for antigen-specific responses. Radioactivity was measured in a Tri-Carb 1600TR counter (Canberra-Packard, Meriden, CT). Inhibitory effects of various mAb to surface CLC molecules were assayed by incubating 48-hr cultured LC-enriched epidermal cells with dialysed mAb in culture medium for 45 min at 4°. Thereafter, cells were placed at 37° and antigen and T cells added. In some experiments, APC were preincubated with 100 μM chloroquine or 100 μM leupeptin (Sigma Chemical Co., St Louis, MO) for 15 min, pulsed with antigen for 3 hr in the presence of the inhibitors, and then washed twice before adding T cells.

RESULTS

Helper T-cell lines specific for HIV-1 antigens

T-cell lines specific for gp120 or p66 were generated from HIV-1 uninfected and unprimed volunteers. Following four cycles of antigen stimulation and IL-2 expansion, these T cells proliferated specifically to either gp120 or p66. The lines exhibited an $\alpha\beta$ T-cell receptor $(TCR)^+$ (WT31), $CD3^+$, $CD4^+$, $CD8^-$ helper phenotype, did not express the high-affinity IL-2 receptor (p75) at the time of the antigen presentation assay, did not respond to unrelated antigen (e.g. tetanus toxoid, purified protein derivative) and required autologous or MHC-compatible APC (data not shown). The p66-specific T-cell line derived from donor 1 was screened with a large panel of peptides encompassing the entire p66 molecule and was shown to respond significantly only to peptides 29, 30 and 43, which therefore were selected for subsequent experiments. These T cells, however, did not proliferate in response to heat-inactivated virus using either PBMC or FLC as APC (data not shown).[25]

Presentation of HIV-1 antigens by LC

In the first experiments, we evaluated the relative efficiency of FLC and peripheral blood monocytes in presenting intact proteins or immunogenic peptides. FLC were more potent than monocytes at presenting p66 (Fig. 1a) or peptide 29 (Fig. 1b). Half-maximal T-cell proliferation required 10 times less antigen when presented by FLC compared to PBMC. Furthermore, FLC stimulated T cells better at each concentration of native protein or immunogenic peptide. Figure 1c shows that on a per cell basis FLC were also more efficient than peripheral blood monocytes at presenting peptide 43. This finding is in agreement with other studies demonstrating that epidermal LC are superior to PBMC in presenting alloantigens or haptens to T cells.[26,27] However, some variability was observed in the relative effciency of FLC obtained from the two donors. FLC from donor 1 were more capable than those obtained from donor 2 (Fig. 1a versus Fig. 2a).

In the following experiments, we examined the antigen presenting function of CLC. As evident in Fig. 2, 48-hr CLC exhibited a markedly augmented capacity to present two different intact proteins (gp120 and p66) or peptide 29, and they were more efficient than FLC, PBMC or adherent monocytes. As few as 250 CLC (APC:T cells ratio, 1:80) could trigger a significant T-cell response, and, on a per cell basis, they were 2–8-fold and 10–20-fold more effective than FLC or PBMC, respectively, in stimulating specific T cells. CLC were also more effective in terms of the amount of antigen required for T-cell activation. CLC derived from either donors showed comparable increased APC function. Addition of GM-CSF to the culture medium did not significantly change the APC potency of CLC (data not shown).

FLC and CLC possess an antigen-processing pathway that is sensitive to chloroquine or leupeptin

Inasmuch as previous experiments had shown that CLC can efficiently present soluble proteins, we wanted to confirm that these cells had utilized the exogenous pathway of antigen

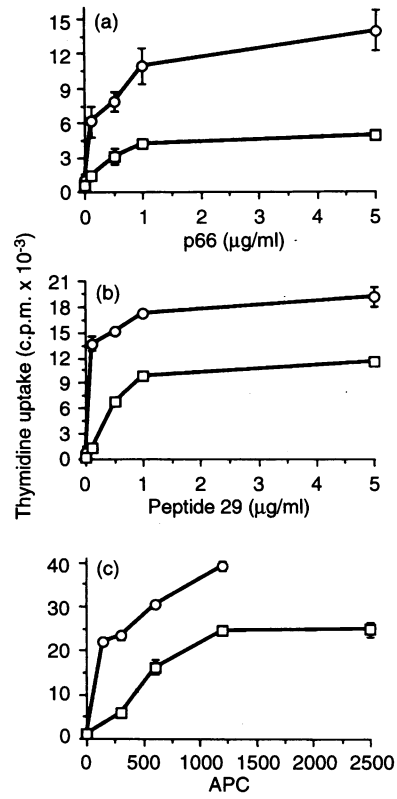

Figure 1. FLC are more efficient than peripheral blood monocytes in presenting both intact p66 and immunogenic peptides. (a and b) Epidermal cells enriched for LC (600 $CD1a^+$ cells/well) (circles) or PBMC (600 monocytes/well) (squares) were pulsed with increasing concentrations (0·1–5 μg/ml) of either intact p66 or peptide 29 and co-cultured with 20×10^4 T cells for 3 days. (c) Increasing numbers of LC or monocytes were pulsed with peptide 43 (2 μg/ml) and co-cultured with 20×10^4 T cells for 3 days. Experiments were performed using cells from donor 1. Results are given as mean ± SD.

processing. To this end, we pulsed CLC with antigen in the presence of chloroquine or leupeptin. Chloroquine is a weak base that concentrates and inhibits acidification of intracellular compartments where antigen processing takes place.[28] Leupeptin is an inhibitor of cysteine and serine proteases, which represent a major class of enzymes for antigen degradation as well as for invariant chain breakdown.[29,30] Chloroquine and leupeptin blocked to a similar extent the presentation of p66 by FLC or CLC; in contrast, they did not affect presentation of an immunogenic peptide (Fig. 3). These results confirm that FLC and CLC possess analogous antigen-processing mechanisms based on intracellular acidification and protease activities.[19] Chloroquine and leupeptin also inhibited similarly the presentation of p66 by monocytes and EBV-B cells (data not shown).

Costimulatory signals

Activation of T lymphocytes by APC requires the interaction of MHC/antigen complexes with the TCR as well as the binding of costimulatory molecules with receptors on T cells. With the exception of ICAM-3 (CD50), FLC did not display significant numbers of costimulatory molecules.[11,12] After short-term

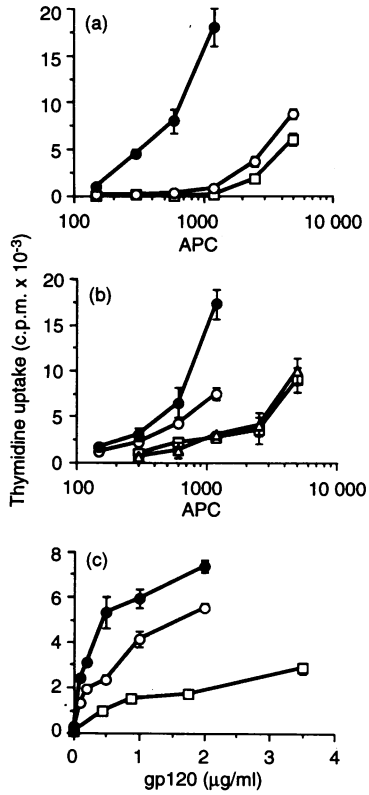

Figure 2. CLC display enhanced antigen-presenting capacity compared to FLC. FLC (open circles) or 48-hr CLC (solid circles), peripheral blood monocytes (open squares) or adherent monocytes (open triangles) were pulsed with (a) p66 (1 μg/ml) (donor 2), (b) peptide 29 (1 μg/ml) (donor 1) or (c) increased amounts of gp120 (500 APC/well) (donor 2) and co-cultured with 20×10^4 specific T cells for 3 days. In (a) and (b), background proliferation (in the absence of APC) was less than 400 or 800 c.p.m., respectively. Results are given as mean ± SD.

culture, LC expressed and then up-regulated ICAM-1 (CD54), LFA-3 (CD58) and B7–1 (CD80).[11,13] Although some studies have established the importance of these membrane molecules in the LC-dependent stimulation of allogeneic T cells,[11,31] few data are available concerning the role of costimulatory molecules in LC-driven antigen-specific T-cell activation.[11] In the next set of experiments, incubation of CLC with mAb against HLA-DR blocked T-cell proliferation completely (95% inhibition), whereas mAb to ICAM-1, ICAM-3, LFA-3, B7–1 or β_2-integrins reduced T-cell proliferation between 50% and 90%. As expected, mAb against MHC class I molecules did not inhibit the T-cell response (Fig. 4). Incubation of CLC with isotype-matched (IgG1 or IgG2a) mAb had no effect (data not shown).

DISCUSSION

In this study, we have shown that CLC retain the ability to process MHC class II-restricted intact protein antigens[19] and augment markedly their antigen-presenting function to specific $CD4^+$ T lymphocytes. T-cell lines rather than cloned lines were used because they more closely resemble the polyclonal circumstances *in vivo*. The increased APC potency is probably not related to antigen uptake or degradation because it was

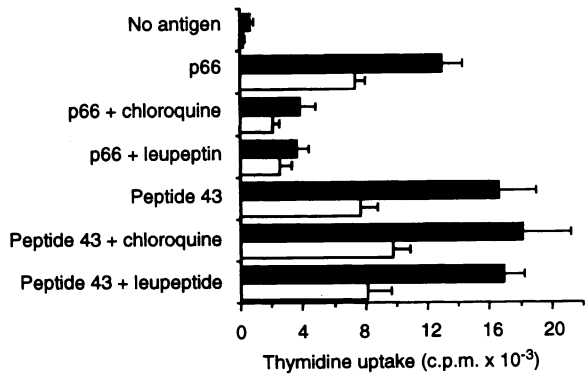

Figure 3. Chloroquine and leupeptin inhibit processing of native p66 by CLC. Epidermal cells enriched for LC (500 $CD1a^+$ cells/well) either freshly procured (white bars) or 48-hr cultured (hatched bars) were treated with chloroquine (100 μM) or leupeptin (100 mM) for 15 min, then pulsed with p66 or peptide 43 (1 μg/ml) for 3 hr in the presence of inhibitors. After washing, T cells were added at 20×10^4 cells/well. Cells were obtained from donor 1. Results are given as mean ± SD.

confirmed for both native proteins and immunogenic peptides; rather, the high antigen-presenting efficiency of CLC can be explained better as a result of up-regulated expression of membrane MHC and adhesion/costimulatory molecules, including ICAM-1, LFA-3 and B7–1.[11,13] The relevance and

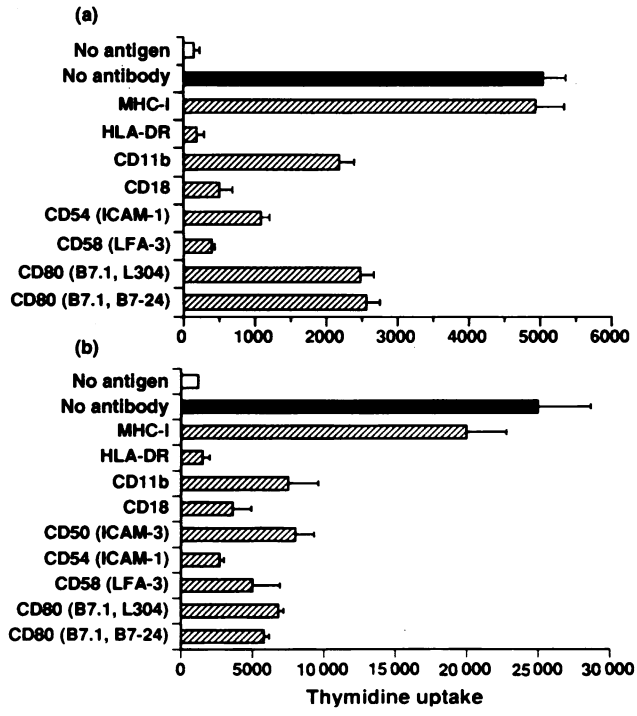

Figure 4. Costimulatory molecules involved in the presentation of (a) p66 and (b) gp120 by CLC to specific $CD4^+$ T-cell lines. Epidermal cells enriched for LC (p66, 625 $CD1a^+$ cells/well; gp120, 1250 $CD1a^+$ cells/well) were cultured for 48 hr, incubated at 4° for 45 min with saturating concentrations of dialysed mAb, pulsed with antigen (1 μg/ml) and then co-cultured with 20×10^4 specific T cells for 3 days. The experiments were performed using cells from donor 2. Results are given as mean ± SD.

the number of the accessory molecules involved in antigen-specific T-cell activation by CLC was demonstrated in blocking experiments with specific mAb.

Increased expression of adhesion and costimulatory molecules associated with functional improvement during culture is not unique of epidermal LC, having also been demonstrated for human peripheral blood DC,[32,33] rat lung DC[34] and mouse splenic DC.[35,36] The significance and the factors that drive this activation process of DC are not completely known. Cytokines released by DC themselves or other cell types probably play a primary role. Keratinocyte-conditioned medium and GM-CSF improve survival and enhance the accessory function of mouse LC in culture.[37] Viability of cultured peripheral blood DC is maintained by monocyte-conditioned medium, GM-CSF and tumour necrosis factor-α (TNF-α), but only the former augments their antigen-presenting capacity.[33,38] IL-1β is an additional cytokine with potent activities on epidermal LC.[39]

The vast majority of knowledge on antigen processing derives from studies performed on B cells or macrophages, and few data are available concerning LC or DC, mostly due to difficulties in obtaining sufficient numbers of purified cells. The early observation that CLC and cultured DC were unable to process protein antigens stimulated studies on the mechanisms of antigen processing by these APC. It was thus shown that FLC and CLC endocytose similarly exogenous proteins,[40] and display lysosomes as well as other vesicles with acidic interior.[41] Biochemical studies have also confirmed that both FLC and CLC possess similar amounts of bafilomycin-sensitive ATPase activity, which creates the acidic milieu in the lumen of vacuolar compartments.[42] However, while the synthesis of class II molecules and invariant chain is very active in FLC, it decreases dramatically and selectively with culture and virtually ceases after 1–3 days; in addition, the turnover of metabolically labelled class II molecules in LC appears particularly slow, a phenomenon that may explain the apparent paradox of blocked new synthesis and increased membrane expression.[40,43,44] Here we show that CLC as well as FLC can present, and thus process, different intact proteins (gp120 and p66) through a pathway that can be inhibited by chloroquine or leupeptin. In contrast, presentation of immunogenic peptides by LC was not diminished under these conditions. These results suggest that antigen processing in both FLC and CLC requires acidification of intracellular organelles and protease activity, and that immunogenic peptides can bind directly to class II molecules. In the mouse system, CLC and cultured DC populations down-regulate their processing capacity, and the residual antigen-processing activity has been explained by incomplete maturation of a small fraction of cells in culture.[18] In contrast, in the human system, CLC are similarly[19] or more potent (our results) APC than FLC. It is therefore unlikely that a small residual fraction of immature LC could account for this acitivity, especially considering the low numbers of LC or the small amount of antigens employed in the antigen-presentation assays. In addition, our LC preparations expressed homogeneously B7·1 and high levels of HLA-DR, indicating uniform *in vitro* maturation (data not shown).

Current understanding of processing of exogenous protein antigens encompasses uptake and degradation of antigen, binding of immunogenic peptides to newly synthesized class II molecules deprived of the invariant chain, and then transport of class II–peptide complexes to the cell surface. Most of the antigen-processing events occur within a specialized acidic and proteolytic subcellular compartment that is intersected in the endocytic route, contains abundant MHC class II and possesses the characteristics of lysosomes (MIIC).[30,45,46] In B cells, peptides bind primarily nascent class II molecules rather than those internalized from the plasma membrane.[47] In other studies, however class II endocytosed from the plasma membrane appear to contribute significantly to antigen presentation.[48,49] The inability of CLC to synthesize new MHC class II molecules raises the possibility that the latter route is dominant in these cells. Alternatively, immunogenic peptides generated within CLC may bind to class II molecules previously synthesized and stored in intracellular organelles or to empty class II on the cell surface. These questions and, in general, antigen processing by LC and DC need to be examined in more detail. The availability of functional DC lines from human cord blood, peripheral blood or bone marrow precursors should greatly facilitate these studies.[50–53]

Finally, the high efficiency of epidermal LC to present HIV-1 antigens, together with the observation that LC *in vivo* can be infected by HIV-1 without significant compromise of their presenting capacity,[8–10,54] suggests that LC can actively participate in the generation and maintenance of T-helper cell immunity to HIV-1 infection.[5] In this respect, LC of mucosal epithelia can be among the first targets of HIV entry following sexual contact, and, thus, they may play a primary role in priming HIV-specific $CD4^+$ T cells.

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REFERENCES

1. Steinman R.M. (1991) The dendritic cell system and its role in immunogenicity. *Annu Rev Immunol* **9,** 271.
2. Croft M., Duncan D.D. & Swain S.L. (1992) Response of naive antigen-specific $CD4^+$ T cells *in vitro*: characteristics and antigen-presenting cell requirements. *J Exp Med* **176,** 1431.
3. Levin D., Constant S., Pasqualini T., Flawell R. & Bottomly K. (1993) Role of dendritic cells in the priming of $CD4^+$ T lymphocytes to peptide antigen *in vivo*. *J Immunol* **151,** 6742.
4. Paglia P., Girolomoni G., Robbiati F., Granucci F. & Ricciardi-Castagnoli P. (1993) Immortalized dendritic cell line fully competent in antigen presentation initiates primary T cell responses *in vivo*. *J Exp Med* **178,** 1893.
5. Manca F., Li Pira G., Fenoglio D. *et al.* (1994) Dendritic cells are potent antigen-presenting cells for *in vitro* induction of primary human $CD4^+$ T-cell lines specific for gp120. *J Acquir Immune Defic Syndr* **7,** 15.
6. Romani N. & Schuler G. (1992) The immunologic properties of epidermal Langerhans' cells as a part of the dendritic cell system. *Springer Semin Immunopathol* **13,** 265.
7. Moll H. (1993) Epidermal Langerhans' cells are critical for immunoregulation of cutaneous leishmaniasis. *Immunol Today* **14,** 383.
8. Giannetti A., Zambruno G., Cimarelli A. *et al.* (1993) Direct detection of HIV-1 RNA in epidermal Langerhans' cells of HIV-infected patients. *J Acquir Immune Defic Syndr* **6,** 329.

9. CIMARELLI A., ZAMBRUNO G., MARCONI A., GIROLOMONI G., BERTAZZONI U. & GIANNETTI A. (1994) Quantitation by competitive PCR of HIV-1 proviral DNA in epidermal Langerhans' cells of HIV-infected patients. *J Acquir Immune Defic Syndr* **7,** 230.
10. RAPPERSBERGER K., GARTNER S., SCHENK P. *et al.* (1988) Langerhans' cells are an actual site of HIV-1 replication. *Intervirology* **29,** 185.
11. TEUNISSEN M.B.M., RONGEN H.A.H. & BOS J.D. (1994) Function of adhesion molecules LFA-3 and ICAM-1 on human epidermal Langerhans' cells in antigen-specific T-cell activation. *J Immunol* **152,** 3400.
12. ZAMBRUNO G., COSSARIZZA A., OTTANI D. *et al.* (1995) Functional ICAM-3 is expressed by freshly isolated epidermal Langerhans' cells and is not regulated during culture. *J Invest Dermatol* **105,** 215.
13. GIROLOMONI G., ZAMBRUNO G., MANFREDINI R. *et al.* (1993) Expression of B7 costimulatory molecule in cultured human epidermal Langerhans' cells is regulated at the mRNA level. *J Invest Dermatol* **103,** 54.
14. DAI R., GRAMMER S.F. & STREILEIN J.W. (1993) Fresh and cultured Langerhans' cells display differential capacities to activate hapten-specific T cells. *J Immunol* **150,** 59.
15. AIBA S. & KATZ S.I. (1989) The ability of cultured Langerhans' cells to process and present protein antigens is MHC-dependent. *J Immunol* **146,** 2479.
16. DE BRUIJN M.L.H., NIELAND J.D., HARDING C.V. & MELIEF C.J.M. (1992) Processing and presentation of intact hen egg-white lysozyme by dendritic cells. *Eur J Immunol* **22,** 347.
17. ROMANI N., KOIDE S., CROWLEY M. *et al.* (1989) Presentation of exogenous protein antigens by dendritic cells to T cell clones. *J Exp Med* 169, 1169.
18. KOCH F., TROCKENBACHER B., KÄMPGEN E. *et al.* (1995) Antigen processing in populations of mature murine dendritic cells is caused by subsets of incompletely matured cells. *J Immunol* **155,** 93.
19. COHEN P.J. & KATZ S.I. (1992) Cultured human Langerhans' cells process and present intact protein antigens. *J Invest Dermatol* **99,** 331.
20. MANCA F., WALKER L., NEWELL A., CELADA F., HABESHAW J.A. & DALGLEISH A.G. (1992) Inhibitory activity of HIV envelope gp120 dominates over its antigenicity for human T cells. *Clin Exp Immunol* **88,** 17.
21. MANCA F., HABESHAW J., DALGLEISH A.G., FENOGLIO D., LI PIRA G. & SERCARZ E.E. (1993) Role of flanking variable sequences in antigenicity of consensus regions of HIV gp120 for recognition by specific human T helper clones. *Eur J Immunol* **23,** 269.
22. MYERS G., RABSON A.B., BERZOFSKY J.A., SMITH T.F. & WONG-STAAL F. (1990) *Human Retrovirus and AIDS. A Compilation and Analysis of Nucleic Acid and Amino Acid Sequences.* Los Alamos National Laboratory, Los Alamos.
23. MANCA F., HABESHAW J. & DALGLEISH A.G. (1991) The naive repertoire of human T helper cells specific for gp120, the envelope glycoprotein of HIV. *J Immunol* **146,** 1964.
24. BIRD A.G., MCLACHLAN S.M. & BRITTON S. (1981) Cyclosporin A promotes spontaneous outgrowht *in vitro* of Epstein–Barr virus-induced B cell lines. *Nature* **289,** 300.
25. MANCA F., FENOGLIO D., VALLE M.T. *et al.* (1995) Human $CD4^+$ T cells can discriminate the molecular context of immunodominant epitopes of HIV gp120 and HIV p66. *J Acquir Immune Defic Syndr* **9,** 227.
26. SONTHEIMER R.D. (1983) The mixed epidermal cell-lymphocyte reaction. *J Immunol* **130,** 2612.
27. RES P., KAPSENBERG M.L., BOS J.D. & STIEKEMA F. (1987) The crucial role of human dendritic antigen-presenting cell subsets in nickel-specific T-cell proliferation. *J Invest Dermatol* **88,** 550.
28. GIROLOMONI G., CRUZ P.D. & BERGSTRESSER P.R. (1990) Internalization and acidification of surface HLA-DR molecules by epidermal Langerhans' cells: a paradigm for antigen processing. *J Invest Dermatol* **94,** 753.
29. VIDARD L., ROCK K.L. & BENACERRAF B. (1992) Eterogeneity in antigen processing by different cell types of antigen-presenting cells. Effect of cell culture on antigen processing ability. *J Immunol* **149,** 1905.
30. BÉNAROCH P., YILLA M., RAPOSO G. *et al.* (1995) How MHC class II molecules reach the endocytic route. *EMBO J* **14,** 37.
31. SIMON J.C., CRUZ P.D., TIGELAAR R.E., SONTHEIMER R.D. & BERGSTRESSER P.R. (1991) Adhesion molecules CD11a, CD18, and ICAM-1 on human epidermal Langerhans' cells serve a functional role in the activation of alloreactive T cells. *J Invest Dermatol* **96,** 148.
32. THOMAS R., DAVIES L.S. & LIPSKY P.E. (1993) Isolation and characterization of human peripheral blood dendritic cells. *J Immunol* **150,** 821.
33. O'DOHERTY U., STEINMAN R.M., PENG M. *et al.* (1993) Dendritic cells freshly isolated from human blood express CD4 and mature into typical immunostimulatory dendritic cells after culture in monocyte-conditioned medium. *J Exp Med* **178,** 1067.
34. HOLT PG, OLIVIER J, BILYK N. *et al.* (1993) Downregulation of the antigen presenting function(s) of pulmonary dendritic cells *in vivo* by resident alveolar macropahges. *J Exp Med* **177,** 397.
35. GIROLOMONI G., SIMON J.C., BERGSTRESSER P.R. & CRUZ P.D. (1990) Freshly isolated spleen dendritic cells and freshly isolated epidermal Langerhans' cells undergo similar phenotypic and functional changes during short term culture. *J Immunol* **145,** 2820.
36. GIROLOMONI G., ZAMBRUNO G., MANCA V., BERGSTRESSER P.R., GIANNETTI A. & CRUZ P.D. (1992) Spleen dendritic cells exhibit altered morphology and increased allostimulatory capacity after short term culture. *Exp Dermatol* **1,** 129.
37. KOCK F., HEUFLER C., KÄMPGEN E., SCHNEEWEISS D., BÖCK G. & SCHULER G. (1990) Tumor necrosis factor α maintains the viability of murine epidermal Langerhans' cells in culture, but in contrast to granulocyte/macrophage colony-stimulating factor, without inducing their functional maturation. *J Exp Med* **171,** 159.
38. MARKOWICZ S. & ENGLEMAN E. (1990) Granulocyte–macrophage colony stimulating factor promotes differentiation and survival of human peripheral blood dendritic cells *in vitro*. *J Clin Invest* **85,** 955.
39. ENK A.H., ANGELONI V.L., UDEY M.C. & Katz S.I. (1993) An essential role for Langerhans' cell-derived IL-1β in the initiation of primary immune responses in skin. *J Immunol* **150,** 3698.
40. PURÉ E., INABA K., CROWLEY M.T. *et al.* (1990) Antigen processing by epidermal Langerhans' cells correlates with the level of biosynthesis of major histocompatibility complex class II molecules and expression of invariant chains. *J Exp Med* **172,** 1459.
41. STÖSSEL H., KOCH F., KÄMPGEN E. *et al.* (1990) Disappearance of certain acidic organelles (endosomes and Langerhans' cell granules) accompanies loss of antigen processing capacity upon culture of epidermal Langerhans' cells. *J Exp Med* **172,** 1471.
42. GIROLOMONI G., STONE D.K., BERGSTRESSER P.R. & CRUZ P.D. (1991) Vacuolar acidification and bafilomycin-sensitive proton translocating ATPase in human epidermal Langerhans' cells. *J Invest Dermatol* **96,** 735.
43. KÄMPGEN E., KOCH N., KOCH F. *et al.* (1991) Class II major histocompatibility complex molecules of murine dendritic cells: synthesis, sialylation of invariant chain, and antigen processing capacity are down-regulated upon culture. *Proc Natl Acad Sci USA* **88,** 3014.
44. BECKER D., RESKE-KUNZ A.B., KNOP J. & RENSKE K. (1991) Biochemical properties of MHC class II molecules endogenously synthesized and expressed by mouse Langerhans' cells. *Eur J Immunol* **21,** 1213.
45. KLEIJMEER M.J., OORSCHOT V.M.J. & GEUZE H.J. (1994) Human resident Langerhans' cells display a lysosomal compartment enriched in MHC class II. *J Invest Dermatol* **103,** 516.
46. MOMMAAS A.M., MUDLER A.A., OUT C.J. *et al.* (1995) Distribution of HLA-class II molecules in epidermal Langerhans' cells *in situ*. *Eur J Immunol* **25,** 520.

47. Davidson H.W., Reid P.A., Lanzavecchia A. & Watts C. (1991) Processed antigen binds to newly synthesized MHC class II molecules in antigen-specific B lymphocytes. Irreversible association of peptides with class II MHC molecules in living cells. *Cell* **67,** 105.
48. Roche P.A., Teletski C.L., Stang E., Bakke O. & Long E.O. (1993) Cell surface HLA-DR-invariant chain complexes are targeted to endosomes by rapid internalization. *Proc Natl Acad Sci USA* **90,** 8581.
49. Pinet V., Vergelli M., Martin R., Bakke O. & Long E.O. (1995) Antigen presentation mediated by recycling of surface HLA-DR molecules. *Nature* **375,** 603.
50. Caux C., Dezutter-Dambuyant C., Schmitt D. & Banchereau J. (1992) GM-CSF and TNFα cooperate in the generation of dendritic Langerhans' cells. *Nature* **360,** 258.
51. Reid C.D., Stackpoole A., Meager A. & Tikerpae J. (1992) Interactions of tumor necrosis factor with granulocyte–macrophage colony stimulating factor and other cytokines in the regulation of dendritic cell growth *in vitro* from early bipotent $CD34^+$ progenitors in human bone marrow. *J Immunol* **149,** 2681.
52. Sallusto F. & Lanzavecchia A. (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α. *J Exp Med* **179,** 1109.
53. Romani N., Gruner S., Brang D. *et al.* (1994) Proliferating dendritic cell progenitors in human blood. *J Exp Med* **180,** 83.
54. Blauvelt A., Clerici M., Lucey D.R. *et al.* (1995) Functional studies of epidermal Langerhans' cells and blood monocytes in HIV-infected persons. *J Immunol* **154,** 3506.