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In vitro primary sensitization and restimulation of hapten-specific T cells by fresh and cultured human epidermal Langerhans' cells

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SUMMARY

We examined the capacity of human Langerhans' cells (LC) to sensitize autologous T cells to the trinitrophenyl hapten (TNP) *in vitro*. Two-day cultured Langerhans' cells, but not freshly prepared Langerhans' cells, can induce *in vitro* primary proliferative reactions to the TNP hapten. Using a CD45RA$^+$ naive T-cell subset, similar results were found, therefore making the possibility of a previous *in vivo* T-cell contact with the hapten unlikely. The primary *in vitro* response was strongly inhibited by monoclonal antibodies to major histocompatibility complex (MHC) class I and II, CD4 antigens and ICAM-1 and LFA-3 adhesion molecules. Furthermore, we found that fresh LC can prime T cells to TNP, as revealed by a significant secondary T-cell proliferation after restimulation of the recovered T lymphocytes by fresh hapten-modified autologous LC. Nevertheless, the ability of these fresh LC to stimulate *in vitro* secondary hapten-specific T-cell proliferation was very limited in comparison with that of 2-day incubated Langerhans' cells. After secondary stimulation with TNP-cultured LC, sensitized T cells could be non-specifically expanded without losing hapten specificity. The TNP-specific T-cell lines were mostly of the CD4$^+$ phenotype. The present findings extend previous studies in the mouse, showing that cultured LC are potent antigen-presenting cells (APC) in primary hapten-dependent proliferation assays. Furthermore, this *in vitro* priming assay, using cultured human Langerhans' cells as APC, might be useful to analyse the early steps of T-cell sensitization and subsequently to develop *in vitro* predictive tests allowing detection of sensitizing compounds.

INTRODUCTION

There is now considerable evidence showing that epidermal Langerhans' cells (LC), a population of non-lymphoid dendritic cells which constitutively express major histocompatibility complex (MHC) class II molecules, play a key role in the development of contact hypersensitivity reactions.[1-3] These cells, which represent the antigen-presenting cells (APC) of epidermis, pick up the haptens within the epidermal layer and then migrate to draining lymph nodes where antigen presentation to specific T cells occurs.[4]

Recent studies reported that after 2–3-day *in vitro* incubation, both murine and human LC undergo profound phenotypic changes, as an enhancement in the expression of MHC class I and II antigens, LFA-3 and ICAM-1 molecules, a concomitant decrease of CD1a antigens and a loss of FcγRII.[5-7] Furthermore, cultured LC (cLC) lose or markedly reduce their specific cytoplasmic organelles: the Birbeck granules. Therefore, after a 2–3-day *in vitro* incubation, LC seem to acquire most of the features of lymphoid dendritic cells.[5,6] In the murine system and in at least some strains of mice, together with these phenotypic modifications, the incubated LC become substantially more potent accessory cells than fresh LC (fLC),[8-10] while they are relatively inefficient in processing protein antigens.[11-13] Whether human LC also undergo functional modifications during *in vitro* incubation has been far less studied. Nevertheless, it was suggested that epidermal LC are immature elements of the dendritic cell system,[9] and that cultured LC may represent the *in vitro* counterparts of antigen-bearing LC that have migrated to regional lymph nodes.[9,12]

The present study was aimed to develop *in vitro* sensitization of human naive T cells to the hapten trinitrophenyl (TNP). Although previous papers have reported *in vitro* priming of naive T cells to haptens, these studies used peripheral blood cells as APC.[14,15] We were interested in using in this assay, the

Abbreviations: APC, antigen-presenting cells; CS, contact sensitivity; DC, dendritic cells; FITC, fluorescein isothiocyanate; GM-CSF, granulocyte–macrophage colony-stimulating factor; cLC, cultured Langerhans' cells; fLC, freshly prepared Langerhans' cells; pLC, highly enriched Langerhans' cells; mAb, monoclonal antibodies; PBMC, peripheral blood mononuclear cells; TcR, T-cell receptor; TNBS, trinitrobenzene sulphonic acid; TNP, trinitrophenyl.

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physiological APC involved in the process of contact hypersensitivity reactions, i.e. the epidermal Langerhans' cells. Since LC modifications after *in vitro* culture might be relevant to changes that occur *in vivo* during the sensitization phase of contact hypersensitivity reactions, we analysed the capacity of both freshly isolated and 2-day incubated human LC to elicit *in vitro* primary T-cell sensitization to the hapten TNP.

MATERIALS AND METHODS

Medium

The culture medium was RPMI-1640 medium (Gibco BRL, Grand Island, NY) supplemented with 10% heat-inactivated human AB serum (Centre de Transfusion Sanguine, Lyons, France), 100 μg/ml gentamicin, 2 mM L-glutamine and 1 μg/ml indomethacin (Sigma, St Louis, MO): hereafter designated complete medium.

Langerhans' cell-enriched epidermal cell suspensions

Epidermal cell suspensions were obtained from normal human skin (plastic surgery) by trypsinization: 0·05% trypsin (Difco Laboratories, Detroit, MI), 18 hr at 4°. They were either frozen (with dimethyl sulphoxide at a final concentration of 10% in RPMI-1640 medium supplemented with 20% AB serum) or enriched in LC by density gradient centrifugation on Lymphoprep (Flobio, Courbevoie, France). Cells from the interface were either used as freshly prepared LC-enriched epidermal cells (fLC: 8–30% LC) or incubated (10^6/ml) for 48 hr at 37° in culture medium containing 200 U/ml human recombinant granulocyte–macrophage colony-stimulating factor (GM-CSF; Genzyme, Cambridge, MA). After incubation, viable cells (cLC: 15–30% LC) were recovered by Lymphoprep density centrifugation. In some experiments, highly enriched LC suspensions (pLC: 75–80% LC) were obtained after three successive density centrifugations of epidermal cell suspensions on Lymphoprep. These LC were used as stimulating cells, either as freshly prepared APC (fpLC) or after 2 days of culture with GM-CSF (cpLC). Enrichment for LC (HLA-DR$^+$ cells) in both freshly prepared and cultured epidermal cell suspensions was quantified by FACS.

Chemical modification of antigen-presenting cells

Modification of APC with the trinitrophenyl hapten was performed according to the method of Shearer.[16] Briefly, cell pellets were resuspended in Hanks' balanced salt solution (HBSS) (pH 7·2) containing 5 mM 2,4,6 trinitrobenzenesulphonic acid (TNBS; Sigma). For control of hapten specificity, APC were also conjugated to 100 μg/ml of fluorescein isothiocyanate (FITC; Sigma). Previous experiments showed that these doses were non-toxic for the cells. Cells were incubated with these haptens for 10 min at 37°, and then washed extensively before use as modified APC in T-cell proliferation assays.

T cells

Autologous T cells were isolated from peripheral blood as described previously.[17] The T-cell population contained 95% or more CD3-positive cells, as assessed by FACS. In some experiments, the CD45RA$^+$ subpopulation (naive T cells) was separated from the CD45RO$^+$ subset (memory T cells) by a panning procedure. Briefly, T cells were stained with anti-CD45RO monoclonal antibody (mAb) (UCHL1; Dakopatts, Copenhagen, Denmark), washed and added to a Petri dish coated with goat anti-mouse immunoglobulin (Ig) (Zymed, San Francisco, CA). The unbound CD45RA$^+$ cells were recovered by gentle swirling and used as responder cells in our assay. The efficacy of the panning procedure was assessed by FACS analysis and the naive T-cell population contained 88–90% CD45RA$^+$ cells.

Sensitizing cultures

Hapten-modified fresh LC (TNP-fLC) or 2-day incubated LC (TNP-cLC) were cultured with autologous T cells in 2 ml of complete medium in a 5 ml tube (Falcon 2054, Cockeysville, MD) for 9 days: the purified T cells were incubated with LC at 37° at a ratio of about 100:1. Viable T cells were then recovered and used for restimulation assays. To determine primary proliferation, this sensitizing culture was also set up in 96-well U-bottomed microtitre plates: T cells were cultured at 10^5 cells per well with 10^3 fresh or incubated hapten-coupled LC in 200 μl of complete medium. A kinetic study of T-cell proliferation was performed by pulsing the cells with 1 μCi of [^{3}H]thymidine (1 Ci/mmole; Dositek, Orsay, France) for the final 18 hr of culture. This primary sensitization was also analysed by using highly enriched LC suspensions and a graded responder/stimulator cell ratio.

Blocking experiments

A panel of mAb was used to block the primary proliferative T-cell response to hapten-modified highly enriched cLC: BL2 (anti-HLA-DR), BL6 (anti-CD1a), BL4 (anti-CD4), B9.2 (anti-CD8) (all from Immunotech, Marseilles, France); W6/32HL (anti-HLA-A,B,C) (Sera-Lab, Crawley Down, U.K.); G26 (anti-LFA-3; CD58) (Behring Diagnostic, Rueil-Malmaison, France); and RR/1 (anti-ICAM-1; CD54) (kindly provided by Dr T. A. Springer, Boston, MA). As controls, we used isotype-matched mouse Ig with undetermined specificity (Sigma). Monoclonal antibodies were added at the initiation of the sensitizing culture and T-cell proliferation was assessed as described above.

Restimulation assays

After 9 days of primary cultures, secondary cultures of *in vitro* primed T cells were carried out in 96-well microtitre plates. Viable T cells were recovered by density gradient centrifugation and restimulated (10^5 T cells) for 3 days with thawed and TNP-modified autologous fresh or 2-day incubated LC (5×10^3 LC). T-cell proliferation was determined by addition of [^{3}H]thymidine for the final 18 hr of culture. Specificity of T-cell proliferation was assessed by the use of either non-modified LC, or LC treated with an irrelevant hapten (FITC).

Generation of hapten-specific T-cell lines

After secondary *in vitro* stimulation with TNP-modified cLC, T lymphocytes were weekly expanded with irradiated (40 Gy) allogeneic peripheral blood mononuclear cells (PBMC), 1 μg/ml phytohaemagglutinin (PHA; Difco Laboratories) and 10 U/ml human recombinant interleukin-2 (IL-2; Boehringer Mannheim, Mannheim, Germany). IL-2 (10 U/ml) was also added at day 3 after this stimulation. After 1 month, hapten specificity of T-cell lines was determined as done in secondary responses by stimulation with fresh or incubated hapten-conjugated LC.

FACS analysis

Mouse mAb at the appropriate concentration were used for identification of specific cell types. FITC-coupled anti-HLA-DR mAb (Becton Dickinson, San Jose, CA) was used to quantify LC enrichment. For analysis of T-cell surface antigens, FITC-coupled IOT3, IOT4a and IOT8a mAb (anti-CD3, CD4 and CD8, respectively) were obtained from Immunotech. Anti-CD45RA mAb (2H4) was from Coultronics, Margenay, France. Isotype-matched irrelevant mAb were purchased from Becton Dickinson. TcR1$\alpha\beta$ (T-cell receptor) mAb was from Becton Dickinson, anti-TcR$\gamma\delta$ was an ascite kindly provided by T. Hercend (Institut G. Roussy, Villejuif, France) and FITC goat anti-mouse IgG (H+L) was from Zymed. For direct immunofluorescence, cells were labelled with FITC-conjugated mAb. For indirect immunofluorescence, cells were first incubated with the primary mAb and subsequently with FITC goat anti-mouse IgG. All incubation steps were performed for 30 min at 4° and cells were washed twice in between successive steps. Cells labelled with irrelevant isotype-matched primary mAb or with FITC-conjugated secondary antibody alone were used as controls. FACS analysis was performed on a FACStar Plus cytometer (Becton Dickinson) and results are expressed as the percentage of positive cells above background fluorescence.

RESULTS

TNP-cLC can induce *in vitro* T-cell primary proliferative response

As shown in Fig. 1a, addition of TNP-modified fresh LC (10^3 LC) to autologous T cells did not induce any detectable T-lymphocyte proliferation. To investigate the ability of cultured LC to sensitize T lymphocytes to TNP, freshly prepared human epidermal cells (8–30% LC) were incubated for 2 days in complete medium supplemented with GM-CSF (200 U/ml), since this cytokine was reported to enhance murine LC viability during *in vitro* incubation.[18] Indeed, after incubation in medium alone, about 10% of viable LC were recovered, whereas the yield was doubled in the presence of GM-CSF. Contrary to fresh LC, we observed that upon stimulation wtih a similar number of TNP-modified 2-day incubated LC (10^3 LC), the T-cell population was able to proliferate (Fig. 1a). This *in vitro* primary proliferative response was evident on day 3 of culture and became maximal 2 days later. By contrast, there was no significant autologous response to either fresh or cultured non-modified LC.

In these experiments, enrichment for both fresh and cultured LC was mostly below 30% and it might be possible that the residual keratinocytes could somehow influence the *in vitro* T-cell immune response. We have repeated primary sensitization of T cells using a more efficient LC-enrichment method. Using highly enriched LC suspensions (pLC: 75–80% LC), the results obtained (Fig. 1b) were similar to those first described: TNP-fpLC were unable to induce *in vitro* T-cell proliferation whereas TNP-cpLC led to a substantial and dose-dependent T-cell response. Furthermore, these results were obtained irrespective of whether pLC had been incubated in the presence of GM-CSF or in medium alone (data not shown). We also performed some experiments using LC-depleted keratinocyte suspensions (< 1% LC) as APC. As one would expect, no primary proliferative response of T cells to TNP-modified keratinocytes was observed (data not shown). These results clearly demonstrate that, among epidermal cells, LC are responsible for the observed proliferative response. One cannot completely exclude, however, that cytokines derived from contaminating keratinocytes may potentiate LC APC function.

The primary T-cell response to TNP-cLC was regularly found in experiments using 10 different donors. It was therefore unlikely that these results reflected an *in vivo* sensitization of the

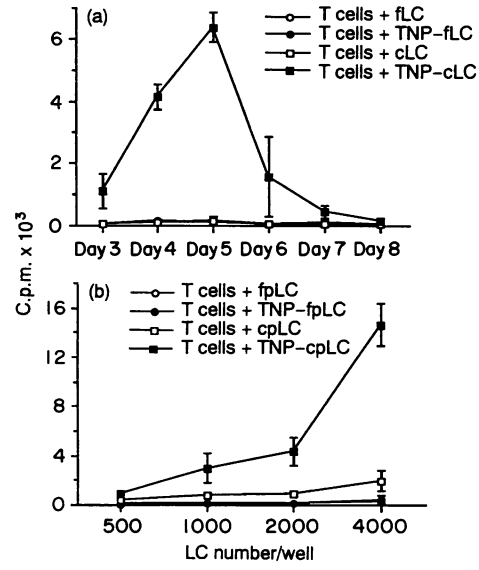

Figure 1. (a) Kinetics of T-cell primary response to TNP-modified fresh or 2-day incubated human LC. T lymphocytes (10^5) were incubated with either 10^3 fresh (fLC) or 2-day incubated LC (cLC), modified or not with TNP. T-cell proliferation was assessed by a [^{3}H]thymidine pulse for the final 18 hr of culture. Results are expressed as c.p.m. ± SD of triplicate cultures. (b) T-cell primary response to a graded number of TNP-modified fresh or cultured LC. T lymphocytes (10^5) were cultured for 5 days with highly enriched fresh (fpLC) or 2-day incubated (cpLC) Langerhans' cells (75–80% LC), treated or not with TNP. T-cell proliferation was assessed by [^{3}H]thymidine incorporation for the final 18 hr of culture. Results are expressed as c.p.m. ± SD of triplicate cultures.

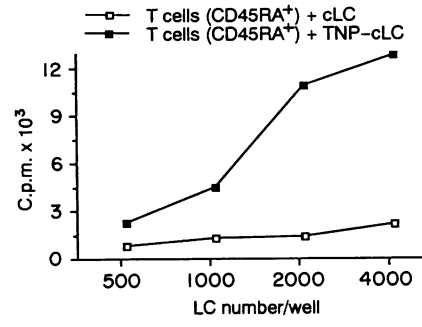

Figure 2. Naive T-cell sensitization to TNP by cultured LC. CD45RA$^+$ T lymphocytes (10^5) were cultured for 5 days with graded numbers of either non-modified or TNP-treated 2-day incubated LC. T-cell proliferation was assessed by a [^{3}H]thymidine pulse for the final 18 hr of culture.

Table 1. Effect of mAb on T-cell primary proliferative response to TNP-modified cultured LC*

Specificity	Clone	Proliferation†‡
Anti-HLA-DR	BL2§	2171±283 (87)
Anti-HLA-A,B,C	W6/32HL§	1860±389 (89)
Anti-CD4	BL4§	3815±430 (72)
Anti-CD8	B9.2§	10,715±1635 (37)
Anti-CD1a	BL6§	17,214±3302 (1)
Anti-ICAM-1 (CD54)	RR/1¶	4162±1503 (76)
Anti-LFA-3 (CD58)	G26¶	3880±1063 (77)
Control mAb	MOPC21	17,092±1957
No mAb		17,923±1605

* T lymphocytes (10^5) were incubated for 5 days with 2×10^3 highly enriched TNP-modified cultured LC. mAb were added either at 10 μg/ml (§) or at 1 μg/ml (¶) at the beginning of the culture and T-cell proliferation was assessed by [^{3}H]thymidine incorporation during the 18 final hr of culture.

† Results are expressed as c.p.m.±SD of triplicate culture.

‡ In parentheses is the percentage inhibition of control proliferation observed with irrelevant mAb.

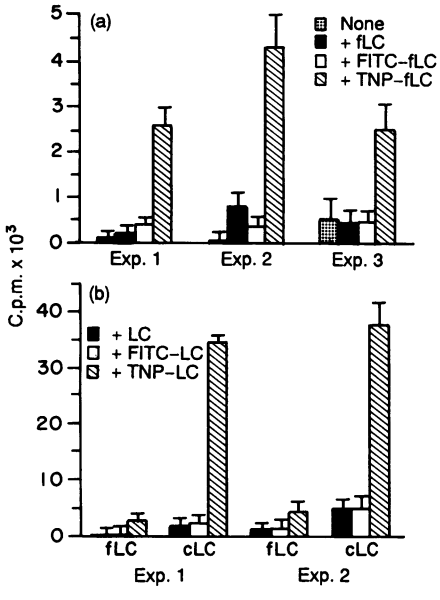

Figure 3. (a) Evidence of *in vitro* T-cell sensitization to TNP using hapten-modified fresh LC. T cells were incubated with TNP-modified fLC for 9 days. Viable lymphocytes (10^5) were then restimulated or not for an additional 3 days with non-treated, FITC- or TNP-modified fresh LC (5×10^3 LC). T-cell proliferation was assessed by [^{3}H]thymidine incorporation for the final 18 hr of culture. Results are expressed as c.p.m.±SD of triplicate cultures. (b) Evidence of *in vitro* T-cell sensitization to the TNP hapten using either fresh or cultured TNP-modified LC. Viable T cells were recovered from a 9-day primary culture with TNP-modified fLC. T cells (10^5) were then restimulated for 3 days with fresh or 2-day incubated LC (5×10^3 LC) which have been either non-treated, TNP- or FITC-coupled. T-cell proliferation was assessed by [^{3}H]thymidine incorporation for the final 18 hr of culture. Results are expressed as c.p.m.±SD of triplicate cultures.

T cells to the hapten. To rule out this possibility, naive T cells were separated from memory T cells by the use of specific cell surface marker:[19] CD45RA$^+$ T lymphocytes were enriched from T-cell suspensions by a panning method and used as responder cells. As shown in Fig. 2, these naive T cells were quite able to proliferate in the presence of TNP-cLC, therefore confirming *in vitro* naive T-cell sensitization to the hapten.

Inhibitory effect of mAb on T-cell primary response to TNP-cLC

Several mAb were tested at a concentration of 1 or 10 μg/ml for their ability to suppress the T-cell primary response to TNP-cLC. As shown in Table 1, when added at the beginning of the sensitizing culture, anti-class II and I as well as anti-CD4 and CD8 mAb were strongly inhibitory. A clear inhibitory effect was also observed using anti-ICAM-1 and LFA-3 molecules. By contrast, proliferation in the presence of anti-CD1a mAb was similar to that observed with the irrelevant control mAb.

In vitro secondary response to TNP-modified fresh LC

In order to determine whether the T cells recovered after a 9-day primary culture with TNP-modified fresh LC could have been primed without proliferating, these lymphocytes were restimulated *in vitro* with thawed and TNP-modified fresh autologous LC. Figure 3a shows data of independent triplicate experiments, each of them performed with different healthy donors. We regularly observed that, after a primary culture with fresh TNP-LC, a low but significant secondary T-cell proliferation was obtained following restimulation of T lymphocytes with hapten-conjugated fresh LC. This response was TNP specific, as shown by the absence of T-cell proliferation in the presence of fresh LC either non-modified or treated with an irrelevant hapten such as FITC, therefore providing evidence for *in vitro* T-cell sensitization to TNP-fLC.

cLC are more potent APC than fLC for *in vitro* primed T lymphocytes

We then compared the ability of both fresh and 2-day incubated LC to present TNP to *in vitro*-primed T cells: T lymphocytes were incubated with TNP-modified fLC for 9 days. Viable T cells were then restimulated for an additional 3 days with hapten-treated fresh or cultured LC. As shown in Fig. 3b, as expected, it was possible to significantly reveal the sensitization to TNP with fresh LC (sketched columns for fLC). However, the magnitude of the hapten-specific proliferative reaction was considerably enhanced by the use of TNP-cLC (sketched columns for cLC). This secondary T-cell response, when elicited by human hapten-modified cultured LC, was usually 10-fold much greater than that using fresh LC for restimulation. It should be noted that in these two representative experiments, the autologous response to non-treated cultured LC was superior to that obtained in the presence of non-modified fresh LC.

Development of hapten-specific T-cell lines

After secondary stimulation with hapten-modified cLC, we tried to develop T-cell lines by weekly expansion with non-specific mitogenic stimulations (PHA, IL-2 and irradiated allogeneic PBMC). After 1 month, these T cells were tested for

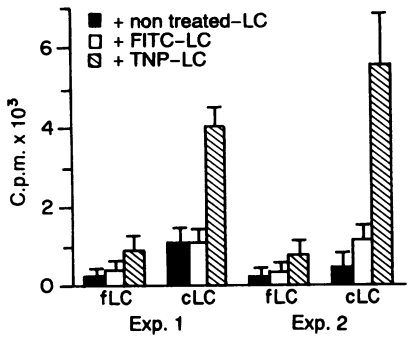

Figure 4. Hapten-specific proliferation of a T-cell line. T cells, *in vitro* primed by TNP-fLC and further restimulated by TNP-cLC, were expanded with PHA, IL-2 and irradiated allogeneic PBMC for 1 month. This T-cell population was then tested for hapten specificity with both fresh and 2-day cultured LC. T-cell proliferation was assessed by [^{3}H]thymidine incorporation for the final 18 hr of culture. Results are expressed as c.p.m. ± SD of triplicate cultures.

hapten specificity. Figure 4 shows that T-cell lines kept their specificity towards the sensitizing TNP hapten and were able to proliferate with TNP-fLC. However, as reported in secondary responses, T cells exhibited stronger proliferative reactions when stimulated by TNP-cultured LC. FACS analysis of a cell line surface antigens revealed a heterogeneous T-cell population, composed mainly of CD4 positive cells: 79% CD4$^+$ T cells versus 20% CD8$^+$ T cells. No significant increase of TcR$\gamma\delta$-bearing T cells (5%) was noticed in this population, compared to the percentage of TcR$\gamma\delta^+$ T cells usually observed in peripheral blood. TcR$\alpha\beta^+$ cells (94%) were the major population of the T cells.

DISCUSSION

Primary contact of T cells with an antigen on APC results in the development of antigen-specific T lymphocytes, which upon secondary exposure to this immunogen will initiate a more rapid and vigourous immune response. It has now become evident that in contact sensitivity (CS), a form of delayed-type hypersensitivity reaction involving epicutaneously applied haptens, the APC function is achieved by LC,[20] the dendritic cells (DC) from epidermis. It was shown that following topical exposure of mice to sensitizing chemicals, antigen-bearing LC leave the skin and rapidly accumulate in the draining lymph nodes.[21] The hapten-bearing DC isolated from lymph nodes were able to induce CS in naive syngenic mice.[22] Lastly, that these antigen-bearing lymph node DC are derived from epidermal Langerhans' cells and participate in the initiation of the T-cell response has been demonstrated directly in the murine system by a study using nude mice bearing allogeneic skin grafts.[4]

In a recent report,[23] Hauser *et al.* first used murine cultured LC to generate *in vitro* hapten-specific T cells from non-sensitized mice. In the present study, we provide the first direct evidence for the ability of human Langerhans' cells to induce *in vitro* primary sensitization of naive T cells to hapten.

We showed that both freshly prepared and cultured human LC were able to sensitize resting T lymphocytes to TNP *in vitro*. This was evidenced by the capacity of T cells, recovered from an *in vitro* primary culture, to proliferate in secondary responses when restimulated with TNP-modified LC. We observed, however, that cultured LC were far more efficient APC than fresh LC in eliciting *in vitro* TNP-specific T-cell responses. Indeed, under secondary stimulation, cLC supported a greatly enhanced T-cell proliferation to the relevant hapten, compared to fresh LC. Moreover, we showed that, contrary to fLC, cLC were able to induce substantial primary proliferative T-cell response to TNP. This was confirmed by using a CD45RA$^+$ T-cell subset as responder, therefore demonstrating *in vitro* naive T-cell sensitization to the hapten.

When comparing the stimulating activity of similar numbers of TNP-modified fresh LC, cultured LC, B cells or monocytes from the same donor, we found that among these APC, only cLC were capable of inducing significant primary *in vitro* T-cell sensitization (data not shown), thus emphasizing the efficiency of cLC APC function.

Several mechanisms could be considered to explain the potent APC function of cLC, compared to fresh LC, in generating *in vitro* hapten-specific T-cell proliferative responses. Cultured human LC are known to increase the expression of MHC class I and II molecules at the cell surface.[5,6,17] In the case of non-peptidic antigens, the nature of the antigenic complex which is recognized by the T-cell receptor is not exactly elucidated, but many reports have indicated the importance of MHC class I and II molecules in this process, and the present blocking experiments with mAb are in line with this view. Previous studies suggested that TNP can directly bind to MHC antigens at the cell surface.[24] More recent studies performed with TNP-specific cytotoxic T-cell clones indicated that, as for nickel,[25] a significant proportion of TNP determinants for T cells were anchored to MHC molecules via peptides.[26] The enhanced density of MHC class I and II molecules on cultured LC might be therefore a decisive factor in terms of TNP-specific T-cell activation. It should be noted, however, that in the murine system, TNP-modified dendritic cells enriched from spleen were fully capable of generating primary *in vitro* T-cell sensitization, whereas a B-cell line was not.[27] As these two cell populations exhibited similar HLA class II antigen expression, it was concluded that APC function was not simply related to MHC class II molecule density.

It has now become evident that recognition of the antigenic complex by the TcR is in most cases insufficient to result in T-cell activation and proliferation. Especially in primary immune responses, the APC must convey additional signals to the T cells in the form of ligand–receptor interactions or delivery of soluble cytokines.[28] There is now a body of data showing that interaction of ICAM-1 and LFA-3 molecules with their specific ligand (LFA-1 and CD2, respectively) on the T cells can serve as co-stimulatory signals in T-cell activation.[29] It is noteworthy that these antigens, weakly expressed on freshly prepared human LC,[6,30] were reported to be significantly increased after *in vitro* incubation.[5,6] Using electron microscopy analysis, we confirmed these findings[17] and the present blocking experiments using mAb support a role for these adhesion molecules in cLC-enhanced APC function.

An alternative explanation for the enhanced cLC APC function might be that, compared to fresh LC, cLC were shown to cluster more efficiently to the T cells even in the absence of antigen,[31] which is a characteristic feature of mature DC.[32] The putative molecules responsible for this initial antigen-independent clustering are still unknown and, in the mouse, this event cannot be blocked with anti-LFA-1 mAb.[33] It may be possible,

therefore, that as yet undefined additional adhesion molecules on cultured human LC facilitate their interaction with the T cells.

In the present system, using TNP-cLC as APC in secondary responses, we showed that *in vitro*-sensitized T cells can be expanded without losing hapten specificity and that the TNP-specific T-cell lines were mostly of the $CD4^+$ phenotype. Earlier studies have provided evidence that in the mouse, CS involved both $CD4^+$ and $CD8^+$ effector T lymphocytes. On the one hand, striking evidence has accumulated showing that $CD4^+$ cells mediate contact sensitivity to TNP and that these cells are capable of transferring CS to normal syngeneic mice.[34,35] On the other hand, *in vivo* T-cell subset depletions with mAb have demonstrated that the $CD8^+$ population has also an effector role in CS.[36] Whether in the present *in vitro* model, both $CD4^+$ and $CD8^+$ T-cell populations can have an effector role remains to be determined. Development of T-cell clones from TNP-specific T-cell lines is in progress to clarify this point.

An as yet unresolved question about hapten presentation by LC is whether or not hapten requires uptake and processing before presentation by LC. A TNP-modified digest of bovine serum albumin (BSA) has been shown to bind to MHC class I molecules and to be capable of stimulating a TNP-specific hybridoma even when the stimulating cells are prefixed with glutaraldehyde, suggesting that this hapten does not require further cellular processing.[26] Otherwise, murine cultured LC were shown to be defective in processing of exogeneous proteins.[11,12] That human cLC are far more effective APC than fresh LC in our system might contradict this assumption. Alternatively, this might be an indirect evidence that hapten presentation does not require cellular processing for T-cell presentation. Further studies are needed to resolve this important point.

In conclusion, this *in vitro* model emphasizes the key role of human LC in the induction of contact hypersensitivity reactions. The finding that *in vitro*-cultured LC are more potent APC than fresh LC support the idea of a functional *in vitro* maturation of human LC, as described in the murine system.[9] This maturation may represent an essential step in the afferent phase of the immune response, leading to hypersensitivity skin reactions.

Lastly, the present assay, showing a potentiation of *in vitro* T-cell responses by using cLC as APC, might prove to be very useful for studying the early steps of T-cell sensitization and subsequently to develop *in vitro* predictive tests allowing detection of sensitizing compounds.

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