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Murine epidermal Langerhans cells are potent stimulators of an antigen-specific T cell response to *Leishmania major*, the cause of cutaneous leishmaniasis*

Cutaneous leishmaniasis is initiated by the bite of an infected sandfly and inoculation of *Leishmania major* parasites into the mammalian skin. Macrophages are known to play a central role in the course of infection because they are the prime host cells and function as antigen-presenting cells (APC) for induction of the cell-mediated immune response. However, in addition to macrophages in the dermis, the skin contains epidermal Langerhans cells (LC) which can present antigen (Ag) to T cells. Therefore, using a murine model of cutaneous leishmaniasis, we analyzed the ability of epidermal cells to induce a T cell response to *L.major*. The results demonstrated that freshly isolated LC, but not cultured LC, are highly active in presenting *L.major* Ag *in vitro* to T cells from primed mice and to a *L.major*-specific T cell clone. Furthermore, freshly isolated LC had the ability to retain *L.major* Ag in immunogenic form for at least 2 days. Their efficiency was much greater than that of irradiated spleen cells, a standard population of APC. LC stimulated both T cell proliferation and production of the lymphokines interleukin (IL)-2 and IL-4. The response was Ag specific and could be induced by lysate of *L.major* parasites and by live organisms. The data suggest that epidermal LC are important APC in cutaneous leishmaniasis. They may perform a critical function by capturing *L.major* Ag in the skin and presenting it either to quiescent T cells circulating through the draining lymph node or locally to T effector cells infiltrating the cutaneous lesion.

1 Introduction

Leishmaniases are infectious diseases caused by protozoan parasites of the genus *Leishmania* that alternate between the promastigote form in the sandfly vector and the obligatory intracellular amastigote form residing in the phagolysosomes of mammalian MΦ. Parasite Ag can be detected on the surface of infected MΦ [1, 2] and, in addition to its role as a host cell, the MΦ thus serves as APC for induction of an Ag-specific T cell response. The T cell-dependent immunity is crucial for the outcome of the disease because activated T cells release various cytokines that promote either the spreading or the elimination of parasites in infected MΦ [3–5]. Depending on the genetic background of the mammalian host and on the species of parasite, the infection may be restricted to the skin with healing occurring spontaneously, it may disseminate progressively and affect the mucous membranes, or it may visceralize and finally result in the death of the infected individual. In all these situations, infection is caused by the bite of an infected sandfly and initially produces a cutaneous disease.

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Abbreviations: **EC:** Epidermal cells **LC:** Langerhans cells

In addition to MΦ residing in the dermis, the epidermis harbors Langerhans cells (LC) which are capable of presenting Ag to T cells [6]. Resident LC constitutively express MHC class II molecules. When freshly isolated from the skin, they can process native Ag and present it to sensitized T cells [7, 8]. Furthermore, they have the unique ability to retain the Ag in an immunogenic form for at least an additional 2 days in culture [9]. Although freshly isolated LC are weak activators of resting T cells [7, 8], they develop into potent stimulators of a primary T cell response after 2 to 3 days of culture [10] in the presence of granulocyte/MΦ-CSF produced by keratinocytes [11]. These observations elaborate the concept that LC capture and process Ag in the skin for subsequent transport via the lymph to the regional LN [12, 13] and presentation to naive, Ag-specific T cells from the circulating pool. Furthermore, LC may present cutaneous Ag *in situ* to sensitized T cells that enter the epidermal compartment [14, 15].

To the best of our knowledge, there is no published information on the ability of LC to present parasite Ag to T cells. We consider the *Leishmania* model to be highly suitable for analysis of this function because the skin is the site of both entry of the parasite and initiation of the immune response. There have been reports on the distribution and turnover of LC during leishmaniasis [16–18] but their role as APC for regulation of the T cell-mediated immune response has not been investigated. In the present study, we used *Leishmania major*, the cause of human cutaneous leishmaniasis, for immunization and experimental infection of genetically susceptible BALB/c mice and we analyzed the efficacy of epidermal cells (EC) to present *L.major* Ag to T cells from these mice *in vitro*. Our data

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demonstrate that epidermal LC are highly active in inducing the proliferation and lymphokine production of *L.major*-specific T cells and may be critical APC regulating the cellular immunity during cutaneous leishmaniasis.

2 Materials and methods

2.1 Mice

Female mice of the inbred strain BALB/c were 6 to 10 weeks of age at the onset of experiments. All mice were purchased from Charles River Breeding Laboratories (Sulzfeld, FRG) and, during experimentation, were maintained under conventional conditions in an isolation facility.

2.2 Parasites, infection of mice and preparation of Ag

The origin and propagation of the *L.major* isolate have been described elsewhere [19]. The cloned virulent line used for this study was confirmed to be *L.major* by isoenzyme analysis (Dr. D. Evans, London School of Hygiene and Tropical Medicine, London, GB) and was maintained by passage in BALB/c mice. Promastigotes were grown *in vitro* in blood agar cultures. Stationary-phase promastigotes were washed in PBS and, for infection of mice, 2×10^7 organisms were injected in a volume of 50 µl i.d. on the dorsum close to the base of the tail. Amastigote suspensions were prepared from skin lesions 2 to 3 weeks later as described [20]. For the preparation of *L.major* lysate, stationary-phase promastigotes were subjected to three cycles of rapid freezing and thawing.

2.3 Culture medium

Click's RPMI 1640 medium (Gibco Laboratories, Eggenstein, FRG) was supplemented with 10 % FCS (Seromed-Biochrom, Berlin, FRG) or 0.5 % heat-inactivated mouse serum, 2 mM L-glutamine (Seromed-Biochrom), 10 mM Hepes buffer (Seromed-Biochrom), 100 µg/ml penicillin (Seromed-Biochrom), 160 µg/ml gentamycin (Seromed-Biochrom), 7.5 % $NaHCO_3$ and 5×10^{-5} M 2-ME.

2.4 Reagents and antibodies

The Ag myoglobin from horse skeletal muscle and CFA were purchased from Sigma Chemical Co., Deisenhofen, FRG. Con A (Serva, Heidelberg, FRG) was used for mitogenic stimulation of T cells. Rat mAb directed against I-A (b,d,q haplotypes) and I-E (d,k haplotypes), from hybridoma M5/114.15.2 [21], and anti-IL-4 mAb, from hybridoma 11B11 [22], were used as culture SN. Anti-IL-2 mAb, from hybridoma S4B6 [23], was used as ascites fluid. A FITC-conjugated goat anti-rat Ab (Medac, Hamburg, FRG) served as second-stage reagent for staining of MHC class II (Ia)-positive EC and their quantitation by fluorescence microscopy.

2.5 APC

Single-cell suspensions of EC were prepared from ear skin by trypsinization procedures as described [24]. A concentration of 1 % trypsin (40 min) was used for processing the ventral, thick ear halves, and 0.6 % trypsin (20 min) for the dorsal, thin ear halves. These preparations contained 2 % to 4 % Ia Ag-bearing LC. For depletion of Ia^+ cells, EC suspensions were incubated with anti-Ia mAb M5/114 for 30 min on ice followed by removal of mAb-coated cells with sheep anti-rat IgG coupled to magnetic beads (Dynabeads M-450; Dynal, Hamburg, FRG) using a magnet. Spleen cells were depleted of erythrocytes by incubation with an ammonium chloride solution (16 mM), and were irradiated with 2500 rad before use.

2.6 T cells

For the generation of Ag-primed LN cells, mice were immunized s.c. at the base of the tail and into the hind footpads with either *L.major* lysate (equivalent to 70×10^6 parasites) or myoglobin (150 µg) in CFA. After 8 days, inguinal and popliteal LN cells were collected and were restimulated with the respective Ag *in vitro* in bulk cultures [25]. This procedure reduces the syngeneic MLR that occurs in the absence of Ag when dendritic cells are co-cultured with primary T cells [26]. In 16-mm macrowells, 5×10^6 LN cells from myoglobin-immunized mice were restimulated with 5 µM myoglobin in 1.5 ml culture medium supplemented with 0.5 % heat-inactivated mouse serum and 2.5×10^6 LN cells from *L.major*-immunized mice were restimulated with parasite lysate (equivalent to 2×10^6 organisms) in culture medium supplemented with 10 % FCS for 10 days. Alternatively, when inguinal LN T cells from mice that had been infected with *L.major* for 2 weeks were used as responder cells, the cultures were supplied with live *L.major* promastigotes (5×10^6 per well) as a source of Ag and were incubated for 8 days. After expansion in bulk cultures, viable cells were isolated by floatation on Ficoll-Paque columns (1.077g/l; Pharmacia, Freiburg, FRG) and were used as responder cells at a dose of 1×10^5/microculture well.

The *L.major*-specific $CD4^+$ T cell clone L1/1 (kindly provided by Dr. M. Lohoff, our institute) was established from parasite-infected BALB/c mice by LD techniques and sequential stimulation with *L.major* lysate and lymphokine-containing culture SN as described [27]. Upon specific stimulation, L1/1 cells secreted IL-3, IL-4, IL-5 and IL-6, but not IL-2 or IFN-γ, thus showing the characteristics of T_h2 type cells. Before use as responder cells at a dose of 2×10^4/microculture well, L1/1 cells were rested for more than 14 days after the last restimulation with *L.major* lysate and irradiated spleen cells.

2.7 Assay for proliferation of T cells

T cells from Ag-primed mice or cloned *L.major*-specific T cells were cultured with various numbers of APC in the presence or absence of Ag. Culture was performed in 96-well flat-bottom microtiter plates (Nunc, Wiesbaden, FRG) in a final volume of 0.2 ml of medium supplemented with 0.5 % heat-inactivated mouse serum. After 48 to 60 h, [^{3}H] dThd (0.5 µCi/well; Du Pont De Nemours, Dreieich, FRG) was added for the final 18 h of culture. The cells were harvested onto filter strips using a semi-automated cell harvester and incorporation of [^{3}H] dThd was measured in a

liquid scintillation counter. Data were expressed as the arithmetic mean cpm ± SD of triplicate cultures.

2.8 Assay for IL-2 and IL-4 activity

T cells from Ag-primed mice or cloned *L.major*-specific T cells were cultured with various numbers of APC in the presence or absence of Ag. After 18 to 20 h, 50-µl aliquots of culture SN were collected and tested for the ability to stimulate proliferation of 3×10^3 cells of the IL-2- and IL-4-dependent mouse T cell line HT-2 [28] in a total volume of 0.1 ml in flat-bottom culture wells. Fourty-eight hours later, responsiveness was measured by using a modified MTT [3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide] assay [29]. One aliquot was incubated in the absence of Ab, to test for both IL-2 and IL-4 activities, and a second aliquot was tested in the presence of anti-IL-2 mAb, for the determination of IL-4 activity, or in the presence of anti-IL-4 mAb, for the determination of IL-2 activity. The detection threshold was 0.1 U/ml for IL-2 as well as for IL-4, as measured by standard titrations of recombinant lymphokines.

3 Results

3.1 EC are able to present Ag in *L.major* lysate to T cells from primed mice

Freshly isolated EC were compared with irradiated spleen cells, a standard population of APC that is generally used to study presentation of *L.major* Ag to mouse T cells. LN cells from BALB/c mice immunized with *L.major* lysate in CFA were used as responder cells. It was evident that EC elicited a strong proliferation of Ag-primed LN T cells in the presence of *L.major* lysate (Fig. 1A). This finding showed that APC derived from the epidermal compartment of the skin have the capacity to present *L.major* Ag and thus are functionally equivalent to irradiated spleen cells, a source of MΦ, dendritic cells and B cells.

Strikingly, the induction of an Ag-reactive T cell proliferation was optimal when freshly isolated EC were incubated in the presence of *L.major* lysate for 1 to 2 days prior to addition of sensitized T cells (Table 1). On the other hand, when freshly isolated EC were directly exposed to primed T cells that had been expanded in bulk cultures, they induced a strong proliferative response that was independent of the addition of Ag (Table 1). However, although the T cells collected from the bulk cultures were washed extensively, it can not be excluded that small amounts of *L.major* Ag had been carried over and, in the microcultures lacking exogenous Ag, could have been processed and presented by fresh EC (day 0) but not by EC that had already been cultured for 1 to 2 days [7]. Indeed, the data in Fig. 2 show that only freshly insolated EC, but not EC that had been cultured in the absence of Ag for 2 days before use as APC were able to present *L.major* lysate to primed T cells.

The capacity of EC to present *L.major* Ag was confirmed with cloned T cells. EC that had been cultured in the presence of parasite lysate for 2 days were potent stimulators of the MHC class II-restricted, *L.major*-specific $CD4^+$ T cell clone L1/1 (Fig. 1B). EC were much more effective in presenting *L.major* Ag to sensitized T cells than irradiated spleen cells, a standard population of APC. The optimal number of EC per microculture was 1.2 to 5×10^4 (Figs. 1 and 2), whereas for spleen cells it was 1×10^5 to 5×10^5 (data not shown). Furthermore, the maximal T cell response induced by EC was usually higher than that elicited by spleen cells.

To verify the Ag specificity of the EC-induced T cell response to *L.major* lysate, fresh EC were cultured with an irrelevant Ag (myoglobin) for 2 days before addition of *L.major*-primed T cells. The data in Table 2 show that EC were highly active in presenting *L.major* Ag to parasite-primed T cells but did not stimulate the same responder cell population in the presence of myoglobin or in the absence of Ag. Conversely, EC failed to present *L.major* lysate to myoglobin-primed T cells. Thus, the T cell stimulatory effect of *L.major*-pulsed EC was Ag specific.

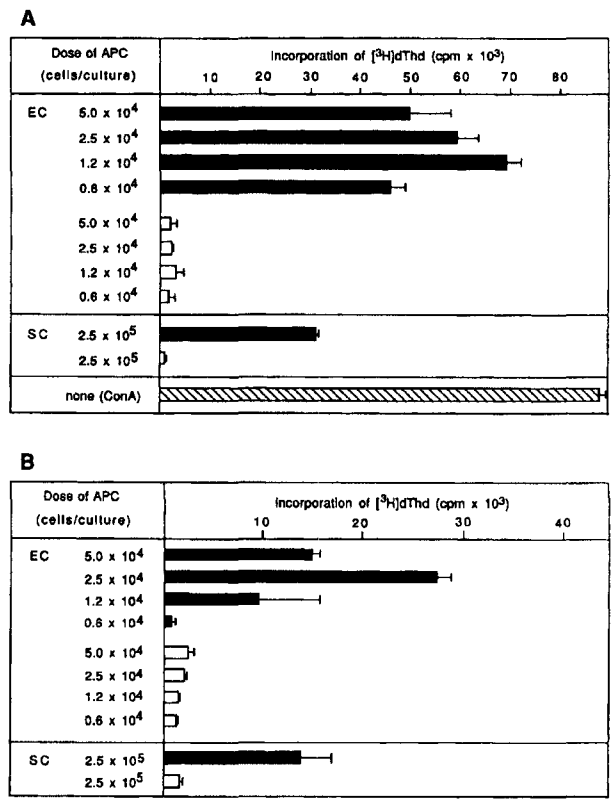

Figure 1. EC present *L.major* Ag to T cells from primed mice (A) and to *L.major*-specific cloned T cells (B). LN cells from mice immunized with *L.major* lysate in CFA were expanded with parasite lysate in bulk cultures and, 10 days later, were used as responder cells at a dose of 1×10^5/microwell (A). Alternatively, 2×10^4 cells/microwell of the *L.major*-specific T cell clone L1/1 were used (B). They were added to graded doses of EC that had been incubated in the absence (white bars) or presence (dark bars) of *L.major* lysate (equivalent to 2×10^5 parasites per microwell) for 2 days, or to irradiated spleen cells (SC) that had been freshly isolated. Cultures containing Con A (2.5 µg/well) served as a control for polyclonal T cell activation. Incorporation of [^{3}H] dThd was determined at 60 to 78 h (A) or at 48 to 66 h (B). The data represent means ± SD of triplicate cultures.

Table 1. Induction of *L.major*-reactive T cell proliferation *in vitro* is dependent on preincubation of EC with Ag[a)]

APC	Preincubation with Ag	Dose of APC (cells/well × 10^{-4})	[^{3}H] dThd incorporation (cpm × 10^{-3})	
			L.major lysate	No Ag
EC	2 days	6	56.7	4.8
		3	59.3	9.8
		1	11.8	3.6
		0.5	0.6	0.4
EC	0 days	3	53.2	49.0
		1	59.9	40.8
		0.5	18.1	6.2
		0.1	0.9	0.5
SC	0 days	25	20.4	2.1

a) LN cells from mice immunized with *L.major* lysate in CFA were restimulated in bulk cultures and 10 days later, 1 × 10^5 cells/microwell were added to graded doses of EC that had been preincubated with Ag (equivalent to 2 × 10^5 parasites) for 2 days or, alternatively, were added simultaneously with Ag to freshly isolated EC or to irradiated spleen cells (SC). [^{3}H] dThd incorporation was determined at 60 to 78 h. Not shown are the data obtained after preincubation of EC for 1 day, which gave results similar to those after a 2-day culture.

3.2 EC present Ag derived from viable *L.major* parasites to T cells from infected mice

To determine the relevance of our findings with parasite lysate for the T cell response to virulent parasites in the course of cutaneous disease, we examined the reactivity of T cells recovered from *L.major*-infected mice to EC cultured with viable *L.major* amastigotes or promastigotes. The results in Fig. 3 show that T cells from LN draining the site of cutaneous infection proliferated vigorously in response to *L.major* Ag presented by EC. For the level of responsiveness, it was irrelevant whether parasite lysate or intact amastigotes or promastigotes were used as the source of Ag for *in vitro* restimulation of T cells. Thus, EC were able to present Ag derived from viable parasites.

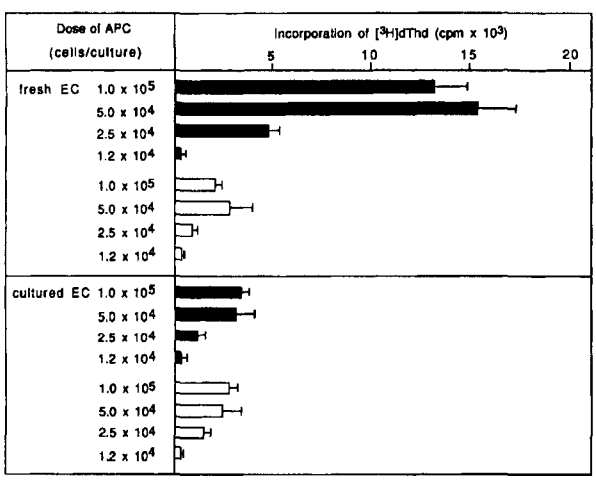

Figure 2. Only freshly isolated EC but not cultured EC present *L.major* Ag to T cells from primed mice. LN cells from mice immunized with *L.major* lysate in CFA were expanded with parasite lysate in bulk cultures. Ten days later, 1 × 10^5 cells/microwell were added to graded doses of EC. Freshly isolated EC (top) were mixed with Ag, and T cells were added 2 days later. Cultured EC (bottom) were mixed with both Ag and T cells 2 days after isolation. Dark bars represent triplicate cultures ± SD set up in the presence of Ag, white bars represent the controls set up in the absence of Ag. Incorporation of [^{3}H] dThd was determined 60 to 78 h after addition of T cells.

3.3 EC induce lymphokine production by *L.major*-reactive T cells

Since the production of lymphokines is a functionally important attribute of MHC class II-restricted T cells, we tested the culture SN of Ag-primed T cells and of cloned *L.major*-specific T cells for IL-2 and IL-4 activity. Bulk populations of primed LN T cells released high amounts of lymphokines in response to EC presenting *L.major* Ag derived either from parasite lysate or from intact amastigotes (Fig. 4A). Similarly, EC stimulated lymphokine production by the *L.major*-specific T cell clone L1/1 (Fig. 4B). In the presence of mAb to IL-2 or to IL-4, the lymphokine activity of short-term cultured LN T cells was partially blocked and, therefore, could be attributed to both lymphokines. On the other hand, the activity of the T_h2 cell clone L1/1 was only affected by treatment with anti-IL-4 but not by anti-IL-2 and thus was confirmed to be IL-4 (Fig. 4).

3.4 The ability of EC to present *L.major* Ag to T cells can be attributed to LC

The above findings document that APC derived from the epidermal compartment of the skin are potent stimulators

Table 2. The T cell stimulatory effect of EC is Ag specific[a)]

Ag for immunization and bulk culture	[^{3}H] dThd incorporation (cpm × 10^{-3} ± SD) after restimulation with		
	L.major	Myoglobin	No Ag
L.major	58.7 ± 4.1	1.9 ± 0.2	2.3 ± 0.2
Myoglobin	2.3 ± 0.2	10.5 ± 0.8	2.2 ± 0.3

a) LN cells from mice immunized with either *L.major* lysate or myoglobin in CFA were restimulated with the respective Ag in bulk cultures. For the subsequent proliferation assay, 1 × 10^5 T cells/microwell were added to 2.5 × 10^4 EC that had been preincubated either with the Ag that had been used for immunization and expansion in bulk cultures or with the irrelevant Ag. *L.major* lysate was used at a concentration of 2 × 10^5 parasites/microwell, myoglobin was used at 5 μM. [^{3}H] dThd was added at 60 to 78 h.

Table 3. LC are required for presentation of *L.major* Ag to T cells[a)]

Treatment of EC	Ag	[^{3}H] dThd incorporation of T cells with graded doses of EC (cpm × 10^{-3} ± SD) 5×10^4	2.5×10^4	1.2×10^4
None	–	1.28 ± 0.48	0.23 ± 0.10	0.22 ± 0.08
	+	**9.62** ± 1.40	**1.34** ± 0.57	0.19 ± 0.06
Beads	–	0.73 ± 0.30	0.31 ± 0.16	0.11 ± 0.03
	+	**6.91** ± 1.06	**1.86** ± 0.38	0.24 ± 0.13
Anti-Ia, beads	–	1.15 ± 0.17	0.35 ± 0.12	0.10 ± 0.02
	+	1.25 ± 0.26	0.34 ± 0.13	0.21 ± 0.14

a) Twenty thousand cells per microwell of the *L.major*-specific T cell clone L1/1 were added to graded doses of EC that had been incubated in the absence or presence of *L.major* lysate (equivalent to 2×10^5 parasites) for 2 days. The EC populations were either untreated or had been depleted of Ia$^+$ LC by sequential incubation with rat anti-Ia mAb and magnetic beads coated with anti-rat Ig Ab. Ia-depleted EC contained <0.1 % Ia$^+$ cells. [^{3}H] dThd was added at 48 to 66 h.

of an Ag-specific T cell response to *L.major*, a parasite causing cutaneous infection. In the normal epidermis, LC are the only cells expressing Ia Ag and, therefore, the sole candidates for induction of an MHC class II-restricted cellular immune response. However, epidermal keratinocytes can bind substantial amounts of Ag [9] and it has been shown that T cell responses can be induced in the absence of MHC products [30]. It was, therefore, important to identify the APC responsible for the EC-mediated T cell activation in our assay system. To test for the role of LC, Ia$^+$ cells were depleted from EC populations by sequential treatment with rat anti-Ia mAb and magnetic beads coated with Ab against rat Ig. Because Ia$^-$ EC were not capable of inducing a T cell response to *L.major* Ag (Table 3), we conclude that LC are responsible for the ability of EC to present *L.major* Ag to T cells.

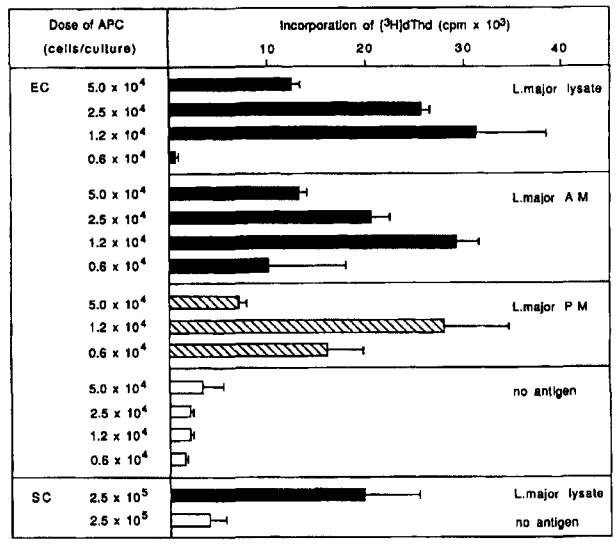

Figure 3. EC present *L.major* Ag to T cells from infected mice. LN cells from genetically susceptible BALB/c mice that had been infected with *L.major* for 14 days were expanded with viable *L.major* promastigotes in bulk cultures and, 8 days thereafter, were restimulated in vitro with lysed parasites or with viable *L.major* promastigotes (PM) or amastigotes (AM). One hundred thousand cells per microwell were added to graded doses of EC that had been preincubated in the absence or presence of the respective source of Ag (2×10^5 parasites or equivalent) for 2 days, or to irradiated spleen cells (SC) that had been freshly isolated. [^{3}H] dThd incorporation was determined at 60 to 78 h.

4 Discussion

Members of the dendritic cell lineage express high levels of MHC class II molecules and are potent stimulators of T cell-mediated immune responses. They occur in low numbers in lymphoid as well as in non-lymphoid organs. In the present study, we examined LC, the dendritic cells of the

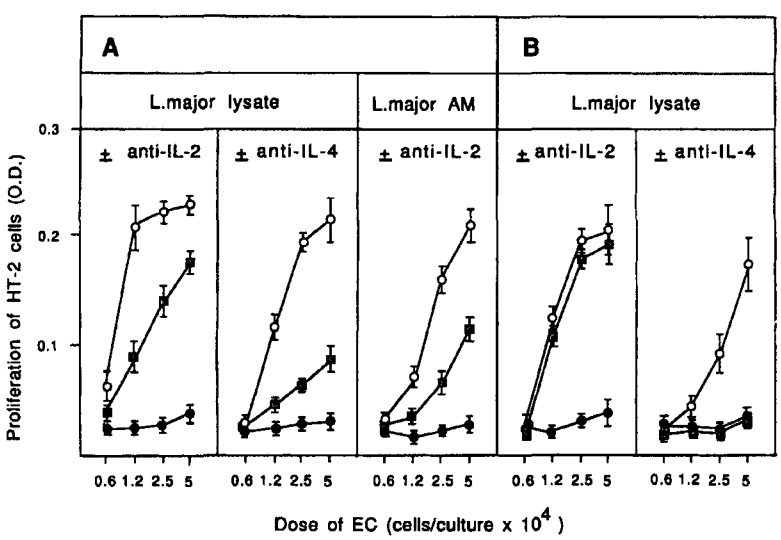

Figure 4. EC induce lymphokine production by *L.major*-reactive T cells. LN cells from mice immunized with *L.major* lysate in CFA were expanded with parasite Ag in bulk cultures and, 10 days thereafter, were used as responder cells at a dose of 1×10^5/microwell (A). Alternatively, 2×10^4 cells/microwell of the *L.major*-specific T_h2 clone L1/1 were used as responder cells (B). The T cells were added to graded doses of EC that had been incubated in the presence (○, □) or absence (●) of *L.major* Ag for 2 days, and after 18 to 20 h, 50-µl aliquots of culture SN were collected and tested for the ability to stimulate the proliferation of the lymphokine-dependent cell line HT-2. One aliquot of the SN from Ag-stimulated cultures was incubated in the absence of Ab (○) and a second aliquot was tested in the presence of either anti-IL-2 or anti-IL-4 mAb (□), as indicated in the figure.

skin, for their ability to present Ag of *L.major*, the protozoan parasite causing cutaneous leishmaniasis. The data demonstrated that epidermal LC are highly active in inducing the *L.major*-specific proliferation and lymphokine production of parasite-primed T cells. Thus, LC may be crucial for the regulation of T cell-mediated immune reactions during cutaneous leishmaniasis.

To assess the efficiency of epidermal APC, we used unselected EC populations which contain 2% to 4% LC. The heterogeneous EC were remarkably active in presenting *L.major* Ag to primed T cells because the doses required for optimal responses were equivalent to merely 250 to 2000 LC per culture. After depletion of LC from the EC population, the T cell-stimulatory effect was completely abrogated confirming that it could indeed be attributed to LC. Thus, LC were 100 to 1000 times more effective than irradiated spleen cells, a standard population of APC that is generally used to study the *in vitro* T cell responsiveness to *L.major* Ag in mice. Presentation of *L.major* Ag by epidermal LC appears to be particularly efficient because, using the same protocol, the proliferation of primed T cells in response to *L.major* was routinely 5 to 10 times higher than the response to myoglobin, and the numbers of LC required for maximal activities were significantly lower than those reported by other authors using exogenous Ag [7–9, 31].

Tissue dendritic cells, *e.g.* LC in the epidermis, are thought to be precursors of dendritic cells in lymphoid organs [14, 15]. The two forms have unique functional properties with lymphoid dendritic cells being superior in the stimulation of allogeneic and of syngeneic T cells as well as of primary immune responses, and epidermal LC being more efficient at processing native Ag [7, 10, 32]. Freshly isolated LC would be the *in vitro* equivalents of intraepidermal LC, and LC cultured for 2 to 3 days would be the *in vitro* equivalents of LC that have left the epidermal compartment for migration to the draining LN while developing into lymphoid dendritic cells [14]. Recent evidence suggests a causal relationship between the ability to process Ag and the presence of numerous acidic organelles such as endosomes in freshly isolated LC [33]. Furthermore, the synthesis of MHC class II molecules is high in fresh LC, but switched off in cultured LC [9, 34, 35]. Interestingly, T cell-stimulating *Leishmania* Ag have been reported to be presented only in association with newly synthesized class II molecules [36]. Our present results are consistent with these concepts because *L.major* Ag could be presented only by freshly isolated EC, but not by cultured EC. This strongly suggests that LC residing in the skin are capable of processing and presenting *L.major* Ag.

In addition to the finding that only freshly isolated EC are able to utilize *L.major* Ag, we could show that their APC function for T cells was conserved when they were preincubated in the presence of Ag for a period of 1 or 2 days. This confirms the observation of Puré et al. [9] that LC have the unique ability to retain Ag in an immunogenic form for at least 2 days. In consideration of the migratory and stimulatory function of LC *in situ*, this unusual feature may be of central importance. It is conceivable that LC capture Ag encountered in the skin and retain it for transport to the regional LN and presentation to T cells [12–15]. Indeed, we have experimental evidence for migration of dendritic APC from the infected skin to the regional LN during cutaneous leishmaniasis (Fuchs, Röllinghoff and Moll; manuscript in preparation).

As compared with the level of endocytosis exhibited by MΦ, dendritic cells show weak endocytic activity [37]. Nevertheless, LC have been shown to internalize and process native Ag [9]. Our finding that EC can present Ag in *L.major* lysate as well as Ag derived from intact amastigotes or promastigotes emphasizes the ability of epidermal LC to take up and process complex structures. Degradation of parasite Ag by LC is furthermore suggested by the fact that cultured LC, which are known to have lost Ag processing capacity [7, 9, 15], could not present *L.major* to Ag-specific T cells. In the experimental system used for the present study, it can not be determined whether LC internalized whole parasites. Alternatively, they may have aquired soluble Ag released by viable parasites [38] or degradation products of parasites that had died in culture. Nonetheless, because all these sources of Ag are likely to be available during natural infection in the skin, either possibility is consistent with the conclusion that LC have the potency of handling *L.major in vivo*. In this context, however, it is noteworthy that LC have been reported to contain *Mycobacterium leprae* organisms in human biopsy specimen [39]. Furthermore, we have recently been able to demonstrate that murine LC can be infected by *L.major* both *in vitro* and *in vivo* (Blank, Fuchs, Röllinghoff and Moll; manuscript in preparation).

Previous studies have shown that a lipophosphoglycan from *L.major* can vaccinate mice against cutaneous leishmaniasis [40, 41]. It is, therefore, of particular interest that EC did not only induce a T cell response to crude *L.major* Ag and to intact parasites, but also stimulated a low level of T cell reaction to highly purified *L.major* lipophosphoglycan (Moll et al., unpublished observation). This preliminary finding extends previous reports by us [42, 43] and by others [44–46] implicating lipophosphoglycan in T cell immunity. The T cell recognition of this host-protective non-protein Ag presented by epidermal LC raises important questions with respect to the mechanisms of its processing and association with MHC molecules. Current work addresses these issues.

In experimental cutaneous leishmaniasis, resistance and susceptibility to disease can be attributed to $CD4^+$ T cell populations with different patterns of lymphokine activity [3–5]. Production of IFN-γ seems to promote protection, whereas IL-4 has been suggested to facilitate survival of the parasites. The intriguing possibility exists that different types of APC selectively stimulate different subsets of $CD4^+$ T cells. In fact, it has been reported that hapten-modified cultured LC preferentially stimulate the generation of IL-4-producing T cell lines of the T_h2 type [47]. Our current data demonstrate that freshly isolated LC are able to present *L.major* Ag to cloned T_h2 cells producing IL-4 as well as to short-term cultured T cells producing both IL-2 and IL-4. In the latter situation, however, additional cycles of restimulation may be required to detect the selection of T_h1 and/or T_h2 type cells by EC presenting *L.major* Ag. In addition, different types of *L.major* Ag may favor the expansion of disparate T cell subsets. The experimental

system established in the current study provides a valuable tool for analysis of these aspects.

This is the first report suggesting an important role of LC during cutaneous leishmaniasis. We propose that LC are responsible for capturing *L.major* Ag in the skin, processing them and transporting them to the LN draining the site of infection. During migration, they develop into potent APC with the capacity to initiate the *L.major*-specific T cell response. Furthermore, they may present parasite Ag to effector T cells infiltrating the cutaneous lesion and may thus be involved in regulation of the local immune response.

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