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Characterization of human skin-derived CD1a-positive lymph cells

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Abstract **The phenotype and function of CD1a$^+$ lymph cells is of considerable interest. By means of microsurgical lymph cannulation human lymph derived from normal skin was sampled. Cells were isolated and processed for immunocytochemistry, electron microscopy, flow cytometry and functional assays. The majority of the cells, (62%), were T cells. The other cells comprised CD1a$^+$ cells (7%), monocytes/macrophages (8%), and B cells (1%); the remainder were erythrocytes or uncharacterized cells. The CD1a$^+$ cells reacted with antibodies against protein S-100, HLA-DR, the Lag antigen, CD4, CD11a, CD11b, CD18, CD25, CD40, CD54, CD80 and CD86. Interestingly, a small prolow portion the of CD1a$^+$ cells (about 5%) reacted with an antibody to CD14. The CD1a$^+$ cells did not react with an antibody against human follicular dendritic cells nor were they CD19-, CD23-, E-cadherin- or factor XIIIa-positive. Both allogenic and antigen-specific T cell proliferation stimulated by antigen-presenting lymph cells were strongly inhibited by adding anti-CD80 and anti-CD86 antibodies. By electron microscopy Birbeck granules were detected in only 22% of the CD1a$^+$ lymph cells and these cells exhibited an extensive ruffling of the surface. These findings demonstrate that CD1a$^+$ lymph cells, which do not express the dermal dendritic cell marker factor XIIIa, resemble dendritic cells formerly designated as 'veiled' as well as lymphoid dendritic cells, suggesting that after migration to the regional lymphoid organs, Langerhans cells form a more differentiated population of dendritic cells specialized in sensitizing T lymphocytes. Our results add further support to the view that resident Langerhans cells may be precursors of lymphoid dendritic cells acquiring the final phenotype in the microenvironment of the lymph node.**

Key words Skin-derived lymph · Dendritic cells · Langerhans cells · Dermal dendritic cells · Lymphoid cells

Abbreviations *BG* Birbeck granules · *DC* dendritic cells · *LC* Langerhans cells

Introduction

Dendritic cells (DC) are highly efficient antigen-presenting cells and thus play a crucial role in the immune system (Teunissen 1992). They originate from the bone marrow and are found as a trace population in nonlymphoid tissue, in the circulation (blood, afferent lymph) and in lymphoid organs. DC demonstrate a typical dendritic and 'veiled' morphology, constitutively express high levels of major histocompatibility complex class II molecules, and show an outstanding capacity to initiate primary immune responses (Romani and Schuler 1992). As a consequence of different, but not yet fully elucidated stimuli, DC migrate from the nonlymphoid tissues, where they reside in the immature state, via the afferent lymphatics or the blood to the T cell-dependent areas of the Iymphoid organs. There, they appear as mature DC (Romani and Schuler 1992).

There is increasing evidence that DC, according to their specific location in different cutaneous compartments, i.e. epidermis, dermis or afferent lymph of the skin, and their state of maturation have distinct morphological, phenotypic and functional properties. Although the presence of such distinctive subsets of DC in cutaneous tissue is becoming increasingly recognized, DC in human afferent skin lymph have so far been poorly characterized.

We have established a human in vivo system for long-term collection of skin-derived lymph (Brand et al. 1992)

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allowing us to study the cells and soluble mediators that are part of the traffic from the skin to the regional lymph nodes. The investigation of such skin lymph is of considerable interest since it offers the possibility of obtaining an insight into signal transmission between skin and lymphoid tissue and helps to elucidate the processes essential for the function of the skin immune system.

In this study, we present data on the cellular composition of human afferent skin lymph with emphasis on the characterization of $CD1a^+$ positive lymph cells.

Materials and methods

Experimental design

A total of 60 different lymph samples from the normal untreated skin of 12 volunteers, aged from 24 to 32 years, were investigated. The study was approved by the Ethics Committee of the Faculty of Medicine of the University of Bern; written informed consent was obtained from the volunteers. The technique used for cannulation of a lymph vessel on the lower leg exclusively draining a defined skin area on the medial and upper part of the foot, has been described previously (Brand et al. 1992). Briefly, a superficial lymph vessel on the lower leg was isolated for 1–2 cm, and dissected free of fat and fibrous tissue. The tip of a specially prepared polyethylene tube was inserted in the distal direction. After closure of the wound, the other end of the polyethylene tube was immersed in 1 ml 0.9% NaCl containing 20 IU heparin in a 10-ml sterile plastic vial. The vial was taped to the calf and the leg was covered with an elastic bandage. Lymph was allowed to flow freely and was collected at 8 a.m. and 5 p.m. each day. The volume was measured and the cells counted. The cells were then separated by centrifugation at 300 *g* for 5 min and immediately processed for immunocytochemistry, electron microscopy and flow cytometry.

Immunocytochemistry

Immunocytochemical analyses were performed on the samples from four volunteers. The lymph cells were washed twice with phosphate-buffered saline. Cytocentrifuge smears with approximately 2×10^4 cells each were prepared. Alternatively, the cells were reacted with mouse anti-CD1a monoclonal antibody (1 : 100, 30 min, 4 °C), washed and then incubated at 4 °C for 30 min with sheep antimouse IgG antibody-coated Dynabeads M-450 (Dynal, Oslo, Norway) before smears were prepared again with 2×10^4 cells per glass slide. The cell smears were air-dried, fixed at room temperature for 10 min in acetone, incubated for 30 min in 4% buffered formalin (for protein S-100), and then immunostained with antibodies using an immunofluorescence or an ABComplex/alkaline phosphatase technique (Dako, Glostrup, Denmark). The monoclonal antibodies used, and their origin and specificity are shown in Table 1.

Immunolabeling of lymph cells for FACS analysis

The conjugated antibodies used for FACS analysis, and their specificity and isotype/form are listed in Table 2. For control purposes, appropriate isotype-matched antibodies were used in parallel. Aliquots containing $5–10 \times 10^4$ cells were stained with Tc-, PE- or FITC-conjugated antibodies (Table 2) in Hank's balanced salt solution (HBSS) containing 5% FCS and 0.1% NaN_3 for 30 min at 4 °C, before being washed twice with HBSS. Finally 500 µl of 0.1% formaldehyde in PBS was then added for FACScan-based analysis.

Cytofluorometric analysis

The lymph samples from eight volunteers were investigated on a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.). From 10 000 to 20 000 events were acquired and analyzed on a Macintosh computer using CellQuest software (Becton Dickinson). $CD1a^+$ cells were analysed for expression of the surface molecules of interest (Table 2). Irrelevant isotype-matched antibodies were used as a negative control.

Electron microscopy

Transmission electron microscopy was performed on lymph cells from three volunteers. Approximately 10^5 lymph cells were incubated for 60 min with 2×10^6 sheep antimouse IgG antibody-coated Dynabeads coupled with mouse anti-CD1a monoclonal antibody (1:100, 120 min, 4 °C).The cell suspension was fixed for 24 h in 2.5% glutaraldehyde buffered with 0.1 *M* sodium phosphate, pH 7.3, and washed twice in the same buffer by sedimenta-

Table 1 Primary antibodies, their specificity, and the immunostaining reaction of $CD1a^+$ lymph cells in human lymph derived from the normal skin of four volunteers (+ positive, (+) present on some cells, – negative)

Antibody against	Clone	Source	Specificity relevant to present study	Immunostaining reaction
CD1a	NA1/34	Dako[a]	Langerhans cells	+
HLA-DR	CR3/43	Dako[a]	MHC class II antigen	+
Protein S-100	Dako z 311	Dako[a]	Langerhans cells	+
Lag antigen		N. Romani[b]	Langerhans cells	(+)
CD4	AT29/3	Serotec[c]	T helper/inducer cells	(+)
MAC 387		Dako[a]	Monocytes, macrophages, granulocytes	–
CD36	OKM-5	Ortho Diagnostic Systems[d]	Monocytes, macrophages	–
CD68	KP1	Dako[a]	Monocytes, macrophages	–
Human follicular dendritic cells	x-11	Serotec[c]	Follicular dendritic cells in lymph nodes, blood dendritic cells	–
CD54	LB-2	Becton Dickinson[e]	Adhesion molecule ICAM-1	(+)

[a] Dako, Glostrup, Denmark
[b] A kind gift from N. Romani, Innsbruck, Austria
[c] Serotec, Oxford, UK
[d] Ortho Diagnostic Systems, Raritan, N.J.
[e] Becton Dickinson, Mountain View, Calif.

Table 2 The antibody conjugates used for FACS analysis, their specificity, isotype/form and the proportion of cells expressing the corresponding antigens in the total lymph cell population and $CD1a^+$ lymph cells, respectively. For quantitative evaluation of the percentages of positive cells the gate sets were defined on the basis of isotype-matched control antibodies (*n.t.* not tested)

Antibody against	Clone	Source	Specificity relevant to present study	Isotype/form	Proportion of lymph cells expressing the corresponding antigen (mean ± SD %)	Proportion of $CD1a^+$ lymph cells expressing the corresponding antigen (mean ± SD %)
CD1a	VIT6B	Caltag[a]	Langerhans cells	Mouse IgG-1 R-PE	7.6 ± 2.2	
CD1a	B-B5	Serotec[b]		Mouse IgG-1 FITC		
CD3	S4.1	Caltag[a]	T cells	Mouse IgG-2a Tc	62.4 ± 9.8	n.t.
CD4	S3.5	Caltag[a]	Helper/inducer T cells	Mouse IgG-2a R-PE	46.8 ± 8.6	n.t.
CD8	3B5	Caltag[a]	Cytotoxic/suppressor T cells	Mouse IgG-2a FITC	14.7 ± 3.2	n.t.
CD11a	YTH81.5	Serotec[b]	α-chain of LFA-1 complex	Mouse IgG-2a R-PE	64.9 ± 21.8	81.2 ± 1.0
CD11b	44	Serotec[b]	α-chain of CR3	Mouse IgG-1 FITC	1.5 ± 0.8	8.9 ± 4.9
CD14	TÜK4	Caltag[a]	Monocytes, dendritic cells	Mouse IgG-2a PE	6.4 ± 3.6	4.8 ± 2.9
CD18	YFC118.3	Serotec[b]	β-chain of LFA-1 complex	Rat IgG-2b R-PE	65 ± 0.8	73.5 ± 6.7
CD19	B-C3		B cells	Mouse IgG-1 R-PE	1.5 ± 0.7	Negative
CD23	B-G6	Serotec[b]	Low-affinity receptor for IgE (FcRII)	Mouse IgG-1 R-PE	0.3 ± 0.2	Negative
CD25	B-B10	Serotec[b]	IL-2 receptor	Mouse IgG-1 R-PE	2.3 ± 1.7	23 ± 2.9
CD40	B-B20	Caltag[a]	B-cells, monocytes, dendritic cells	Mouse IgG-1 R-PE	4.3 ± 0.3	43.4 ± 1.2
CD45	HI30	Caltag[a]	Leucocytes	Mouse IgG-1 FITC	80.9 ± 12.4	n.t.
CD54	B-C14	Serotec[b]	ICAM-1	Mouse IgG1 R-PE	8.9 ± 1.3	26.1 ± 5.5
CD80	L307.4	Becton Dickinson[c]	B7-1	Mouse IgG-1 R-PE	23.8 ± 15.4	89.3 ± 2.6
CD80	BB1	Ancell[d]		Mouse IgM FITC		
CD86	2331(FUN1)	Pharmingen[e]	B7-2	Mouse IgG-1 FITC	8 ± 3.1	85.3 ± 1.7
HLA-DR	TÜ36	Caltag[a]	MHC class II antigen	Mouse IgG-2b Tc	8.9 ± 2.7	97.3 ± 2.2
HLA-DR	TÜ36	Caltag[a]		Mouse IgG-2b R-PE		
E-cadherin	6F9	Serotec[b]	Epithelial cell adhesion molecule	Mouse IgG-1	Negative	Negative
Human follicular dendritic cells	x-11	Serotec[b]	Follicular dendritic cells in lymph nodes, blood dendritic cells	Mouse IgG-1	Negative	Negative
Factor XIIIa		Calbiochem[f]	Dermal dendritic cells	Rabbit (polyclonal, undiluted serum)	Negative	Negative

[a] Caltag, San Francisco, Calif.
[b] Serotec, Oxford, UK
[c] Becton Dickinson, Mountain View, Calif.
[d] Ancell, Bayport, Minn.
[e] Pharmingen, San Diego, Calif.
[f] Calbiochem, La Jolla, Calif.

tion at 1000 *g* in a pointed 1.5-ml centrifuge tube. The final pellet was prepared by centrifugation in a drop of liquefied 1% phosphate-buffered agarose. After solidification of the agarose the pellet was cut into four blocks. These were postfixed in 1% OsO_4 and embedded in Spurr's low-viscosity resin. Thin randomly chosen sections were contrasted with lead citrate and uranyl acetate. Photographic enlargements of whole individual cells photographed at a nominal primary magnification of 12 000 were used for analysis.

Functional assays

Proliferation assays were performed using standard techniques. The cultures were performed in microtitre plates using RPMI-1640 medium (Gibco, Basel, Switzerland) supplemented with L-glutamine, penicillin, streptomycin, and 10% fetal calf serum for 6 days. Tritiated thymidine (0.025 μCi/well) was added 18 h before harvesting and incorporation was measured in a liquid scintillation counter.

Lymph cells ($2.5–5 \times 10^4$) were either irradiated and cocultured with allogenic T cells (5×10^4) or alternatively incubated for 6 days alone, or with tetanus toxoid (Schweiz. Serum- und Impfinstitut, Bern, Switzerland) at 0.4 μg/well. Furthermore, in blocking experiments all the functional assays mentioned above were performed with or without the addition of 5 μl unrelated mouse IgG1 as well as of 5 μl anti-CD80 (clone L307.4; Ortho Diagnostic Systems, Raritan, N.J.) and/or CD86 (clone 2331(FUN1); Pharmingen, San Diego, Calif.) antibodies. The optimal dose of antibodies used for these experiments was chosen based on the results of preliminary experiments. At least three different functional assays were carried out for each experimental protocol.

Results

As reported previously, the total output of cells in individual lymph samples varied substantially among the volun-

teers (Brand et al. 1992). Individual cell counts per sample ranged from 1.8×10^4 to 1.8×10^6 with outputs from 1×10^3 to 1.9×10^5 cells per hour. Up to 20% of the cells in the peripheral lymph were erythrocytes, but their output varied widely among the volunteers and also in different samples from the same volunteer.

Immunocytochemistry

Immunocytochemical analysis of at least 200 randomly selected lymph cells derived from the normal untreated skin of each of the four volunteers revealed that about 60% were $CD3^+$, 4% $CD1a^+$, 5% $CD36^+$, $CD68^+$, MAC 387^+, and 1% $CD19^+$. The $CD1a^+$ cells did not express the monocyte surface markers CD36, CD68 and MAC 387 nor were they stained with antibody X-11 to human follicular DC but they reacted with antibodies against protein S-100, HLA-DR and in part with antibodies to the Lag antigen, ICAM-1, and CD4 (Table 1). Furthermore, as an interesting finding, some of these $CD1a^+$ lymph cells formed clusters with T cells (Fig. 1).

Flow cytometry

The antibodies used, their isotype/form and the mean percentage (± SD) of lymph cells (whole lymph cell population) and $CD1a^+$ lymph cells, respectively, expressing the corresponding antigens are shown in Table 2. Isotype-matched irrelevant antibodies were used as negative control.

About 81% of the lymph cells expressed the leucocyte common antigen CD45. The majority of the lymph cells (about 62%) were T cells (CD4/CD8 ratio about 3:1).

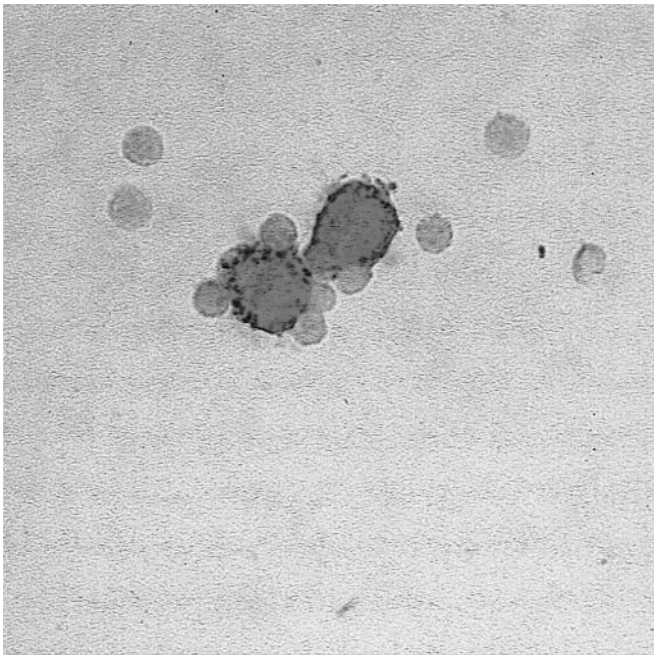

Fig. 1 $CD1a^+$ lymph cells, one forming a cluster with T cells

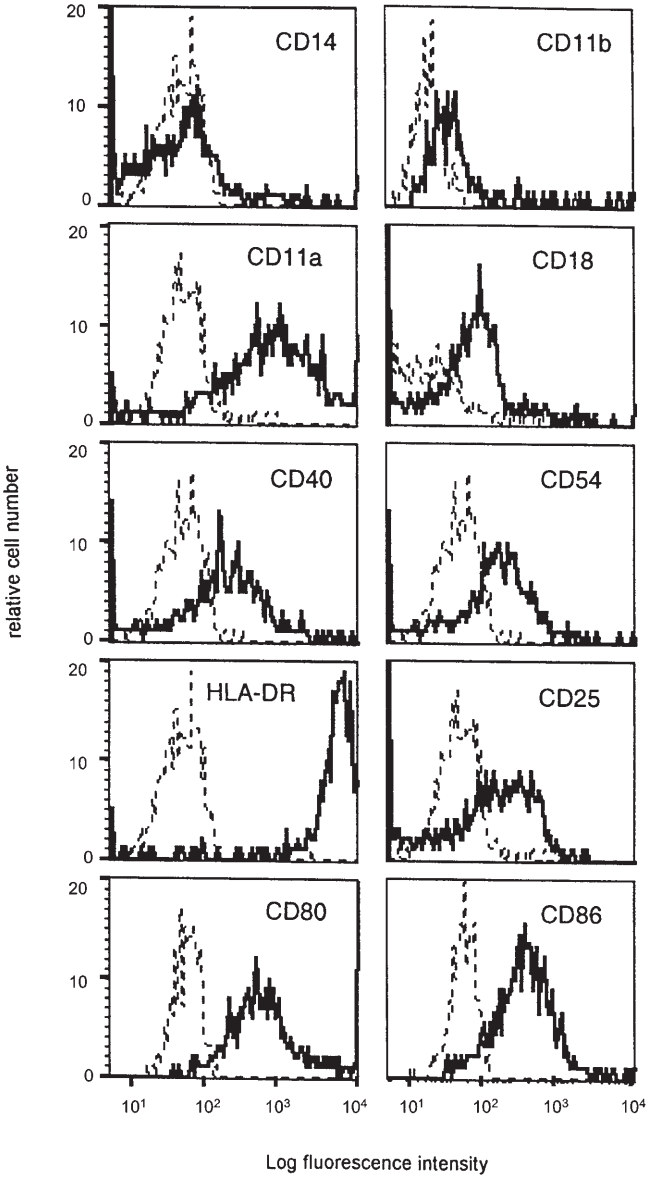

Fig. 2 Cytofluorographic analysis of human skin derived $CD1a^+$ lymph cells. Data of 10 000 to 20 000 lymph cells were obtained and the $CD1a^+$ cells were gated out manually by a combination of forward and side scatter and CD1a gate sets. Histograms from different double-labeling experiments are shown (*dotted lines* isotype controls, *solid lines* specific antibodies expressed on $CD1a^+$ cells)

The other cells comprised about 7% $CD1a^+$ cells, about 8% monocytes/macrophages and about 1% B cells. From the other antigens examined, CD11a/CD18 and CD80 were expressed by 65% and 24% of the lymph cells, respectively, while CD11b, CD23, CD25, CD40, CD54, CD80, CD86 and HLA-DR were present on less than 10% of the cells.

The analysis of $CD1a^+$ cells (Table 2, Fig. 2) revealed that these cells were not stained with antibodies against human follicular DC, factor XIIIa, E-cadherin, CD23 and CD19. However, almost all $CD1a^+$ cells (> 90%) were positive for HLA-DR and a high percentage (> 80%) for

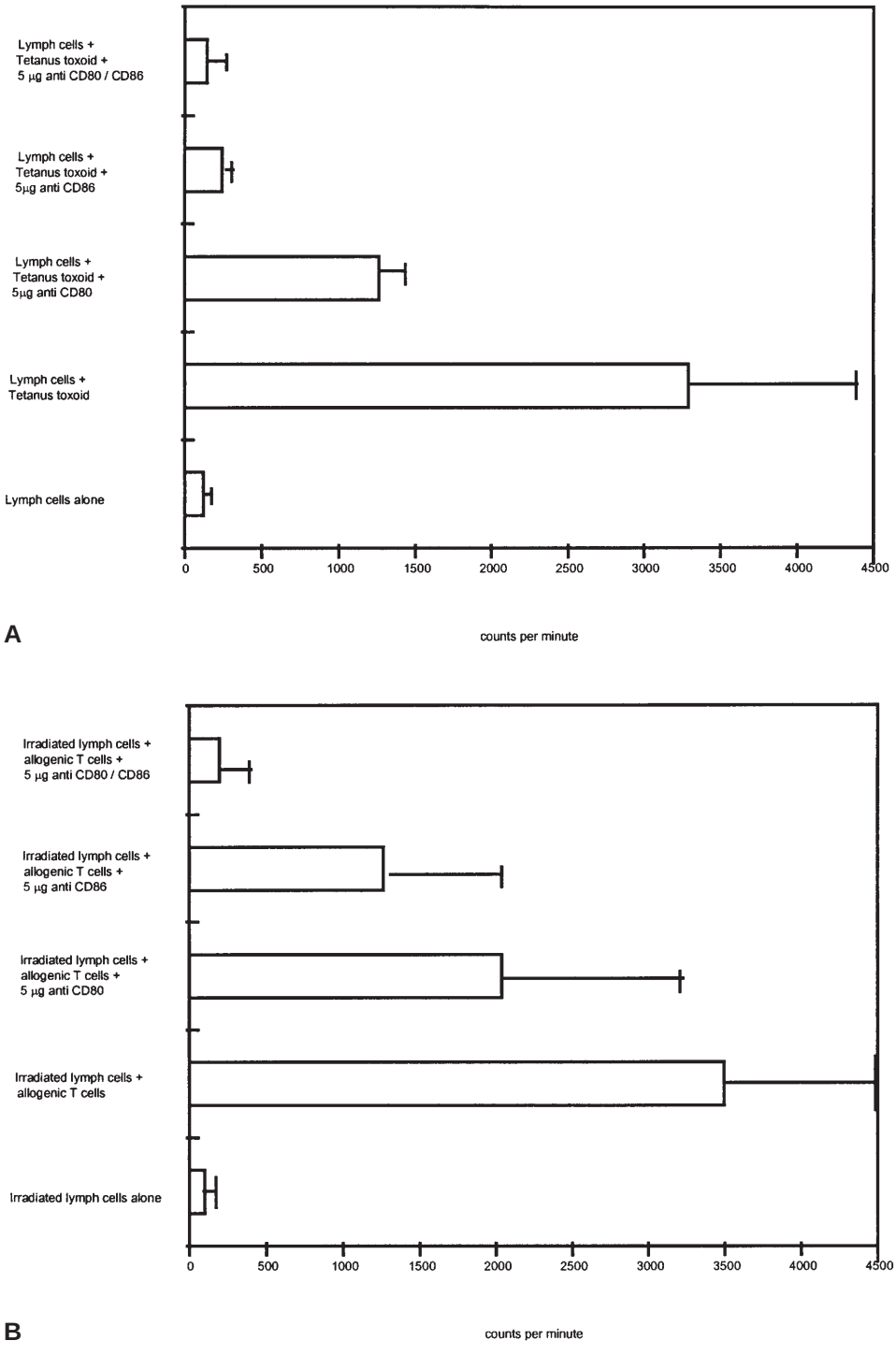

Fig. 3 A, B Antigen specific (**A**) and allogenic (**B**) T cell proliferation stimulated by antigen-presenting lymph cells were cooperatively inhibited by anti-B7-1 and anti-B7-2. Lymph cells (5×10^4) were co-cultured or cultured alone as indicated. Monoclonal antibodies against CD80 and/or CD86 were added at 5 μg/ml. Proliferation was revealed by tritiated thymidine incorporation after 6 days of culture. The results are representative of four experiments. Values are expressed as mean counts per minute from triplicate experiments (*error bars* SD)

CD11a, CD80 and CD86. Furthermore, CD18 was expressed by 74%, CD40 by 43%, CD54 by 26% and CD25 by 23% of $CD1a^+$ cells. Only a minor fraction of the $CD1a^+$ cells (< 9%) were positive for CD11b and CD14.

Electron microscopy

In the examined samples many small- to medium-sized lymphoid cells, a few mononuclear cells, even fewer polymorphonuclear granulocytes and cells with tightly bound Dynabeads were found. Most ultrastructural features of these Dynabead-rosetted cells were indistinguishable from those of epidermal Langerhans cells (LC), which were chosen as control cells, with the following two important differences. First, compared to epidermal LC the surface morphology of the $CD1a^+$ lymph cells at places without attached beads had longer, wider and sometimes branched projections, indicative of extensive ruffling of the surface. Second, in contrast to epidermal

LC which exhibited typical Birbeck granulates (BG) through all sections, only 22% of the lymph cells with attached beads showed this feature.

Functional assays.

To investigate whether B7-1 and B7-2 on antigen-presenting lymph cells mediate allogenic and/or antigen-induced T-cell proliferation, we performed blocking experiments using anti-CD80 and/or anti-CD86 antibodies. The results of a representative experiment is shown in Fig. 3. Both, allogenic and antigen-specific (tetanus toxoid) T-cell proliferation stimulated by antigen-presenting lymph cells were strongly inhibited by adding anti-CD80 and anti-CD86 antibodies (Fig. 3). At the antibody concentrations used, the anti-CD86 antibody seemed to cause a stronger inhibition of T-cell proliferation. Adding both antibodies had additive effects and resulted in similar levels of tritiated thymidine incorporation to that in lymph cells cultured alone. Addition of unrelated IgG1 did not influence the proliferative response (data not shown).

Discussion

Our present investigations were especially focused on identifying morphological, phenotypic and functional features of $CD1a^+$ DC migrating from the skin to the regional lymph nodes. Our results demonstrate that about 7% of the lymph cells expressed the $CD1a^+$ antigen. Almost all $CD1a^+$ cells were positive for HLA-DR, CD11a, CD80 and CD86. Furthermore, CD18 was expressed by 74%, CD40 by 43%, CD54 by 26%, and CD25 by 23%. Only a minor fraction of the $CD1a^+$ cells (9%) were positive for CD11b and CD14. In addition, our results demonstrate that the antibodies against S-100, the Lag antigen, CD40 and CD86 exclusively reacted with $CD1a^+$ cells. Interestingly, the $CD1a^+$ cells did not react with an antibody against factor XIIIa and human follicular DC nor were they E-cadherin- and CD23-positive.

The immunological protection of the skin is provided by the so-called skin-associated lymphoid tissues (SALT) (Streilein 1994), and the immunological function of the epidermis is linked to the presence of LC (Teunissen 1992), the type of DC found in the epidermis (Gerberick et al. 1991; Romani and Schuler 1992; Stingl et al. 1980; Teunissen 1992). These LC constituting 2–4% of the epidermal cell population play a major role in antigen presentation (Braathen and Thorsby 1980; Inaba et al. 1986; Stingl et al. 1980; Teunissen 1992), and together with keratinocytes represent the most peripheral outpost of the immune system. It is generally assumed that epidermal LC are derived from $CD1a^+$ bone marrow cells and travel via the blood circulation to the skin where they enter the epidermis as a result of 'homing' mechanisms that are not yet fully understood (Teunissen 1992). Thereby, the expression of E-cadherin by LC promotes persistence of these cells in the epidermis (Blauvelt et al. 1995; Cumberbatch et al. 1996; Udey 1997; Schwarzenberger and Udey 1996; Tang et al. 1993). As an integral part of immunological surveillance, LC then migrate from the skin to the regional lymph nodes (Brand et al. 1993; Brand et al. 1995; Gerberick et al. 1991; Kripke et al. 1990; Silberberg Sinakin et al. 1976).

LC occur in two states of differentiation. Freshly isolated 'immature' LC (fLC) are highly specialized for processing foreign protein antigens but are poor stimulators of resting T cells (Romani and Schuler 1992). After short-term culture, the 'mature' LC then efficiently stimulate resting antigen-specific T cells, upregulating their stimulatory capacity 30- to 100-fold (Inaba et al. 1986), and compared to fLC, cultured LC (cLC) show downmodulation of E-cadherin (Blauvelt et al. 1995; Tang et al. 1993), increased expression of surface major histocompatibility complex (MHC) class I and class II molecules, intercellular adhesion molecule-l (ICAM-1), leucocyte function-associated antigen-3 (LFA-3), and β2 integrins (Girolomoni et al. 1993; Romani and Schuler 1992). Interestingly, probably as a consequence of maturation/activation during migration, in our experiments no E-cadherin expression could be detected on $CD1a^+$ lymph cells, indicating that the downmodulation of E-cadherin facilitates LC migration (Blauvelt et al. 1995; Tang et al. 1993). Similar to lymphoid DC (Inaba and Steinmann 1986), cLC but not fLC are able to 'cluster' resting T cells in an antigen-independent fashion (Inaba et al. 1986a; Inaba et al. 1989; Romani and Schuler 1992). Cell rosettes consisting of one central LC with attached T cells were also typically found in afferent skin lymph (Fig. 1) (Brand et al. 1995), indicating a maturation of LC migrating in afferent lymph. Furthermore, cultured epidermal LC have been shown to express functional B7 protein, whereas freshly isolated cells do not (Larsen et al. 1992; Symington et al. 1993; Yokozeki et al. 1996; Young et al. 1992). These so-called costimulatory molecules which bind to CD28 or the CD28 homologous CTLA-4, on T lymphocytes (Damle et al. 1992; Galvin et al. 1992; Lanier et al. 1995) deliver, in addition to MHC-peptide complexes, a crucial costimulatory signal for T cell activation (Lanier et al. 1995). If these molecules on antigen-presenting cells are lacking, a functional inactivation of naive T cells may occur (Liu and Linsley 1992). The finding that B7-1 and B7-2 molecules were expressed on all these cells (Table 2) is again indicative of the process of maturation of $CD1a^+$ cells taking place or having already occurred in afferent lymph. Furthermore, in our functional assays, the support of allogenic T-cell proliferation by the antigen-presenting lymph cells as well as the tetanus toxoid-induced lymph cell proliferation was strongly inhibited by adding anti-CD80 and anti-CD86 antibodies to the culture medium (Fig. 3), demonstrating the functional relevance of these two surface molecules for a successful antigen-induced immune response in afferent lymph cells.

Recently, important roles for CD40 and CD40 ligand (CD40L) during activation of DC as well as T cells have also been demonstrated (Banchereau et al. 1994; Caux et al. 1994; Cella et al. 1996; Kato et al. 1996; Koch et al.

1996; Shu et al. 1995). Whereas CD40 was shown to be expressed on B cells, on monocytes/macrophages and on DC, CD40L is induced on activated T cells (Banchereau et al. 1994). Previous reports have demonstrated that cultured DC undergo further maturation with morphological, phenotypic and functional changes after stimulation by CD40L (Caux et al. 1994). In particular, activation of CD40 induces high levels of MHC class II antigens and results in upregulation of accessory molecules such as CD25, CD58, CD80 and CD86 on DC (Caux et al. 1994), and it has also been shown to switch on the production of a distinct set of cytokines such as TNFα, IL-8, MIP-1α and IL-12 (Banchereau et al. 1994; Caux et al. 1994; Cella et al. 1996; Kato et al. 1996; Koch et al. 1996; Shu et al. 1995). Interestingly, FACS analysis of the $CD1a^+$ population in our study revealed that in afferent skin lymph 43% of $CD1a^+$ cells express CD40. This finding may indicate an important functional role for CD40 on $CD1a^+$ cells migrating to the lymph nodes. CD40 triggering may at least in part be responsible for the marked expression of adhesion and costimulatory molecules on $CD1a^+$ lymph cells found in our study, and may result in an increased capacity of DC to stimulate T cells, which are clustered around them. As mentioned above, such cell interactions typically found in afferent skin lymph may even lead to further upregulation of CD40 and CD40L on DC and T cells, respectively, which eventually enhances the further maturation and stimulatory function of migrating DC in vivo.

In addition to such costimulatory molecules the maturation of DC may be strongly influenced by cytokines present in their microenvironment. In this context, GM-CSF has been shown to be one of the key mediators responsible for the viability and maturation of LC (Inaba et al. 1993; Witmer Pack et al. 1987). Thus, mediated by GM-CSF, cultured epidermal LC have been shown to change into immunostimulatory DC, a process which is accompanied by loss of BG, and upregulation of MHC, adhesion and costimulatory molecules (Chang et al. 1994; Inaba et al. 1993; Larsen et al. 1994; Schuler and Steinman 1985; Witmer Pack et al. 1987). In addition, GM-CSF secreted by activated T cells, may upregulate CD40 expression on monocytes/macrophages (Kato et al. 1996). In our laboratory, we have recently demonstrated that GM-CSF is present in human afferent skin lymph at biologically active protein levels (Yawalkar et al. 1996). Hence, one might speculate that maturation of epidermal LC is also influenced by GM-CSF in vivo, i.e. in afferent lymph, resulting in morphological, functional and antigenic profile changes similar to those found in LC cultured in vitro.

Ultrastructurally, the main marker for LC are thought to be the so-called BG (Birbeck et al. 1961). BG are not found in DC in tissues other than skin or SALT, and they are reduced or disappear upon culture of LC (Romani and Schuler 1992; Tuenissen 1992). However, there is increasing controversy concerning the sensitivity of BG as a specific LC marker (Mommaas et al. 1994; Romani and Schuler 1992; Tuenissen 1992; Teunissen et al. 1990). In our electron microscopic analysis all $CD1a^+$ lymph cells exhibited an extensive ruffling of the cell surface, but, similar to the immunocytochemical results using the Lag antibody, BG were detected in only about 22% of the $CD1a^+$ lymph cells. These observations may once again indicate maturation in migrating LC.

In our analysis no cells were demonstrated to express factor XIIIa, thus the $CD1a^+$ lymph cell population may not contain dermal DC, a cell population of the skin, 12–40% of which has been shown to express CD1a and which lacks BG (Nestle et al. 1993). Alternatively, these findings may indicate that factor XIIIa is expressed on DC only during a certain phase of development or in relation to cytokines predominantly found in the dermis. Therefore, the BG^- population may derive from both migrating LC and dermal DC, and the $CD1a^+/CD14^+$ population might represent LC precursors which have failed to enter the epidermis(Misery and Dezutter-Dambuyant 1995).

Until now the exact origin and features of the cells giving rise to the population of DC in the afferent lymph have not been fully elucidated. In the present study we identified $CD1a^+$ dendritic lymph cells, a large fraction of which contained no or markedly fewer BG than epidermal LC. The surface antigens expressed on these cells and their morphological features indicate that this population mainly consists of DC in a state of differentiation/activation similar to LC cultured in vitro (Teunissen 1992). Thereby, the $CD1a^+$ cells resemble DC formerly designated as 'veiled' and also lymphoid DC (Romani and Schuler 1992; Teunissen 1992). Our findings and those of others (Romani and Schuler 1992; Teunissen 1992) suggest that LC must interact in different milieus to function efficiently as antigen presenting cells. Furthermore, our findings indicate that resident 'immature' LC may be mainly engaged in antigen uptake and processing, whereas LC migrating towards the lymph nodes may develop into mature DC, i.e. decrease their ability to process antigens and form a more differentiated population of DC specialized in sensitizing naive T lymphocytes. These results add further support to the view that resident LC and probably also dermal DC are precursors of lymphoid DC acquiring their final phenotype in the microenvironment of the lymph node.

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