A Close-Up View of Migrating Langerhans Cells in the Skin

Patrizia Stoitzner, Kristian Pfaller,* Hella Stössel, and Nikolaus Romani
Departments of Dermatology and *Anatomy and Histology, University of Innsbruck, Innsbruck, Austria

Langerhans cells of the epidermis and dermal dendritic cells screen the skin for invading antigens. They initiate primary immune responses after migrating from sites of antigen uptake to lymphoid organs. The skin is a feasible model to study the morphology and regulation of dendritic cell migration. We therefore used murine skin explant cultures for tracking the pathways of dendritic cell migration by electron microscopy. Several novel observations are reported. (i) In 48 h cultures of epidermal sheets numerous Langerhans cells migrated out between keratinocytes extending long and thin cytoplasmic processes (''veils''). (ii) Langerhans cells in transition from epidermis to dermis were observed by transmission electron microscopy. Where Langerhans cells penetrated the basement membrane, the lamina densa was focally absent. (iii) This was highlighted by scanning electron microscopy, which presented the basement membrane as a tightly packed and dense network of fibrils. (iv) Scanning electron microscopy of the dermis revealed dendritic cells extending their cytoplasmic processes and clinging to collagen fibrils. (v) Entry of dendritic cells into dermal lymphatics was observed by transmission electron microscopy. It occurred by transmigration through intercellular spaces of adjacent endothelial cells. Entry through wide gaps between endothelial cells also seemed to take place. (vi) Dendritic cells inside the afferent lymphatics frequently carried material such as melanosomes and apoptotic bodies. These observations visualize the cumbersome pathway that dendritic cells have to take when they generate immunity. ***Key words: electron microscope/Langerhans cells/migration. J Invest Dermatol 118:117–125, 2002***

Dendritic cells are highly motile antigen-presenting cells. They have optimized the migratory capacity to fulfill their prime task, i.e., to initiate primary immune responses (Banchereau and Steinman, 1998; Banchereau *et al*, 2000; Romani *et al*, 2001). Like sentinels they scan peripheral compartments for invading foreign particles and phagocytose (Inaba *et al*, 1998) or macropinocytose (Sallusto *et al*, 1995) these antigens very effectively. After uptake of antigens they start to mature and migrate to draining lymph nodes to stimulate antigen-specific T cells there. The gap between the sites of antigen uptake and the sites of clonal T cell activation is efficiently bridged by these migratory dendritic cells. They carry immunogenic complexes of major histocompatibility complex and antigenic peptides on their cell surface and possibly also antigenic proteins in antigen retention organelles (Lutz *et al*, 1997) into the T cell areas of lymphoid organs (Austyn, 1996; Steinman *et al*, 1997).

Inflammatory stimuli, such as bacterial lipopolysaccharide, tumor necrosis factor α, and interleukin-1 (Roake *et al*, 1995; Cumberbatch *et al*, 1997), and chemotactic cytokines, like macrophage inflammatory protein 3β (MIP-3β/CCL19), secondary lymphoid tissue chemokine (SLC/CCL21), and interleukin-16 (Saeki *et al*, 1999; Kellermann *et al*, 1999; Stoitzner *et al*, 2001), trigger and guide the migration of dendritic cells from peripheral tissues towards the draining lymphoid organs. As a first step E-cadherin, which mediates Langerhans cell–keratinocyte adhesion, is downregulated by inflammatory cytokines so that Langerhans cells are able to leave the epidermis (Jakob and Udey, 1998). By secretion of matrix metalloproteinases (MMP) dendritic cells can digest extracellular matrices (Kobayashi, 1997), which facilitates their crossing of the basement membrane and migration through the dermis. When they encounter lymphatic vessels they enter the lumen and migration proceeds towards the lymph nodes. This was demonstrated by immunohistochemistry and transmission electron microscopy (TEM) in murine and human skin explant organ culture models where dendritic-cell-filled lymph vessels were originally described as ''cords'' (Larsen *et al*, 1990; Lukas *et al*, 1996; Weinlich *et al*, 1998). Skin in general and the skin explant model in particular represent suitable tools to investigate morphologic and regulatory aspects of dendritic cell migration. Contact hypersensitivity is another widely used experimental model (Enk *et al*, 1993; Cumberbatch *et al*, 1997). In order to learn more about the requirements for efficient dendritic cell migration we were interested in morphologic aspects of emigrating dendritic cells *in situ*. Therefore, we analyzed in detail cultured murine skin explants, primarily by scanning electron microscopy (SEM) but also by TEM.

MATERIALS AND METHODS

Mice Mice of inbred strains BALB/c and C57BL/6 were purchased from Charles River Germany (Sulzfeld, Germany) and used at 8–12 wk of age.

Media and reagents Culture medium was RPMI 1640 supplemented with 10% fetal bovine serum, L-glutamine (Sebac, Stuben, Austria), gentamycin (all from PAA, Linz, Austria), and 2-mercaptoethanol (Sigma Chemical, St. Louis, MO).

Manuscript received July 26, 2001; revised September 17, 2001; accepted for publication September 26, 2001.

Reprint requests to: Dr. Patrizia Stoitzner, Department of Dermatology, University of Innsbruck, Anichstrasse 35, A-6020 Innsbruck, Austria. Email: patrizia.stoitzner@uibk.ac.at

Abbreviation: MMP, matrix metalloproteinase.

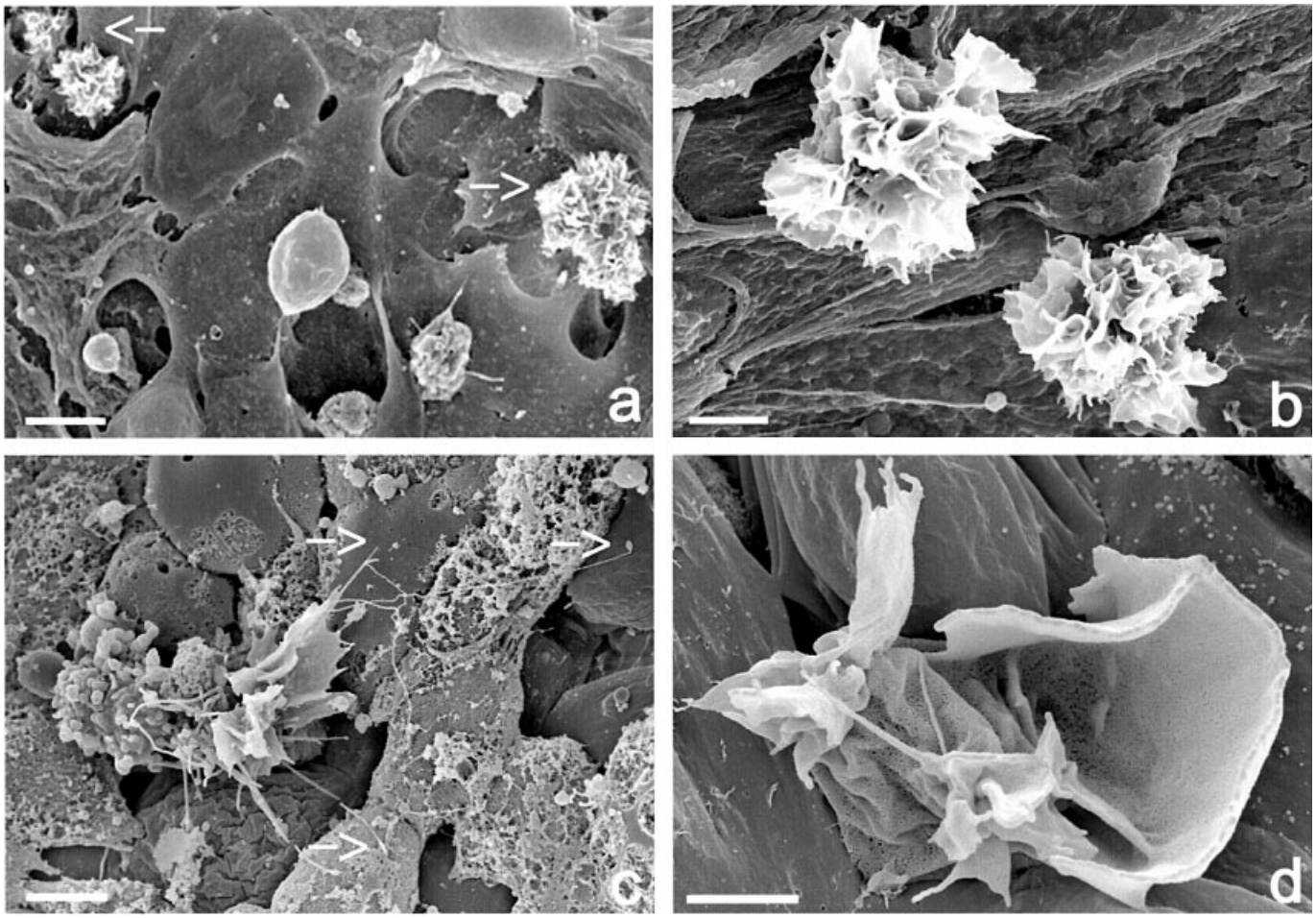

Figure 1. Langerhans cells migrate from epidermal sheet cultures. In a time course experiment we observed Langerhans cells migrating out of epidermal sheets during a 48 h culture. After 48 h (*a*) numerous Langerhans cells (*arrows*) crawled out of the epidermal sheets. The detachment of keratinocytes from each other allowed the Langerhans cells to leave the epidermis through developing gaps (*b*, *d*). The Langerhans cells attached to neighboring keratinocytes with the help of thin cytoplasmic extensions (*arrows*) (*c*). *Scale bars*: (*a*) 10 μm; (*b*) 5 μm; (*c*, *d*) 2.5 μm.

Skin explant culture Mice were sacrificed and ears were cut off at the base. Ear skin was split in dorsal and ventral halves by means of strong forceps and the dorsal halves (i.e., cartilage-free, thinner halves) were cultured in 24-well tissue culture plates (one ear per well) as described previously (Ortner *et al*, 1996; Romani *et al*, 1997; Weinlich *et al*, 1998). Alternatively, epidermis and dermis were separated from each other by means of the bacterial enzyme dispase (Kitano and Okado, 1983), and the epidermal sheets were placed in culture. In most experiments whole skin or epidermis was cultured continuously for 48 h. At the end of the cultures skin explants were further processed for electron microscopy. All observations described are based on the ultrastructural inspection of several explants from three to four separate experiments.

SEM Tissue was fixed immediately after termination of cultures with half-strength Karnovsky's formaldehyde–glutaraldehyde fixative (Karnovsky, 1965), followed by three washes with 0.1 M cacodylate buffer and postfixation in 3% OsO_4 in water. After a short rinse in 0.1 M sodium cacodylate buffer specimens were dehydrated in ascending concentrations of ethanol (50%–100%). Samples were then critical-point dried (CP Dryer, Balzers, Liechtenstein), mounted on aluminum stubs with collodial silver, and subsequently coated with a layer of 5–10 nm of gold–palladium in a sputtering device (Balzers). Specimens were viewed on a Zeiss Gemini 985 scanning electron microscope (Zeiss, Oberkochen, Germany) at 5–8 kV.

TEM Skin organ cultures were minced into small blocks and fixed by submersion for 5 h in half-strength Karnovsky's formaldehyde–glutaraldehyde reagent (Karnovsky, 1965). Further processing was as described previously (Stössel *et al*, 1990). Briefly, specimens were postfixed in 3% aqueous OsO_4, *en bloc* contrasted with veronal-buffered uranyl acetate, and dehydrated in a graded series of ethanols. After dehydration specimens were infiltrated in mixtures consisting of varying proportions of propylene oxide as an apolar solvent and epoxy resin (Epon 812; Serva Feinchemikalien, Heidelberg, Germany). The resin was polymerized at 60°C for 24 h. Ultrathin sections were contrasted with lead citrate and viewed with a Philips EM 400 electron microscope (Fei Company, Eindhoven, The Netherlands) at a voltage of 80 kV.

RESULTS

Langerhans cells emigrate from epidermal sheets in a time-dependent manner Langerhans cell emigration from the epidermis has been repeatedly shown to occur *in vivo* as well as in skin explant cultures (Larsen *et al*, 1990; Lukas *et al*, 1996; Weinlich *et al*, 1998). Here we extend these observations in a morphologic way. Emigration of Langerhans cells from the epidermis occurs irrespective of the presence of the dermis (**Fig 1*a***). When the epidermis was separated from the dermis by means of dispase before the onset of culture, Langerhans cells emigrated into the culture medium cells equally well as in whole skin cultures. SEM of epidermal sheets cultured for 0, 5, 10, or 48 h revealed Langerhans cells coming out of the epidermis in a time-dependent manner (data not shown). Already 5 h after the onset of the explant cultures the first Langerhans cells emigrating from the epidermis could be observed. The numbers of emigrating Langerhans cells increased with duration of the organ culture. Langerhans cells detached from the surrounding keratinocytes. Gaps and holes formed in the keratinocyte layer where the

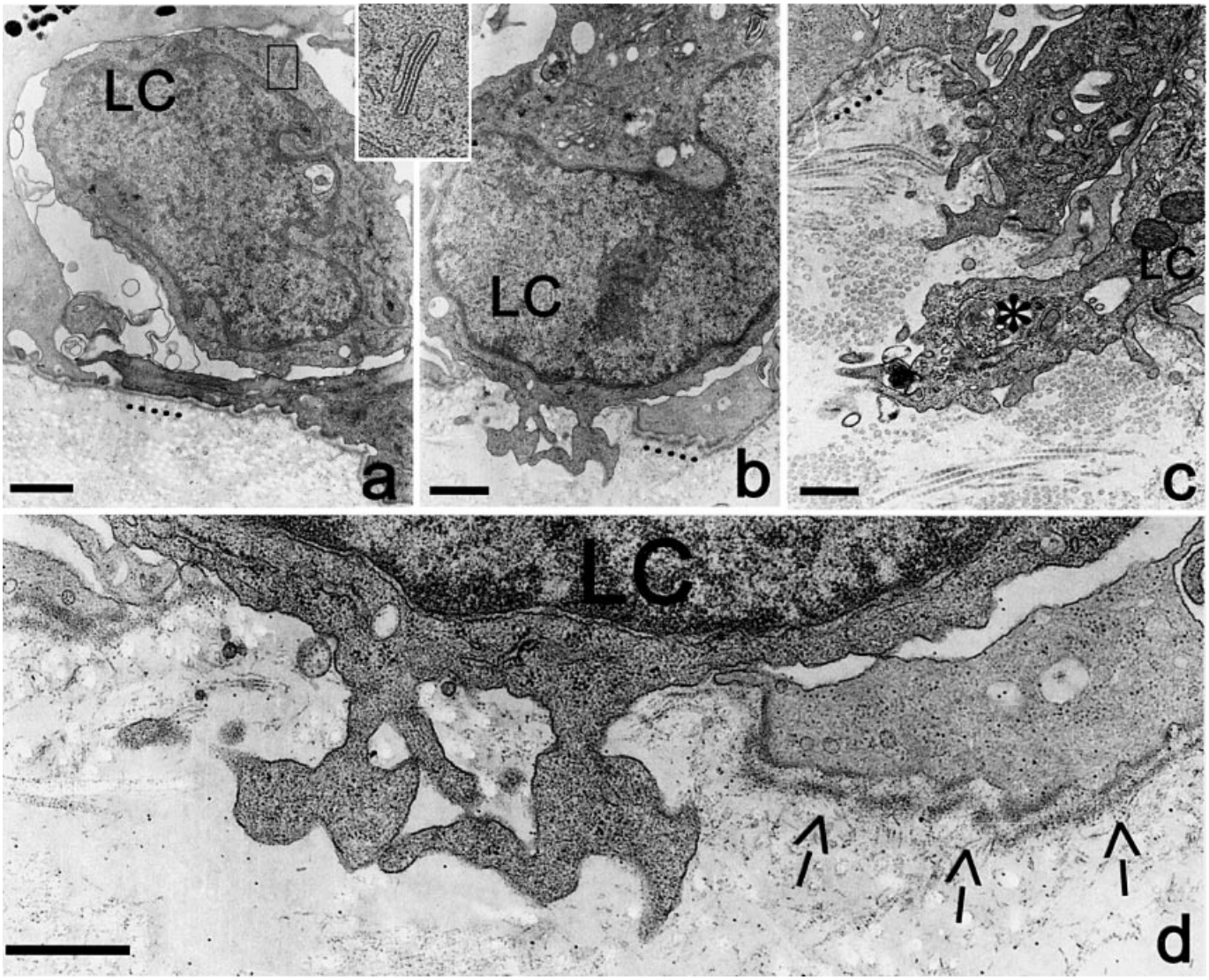

Figure 2. Langerhans cells in transit through the basement. Different sections of skin explants cultured for 48 h suggest a time sequence of events (*a*)–(*c*). A Langerhans cell (LC), identified by a Birbeck granule (*box*, *enlarged in the inset*), is located close to the still intact basement membrane (*dotted line*) (*a*). In *panel b* a pseudopod of the Langerhans cell can be seen to penetrate the basement membrane. Enlargement of this scene in *panel d* highlights the absence of the lamina densa only at the site of direct contact between Langerhans cell and basement membrane (*arrows point at lamina densa*). In *panel c* migration of the Langerhans cell has proceeded further. A long pseudopodium (*asterisk*) has broken its way through the dermo-epidermal junction. *Scale bars*: (*a–c*) 1 μm; (*d*) 0.5 μm.

emigrating Langerhans cells moved through (**Fig 1*b***, ***d***). The emigrating Langerhans cells extended very thin and long pseudopodia with which they seemed to be attached to surrounding keratinocytes (**Fig 1*c***). Langerhans cells displayed pronounced cytoplasmic veils and may be considered – at least partially – mature by this criterion. It should be noted that the dermo-epidermal separation by means of dispase leaves the collagenous part of the basement membrane (lamina densa) on the dermal side. SEM of cultured epidermal sheets also emphasizes that migration is an active process. Langerhans cells do not simply fall out of a disintegrating epidermis. This point is underscored by the fact that in populations of emigrant cells retrieved from the culture medium Langerhans cells are highly enriched up to 70%.

The basement membrane is a major physical obstacle for migrating Langerhans cells After leaving the epidermis Langerhans cells encounter a complex barrier, the basement membrane, which they have to passage on their way to the dermis. By TEM we occasionally found the rare event of a Langerhans cell in transit through the basement membrane (**Fig 2**). We observed that the Langerhans cell extended a pseudopod through the basement membrane. The electron-dense part of the basement membrane (lamina densa) was absent only in the area of physical contact with the emigrating Langerhans cells (**Fig 2*b–d***). The cells displayed ultrastructural features of mature dendritic cells, i.e., few small and runted Birbeck granules or no Birbeck granules at all and a large size. Unfortunately, this event is too rare on ultrathin sections so that it is not possible to completely reconstruct the movement of Langerhans cells through this obstacle, even though this is attempted in **Fig 2**.

The lamina densa stays on the dermis after dermo-epidermal separation by dispase (Kitano and Okado, 1983). We studied this border structure between the skin compartments by SEM. As can be appreciated from **Fig 3**(***a***) the collagen fibrils in the basement membrane are very tightly packed. They form a dense network that would not leave enough space for a transmigrating Langerhans cell.

The dermis does not allow unimpeded movement of migrating Langerhans cells In the dermis the collagen meshwork appears dense but less compact (**Fig 3*b***) than in the

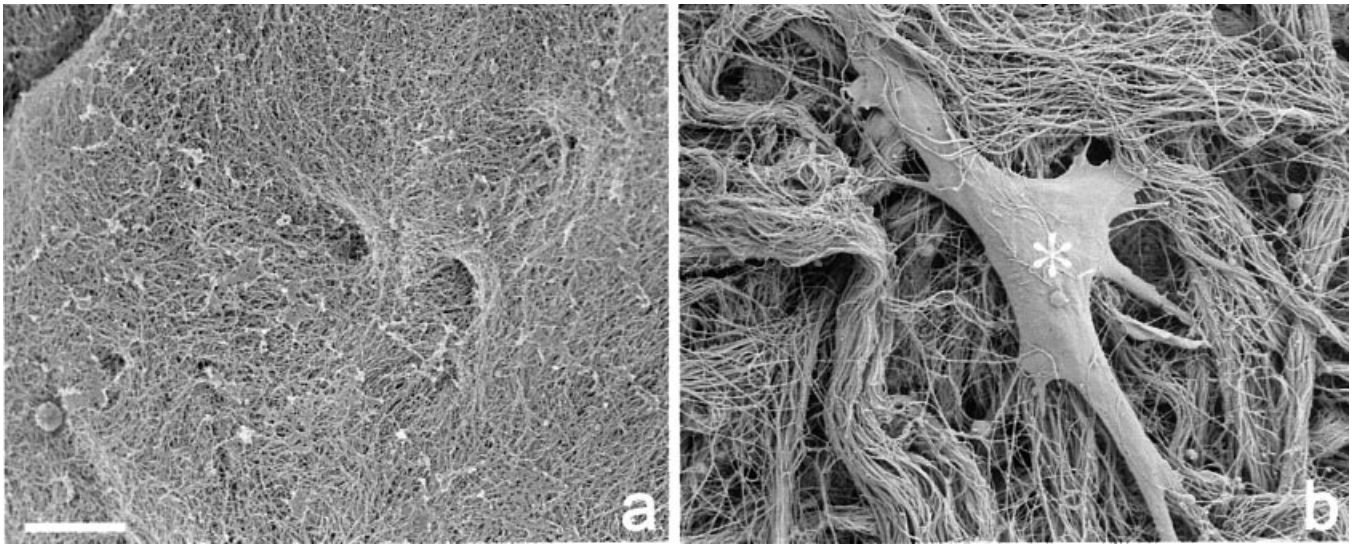

Figure 3. Visualization of the dense collagen meshwork of the basement membrane and the dermis. After enzymatic separation of the epidermis, the basement membrane adherent to the dermal sheets was scanned by electron microscopy. The different packing density of the collagen meshwork in the basement membrane (*a*) and the dermis (*b*) becomes evident in these pictures. Note that in the dermis a fibroblast (*asterisk*) is visible in the collagen meshwork. *Scale bar*: 10 μm.

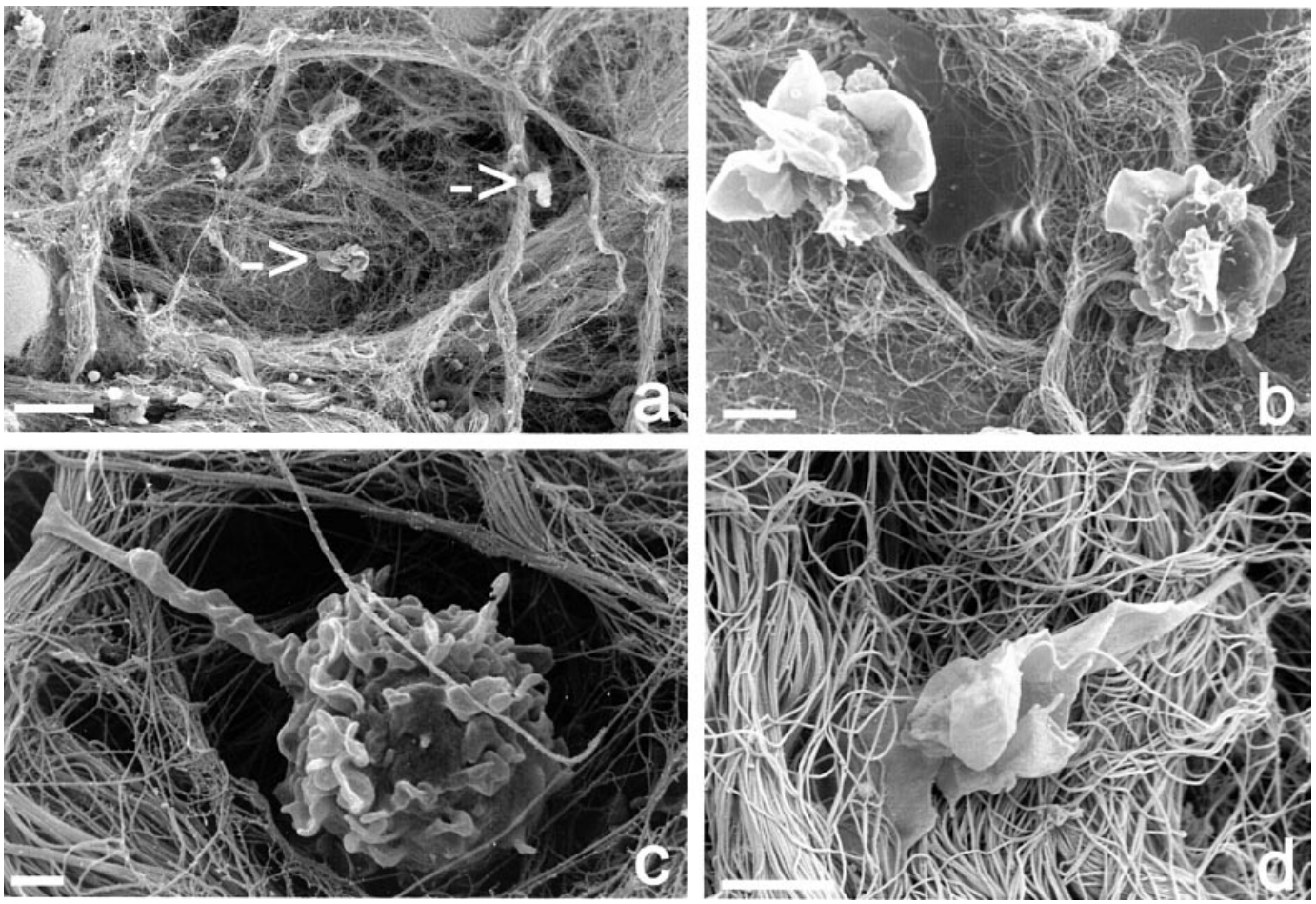

Figure 4. Migration of dendritic cells through the collagen meshwork of the dermis, i.e., after passage through the basement membrane and before entry into lymph vessels. In the scanning electron microscope dendritic cells (*indicated by arrows*) can be observed migrating through the dermal collagen meshwork after skin explant culture for 48 h (*a*). In (*b*) two emigrating dendritic cells, adjacent to a fibroblast (den gibt's ober nicht), move through the dermis. Dendritic cells cling to bundles of collagen fibrils with their veils and pseudopodia (*c*, *d*). *Scale bars*: (*a*) 10 μm; (*b*) 5 μm; (*c*, *d*) 2.5 μm.

lamina densa. Yet, in relation to the size of migrating dendritic cells this connective tissue appears to render movement of dendritic cells difficult. Dendritic cells squeezing through between bundles of collagen fibrils are readily visible (**Fig 4*a*, *b***). Migrating dendritic cells sometimes attached to individual bundles of collagen fibrils with their pseudopodia (**Fig 4*c*, *d***). These observations suggest that the ability of dendritic cells to flexibly change their shape may be essential for their successful movement through the dermis until they encounter a lymphatic vessel.

Migrating dendritic cells enter lymphatic vessels As reported previously for human (Lukas *et al*, 1996) and mouse (Weinlich *et al*, 1998) skin explant cultures dendritic cells accumulated in wide clefts that were lined by a thin endothelium, did not possess a continuous basement membrane, and thus qualified as lymphatic vessels. In immunohistochemistry these structures were originally termed ''cords'' (Larsen *et al*, 1990). In semithin sections no differences were noted in the numbers of lymphatic vessels in cultured and uncultured skin. By TEM of a large number of sections of 48 h explant cultures we did observe entry of dendritic cells into vessels. Dendritic cells moved through the intercellular space of two neighboring endothelial cells (**Fig 5*a*, *b***). As cell–cell contacts between adjacent endothelial cells were often loose, entry of cells may be relatively easy. A tight ''sealing'' of the pore through which the migrating cell entered the vessel was described for dendritic cells acquiring antigens through the intestinal wall (Rescigno *et al*, 2001). Such tight contacts were not observed between migrating dendritic cells and skin lymphatics. In addition, we repeatedly noted distinct gaps in the endothelial layer. The endothelial cells were intact but they were not adjacent to each other, thus forming an interruption that was sometimes very wide (**Fig 5*c***). Clearly, these gaps appeared large enough to allow the entry of a dendritic cell and, indeed, dendritic cells in the vessel lumen were occasionally found close to these gaps, although never really in transit (**Fig 5*c*, *d***). A quantitative comparison of dendritic cells entering the vessel by transmigration between endothelial cells and dendritic cells that had possibly entered via the gaps was not possible. Both events were too rare.

Dendritic cells inside lymph vessels carry antigens The vessels contained cells with the morphology of dendritic cells such as an electron-lucent cytoplasm, veils, and an irregularly shaped nucleus. Frequently we could find Birbeck granules (Birbeck *et al*, 1961), i.e., the Langerin (CD207)-containing cell organelles typical for Langerhans cells (Valladeau *et al*, 2000) and possibly important for antigen processing. This had been described previously (Schuler *et al*, 1991; Weinlich *et al*, 1998). Granules with an electron-dense core, typical for dendritic epidermal T cells (Romani *et al*, 1985), were not found in the cells evaluated. Moreover, as BALB/c mice, which contain only very few dendritic epidermal T cells, were used for most experiments, it is highly unlikely that the cells described here might be dendritic epidermal T cells rather than dendritic cells. Frequently these lymph-borne cells contained substantial amounts of material that they must have taken up earlier. Some dendritic cells had ingested melanosomes (**Fig 6*a*, *c***). Other cells carried various forms of cellular material including apoptotic bodies (**Fig 6*b*, *d***).

DISCUSSION

It has been shown before that Langerhans cells emigrate out of the epidermis after receiving an inflammatory stimulus such as the application of contact allergen (Silberberg-Sinakin *et al*, 1976) or the onset of skin explant culture (Larsen *et al*, 1990). After passage through the basement membrane the migrating dendritic cells enter lymphatic vessels in the dermis and travel to the draining lymph nodes to initiate primary immune responses (Zinkernagel, 1996). Here we present for the first time a three-dimensional view of migrating Langerhans cells with the help of SEM. We were able to follow Langerhans cells on their way out of the epidermis, through the basement membrane and the dermis, and into the dermal lymphatics. This view allows some novel insights to be gained into the mechanism of dendritic cell migration.

Langerhans cell migration appears to be a highly active process After an inflammatory stimulus, here given by the onset of the organ culture, Langerhans cells start to emigrate from the epidermis in a time-dependent way. They squeeze out between the keratinocytes. This may happen in an active way in that they extend long thin pseudopodia, attach them to neighboring keratinocytes, and so seem to pull themselves out of the epidermis. Also in the dermal meshwork of collagen fibrils the migrating dendritic cells appear far from being passive. Again, they frequently stretch out processes and cling onto bundles of collagen. Although we are aware that the study of the dynamics of a process requires serial or real-time analysis – something that is not possible with electron microscopy – it is tempting to speculate that they were actively pulling. Such active dendritic cell movement has been shown in artificial collagen lattices by time-lapse video microscopy (Gunzer *et al*, 2000). Our data suggest that it may also occur *in vivo*.

Migrating Langerhans cells need to ''create a path'' with the help of enzymes and adhesion molecules Our observations suggest critical roles for enzymes and adhesion molecules at several levels.

First, for the emigration of Langerhans cells from the epidermis it is necessary that the intercellular bonds between keratinocytes (e.g., desmosomes) and between keratinocytes and Langerhans cells be loosened. A role for E-cadherin has been proposed (Schwarzenberger and Udey, 1996). Another perhaps additional way is by digestion of cell-adhesion-mediating molecules with special proteases such as the MMPs (Murphy and Gavrilovic, 1999). We have evidence that a broad-spectrum inhibitor of MMPs and, more specifically, monoclonal antibodies against MMP-9 and MMP-2 strongly impaired the emigration of Langerhans cells out of epidermal sheets.[1] As epidermal sheets procured by the use of the enzyme dispase (Kitano and Okado, 1983) possess no more lamina densa these data might suggest a role for the MMPs in helping to loosen contacts between epidermal keratinocytes. Such a function for MMP-9 on epidermal cells has been demonstrated in the context of carcinogenesis (Coussens *et al*, 2000).

Second, the scanning electron micrographs strongly suggest that for the transit through the basement membrane Langerhans cells need to be equipped with enzymatic tools. We[2] and others (Kobayashi, 1997) have shown that migrating Langerhans cells express MMP-9 and MMP-2. Kobayashi *et al* additionally demonstrated that Langerhans cells produce MMP-9, which is able to digest collagen IV (Kobayashi, 1997). TEM highlighted an important qualitative aspect. The basement membrane was absent (presumably ''digested away'') only in the very focused area of cell–basement membrane contact. This mode of action is typical for the MMPs (Basbaum and Werb, 1996).

Third, the scanning electron microscopic view of the dermis suggests that similar cellular tools may also be needed for the second leg of the Langerhans cell journey within the skin, namely from the basement membrane through the collagen thicket until they gain access to draining lymphatic vessels. The pictures suggest that migrating cells contact and temporarily adhere to collagen fibrils. One might speculate that the cells are actively pulling themselves along the fibrils.

Migrating dendritic cells appear to enter dermal lymphatic vessels by endothelial transmigration and through gaps in the endothelium Langerhans cells and dermal dendritic cells *en route* are chemotactically attracted towards the dermal lymphatic vessels by the chemokine SLC/CCL21, which is expressed in lymph endothelial cells *in situ* (Saeki *et al*, 1999). How exactly they

[1]Ratzinger G, Stoitzner P, Lutz MB, *et al*: Effect of matrix metalloproteinases on the migration of murine cutaneous dendritic cells. *Arch Dermatol Res* 292:74, 2000 (abstr.)

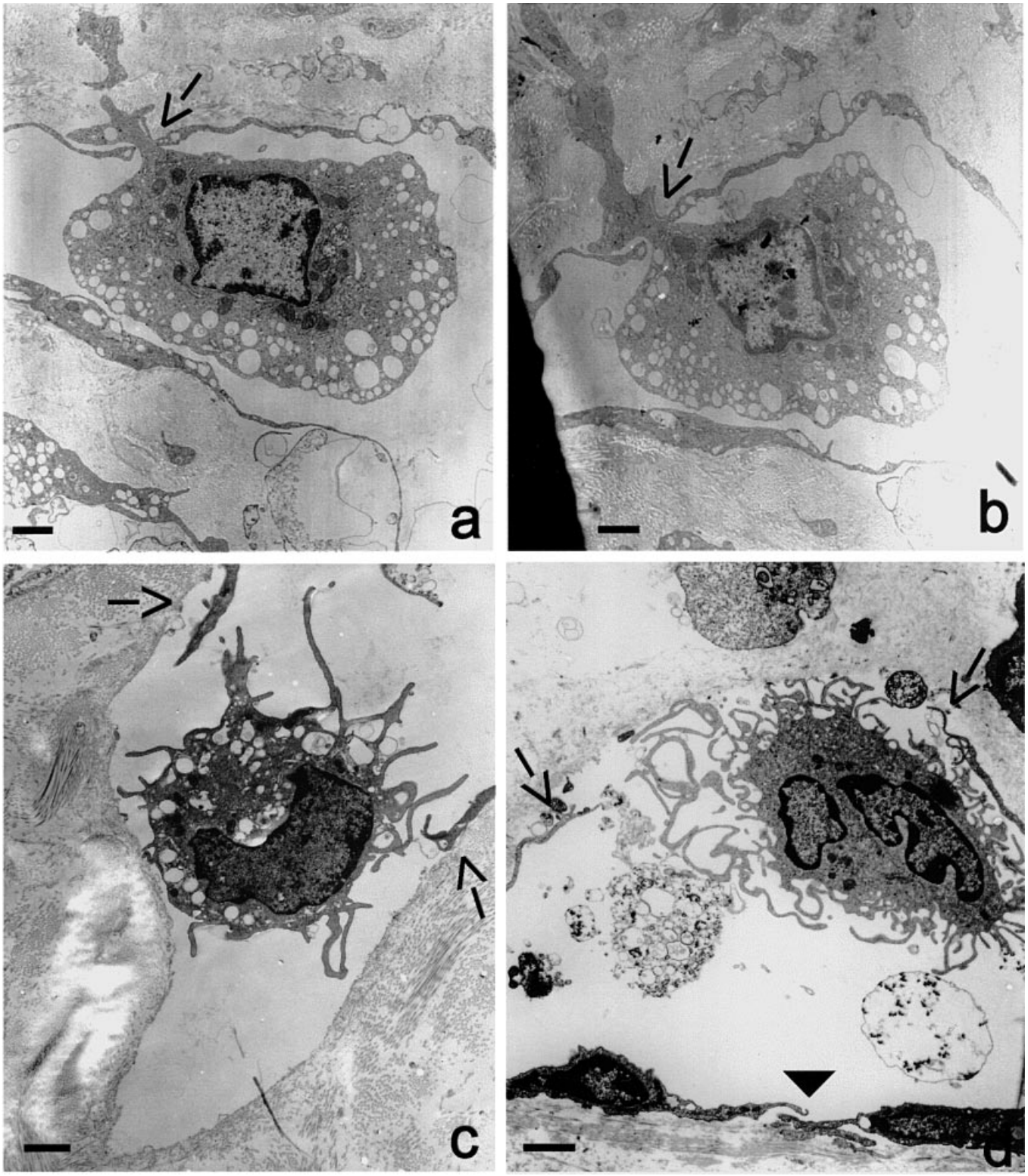

Figure 5. Dendritic cells enter the dermal lymphatics. Skin explants were cultured for 48 h. *Panels a and b* are consecutive ultrathin sections of the same transmigrating dendritic cell. The cell body is already inside the lumen of a lymph vessel; a cytoplasmic process is still extending into the dermal connective tissue. *Arrows* point at an area of close apposition of dendritic cell and endothelial cell surfaces. *Panels c and d* show gaps in the endothelial lining (*ending of endothelium indicated by arrows*). The gaps are wide enough to allow passage of cells. Note the distinct separation of electron-lucent lumen and more electron-dense connective tissue suggesting the presence of a physical barrier. *Panel d* also demonstrates that often endothelial cells are loosely connected to each other (*arrowhead*). *Scale bars*: (*a*, *b*, *c*) 1 μm; (*d*) 2 μm.

get into the lumen of the vessels is not clear. Our observations would suggest two possibilities: entry by transmigration through intercellular spaces and entry through gaps in the endothelial lining.

Transmigration of dendritic cells through intact layers of endothelium occurs. The experiments by Randolph *et al in vitro* (Randolph *et al*, 1998) and *in vivo* (Randolph *et al*, 1999) suggest an

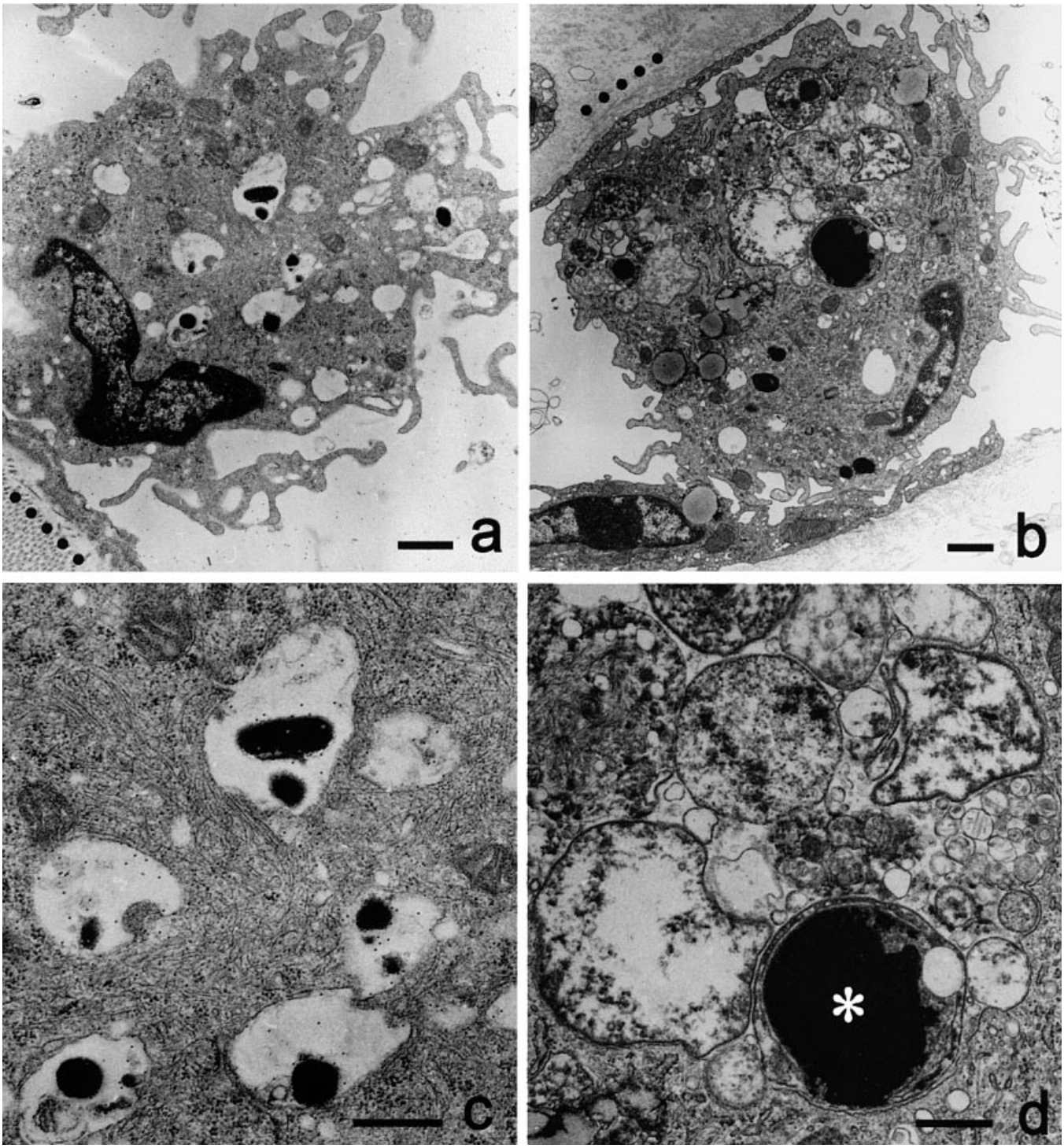

Figure 6. Dendritic cells migrating through lymph carry antigens. Skin explants were cultured for 48 h. The cell in *panels a and c* has ingested melanosomes; the cell in *panels b and d* contains much cellular debris including an apoptotic body (*asterisk*). A *dotted line* indicates lymphatic endothelium. *Scale bars*: (*a*, *b*) 1 μm; (*c*, *d*) 0.5 μm.

additional important role for this type of entry into lymphatic vessels. Inflammatory tissue monocytes may transform into dendritic cells upon ablumenal-to-lumenal transmigration through lymphatic endothelium. Even though the lymph vessel endothelial cells are very thin we noted a close apposition of transmigrating dendritic cells and endothelial cells (**Fig 5*a*, *b***) that may suffice to deliver the necessary signals for cell transformation as described by Randolph *et al*. Transmigration through the endothelium of dermal lymphatics may also be facilitated by the fact that endothelial cells are only loosely connected to each other; sometimes the most distal parts of their elongated cell bodies overlap, forming some sort of flap (**Fig 5*d***). This is clearly in contrast to transmigration through the tightly structured walls of blood vessels.

We have previously noted that in human (Lukas *et al*, 1996) and murine (Weinlich *et al*, 1998) skin explant cultures the lymphatic endothelium shows interruptions that appear large enough for a dendritic cell to go through. In these studies, however, we have not investigated the frequency of the gaps in detail. In this study we

gained the impression that such gaps were frequent. We saw dendritic cells close to the gaps and even in contact with the gaps (**Fig 5*c*, *d***); we did not see dendritic cells going across these gaps, however. Nevertheless, it seems likely that dendritic cells also enter via the gaps. It should be noted that there was always a well-delineated separation of ablumenal connective tissue and the lumen of the vessel, indicating that also the gaps had some sort of a vessel wall, perhaps consisting of electron-lucent lamina lucida materials such as integrins, laminins, etc.

Migrating dendritic cells carry antigens The prime function of dendritic cells in peripheral organs is to take up, process, and transport antigens to lymphoid organs. Observations with dendritic cells from the intestine have suggested that immature dendritic cells may also carry self antigens to the lymph nodes, thereby maintaining tolerance (Huang *et al*, 2000). We show here that this may happen also in the skin: dendritic cells transport self antigens such as melanosomes that they have probably collected from dying keratinocytes in the epidermis. They also carry apoptotic bodies. This would suggest that the described efficient pathway of cross-presentation, which uses apoptotic cells as the preferred form of antigen (Albert *et al*, 1998), may also be operative in the skin. In contrast to Huang *et al* (2000), however, melanosome- and apoptotic-body-containing dendritic cells in lymph vessels looked morphologically mature. Whether this would ultimately result in the generation of immunity of tolerance cannot be judged from ultrastructural observations only. Additionally, our observations complement and extend the work by Hemmi *et al* (2001), who showed recently that melanosomes are transported to draining nodes in two ways. First, we noted melanosome transport under conditions of normal melanogenesis; Hemmi *et al* used experimentally increased melanogenesis. Second, we show uptake and melanosome transport directly in the dermal lymphatics; Hemmi *et al* describe the end result of melanosome transport, i.e., accumulation in the lymph node.

Relevance Dendritic cells are crucial for the generation of immunity to epicutaneously or intracutaneously arriving pathogens or vaccines. The use of *in vitro* generated, tumor-antigen-loaded dendritic cells has come to the stage of clinical evaluation (Thurner *et al*, 1999; Fong and Engleman, 2000; Schuler-Thurner *et al*, 2000; Steinman and Dhodapkar, 2001). When dendritic cells are injected into the skin most of them have been shown to remain at the injection site (Morse *et al*, 1999; Eggert *et al*, 1999; Barratt-Boyes *et al*, 2000). Migration to the lymph nodes is ineffective, and it therefore appears desirable to improve this process and, as a consequence, improve immunogenicity. Better knowledge about the pathways and the regulation of dendritic cell migration in model systems will help to achieve this goal.

This work was supported by a grant of the Austrian Science Fund to N. Romani (P-12163-MED). The continued support of Dr. Peter Fritsch, Chairman of the Department of Dermatology, is greatly appreciated.

REFERENCES

Albert ML, Pearce SFA, Francisco LM, Sauter B, Roy P, Silverstein RL, Bhardwaj N: Immature dendritic cells phagocytose apoptotic cells via $\alpha_v\beta_5$ and CD36, and cross-present antigens to cytotoxic T lymphocytes. *J Exp Med* 188:1359–1368, 1998

Austyn JM: New insights into the mobilization and phagocytic activity of dendritic cells. *J Exp Med* 183:1287–1292, 1996

Banchereau J, Steinman RM: Dendritic cells and the control of immunity. *Nature* 392:245–252, 1998

Banchereau J, Briere F, Caux C, *et al:* Immunobiology of dendritic cells. *Annu Rev Immunol* 18:767–811, 2000

Barratt-Boyes SM, Zimmer MI, Harshyne LA, *et al:* Maturation and trafficking of monocyte-derived dendritic cells in monkeys: Implications for dendritic cell-based vaccines. *J Immunol* 164:2487–2495, 2000

Basbaum CB, Werb Z: Focalized proteolysis: spatial and temporal regulation of extracellular matrix degradation at the cell surface. *Curr Opin Cell Biol* 8:731–738, 1996

Birbeck MS, Breathnach AS, Everall JD: An electron microscopic study of basal melanocytes and high level clear cells (Langerhans cell) in vitiligo. *J Invest Dermatol* 37:51–64, 1961

Coussens LM, Tinkle CL, Hanahan D, Werb Z: MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* 103:481–490, 2000

Cumberbatch M, Dearman RJ, Kimber I: Langerhans cells require signals from both tumour necrosis factor-α and interleukin-1β for migration. *Immunology* 92:388–395, 1997

Eggert AAO, Schreurs MWJ, Boerman OC, *et al:* Biodistribution and vaccine efficiency of murine dendritic cells are dependent on the route of administration. *Cancer Res* 59:3340–3345, 1999

Enk AH, Angeloni VL, Udey MC, Katz SI: An essential role for Langerhans cell-derived IL-1β in the initiation of primary immune responses in skin. *J Immunol* 150:3698–3704, 1993

Fong L, Engleman EG: Dendritic cells in cancer immunotherapy. *Annu Rev Immunol* 18:245–273, 2000

Gunzer M, Friedl P, Niggemann B, Bröcker EB, Kämpgen E, Zänker KS: Migration of dendritic cells within 3-D collagen lattices is dependent on tissue origin, state of maturation, and matrix structure and is maintained by proinflammatory cytokines. *J Leukocyte Biol* 67:622–629, 2000

Hemmi H, Yoshino M, Yamazaki H, *et al:* Skin antigens in the steady state are trafficked to regional lymph nodes by transforming growth factor-β1-dependent cells. *Int Immunol* 13:695–704, 2001

Huang FP, Platt N, Wykes M, Major JR, Powell TJ, Jenkins CD, MacPherson GG: A discrete subpopulation of dendritic cells transports apoptotic intestinal epithelial cells to T cell areas of mesenteric lymph nodes. *J Exp Med* 191:435–443, 2000

Inaba K, Turley S, Yamaide F, *et al:* Efficient presentation of phagocytosed cellular fragments on the major histocompatibility complex class II products of dendritic cells. *J Exp Med* 188:2163–2173, 1998

Jakob T, Udey MC: Regulation of E-cadherin-mediated adhesion in Langerhans cell-like dendritic cells by inflammatory mediators that mobilize Langerhans cells *in vivo*. *J Immunol* 160:4067–4073, 1998

Karnovsky MJ: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J Cell Biol* 27:137A–138A, 1965

Kellermann SA, Hudak S, Oldham ER, Liu YJ, McEvoy LM: The CC chemokine receptor-7 ligands 6Ckine and macrophage inflammatory protein-3β are potent chemoattractants for *in vitro*- and *in vivo*-derived dendritic cells. *J Immunol* 162:3859–3864, 1999

Kitano Y, Okado N: Separation of the epidermal sheet by dispase. *Br J Dermatol* 108:555–560, 1983

Kobayashi Y: Langerhans' cells produce type IV collagenase (MMP-9) following epicutaneous stimulation with haptens. *Immunology* 90:496–501, 1997

Larsen CP, Steinman RM, Witmer-Pack M, Hankins DF, Morris PJ, Austyn JM: Migration and maturation of Langerhans cells in skin transplants and explants. *J Exp Med* 172:1483–1493, 1990

Lukas M, Stössel H, Hefel L, *et al:* Human cutaneous dendritic cells migrate through dermal lymphatic vessels in a skin organ culture model. *J Invest Dermatol* 106:1293–1299, 1996

Lutz MB, Rovere P, Kleijmeer MJ, *et al:* Intracellular routes and selective retention of antigens in mildly acidic cathepsin D/lysosome-associated membrane protein-1/MHC class II-positive vesicles in immature dendritic cells. *J Immunol* 159:3707–3716, 1997

Morse MA, Coleman RE, Akabani G, Niehaus N, Coleman D, Lyerly HK: Migration of human dendritic cells after injection in patients with metastatic malignancies. *Cancer Res* 59:56–58, 1999

Murphy G, Gavrilovic J: Proteolysis and cell migration: creating a path? *Curr Opin Cell Biol* 11:614–621, 1999

Ortner U, Inaba K, Koch F, Heine M, Miwa M, Schuler G, Romani N: An improved isolation method for murine migratory cutaneous dendritic cells. *J Immunol Meth* 193:71–79, 1996

Randolph GJ, Beaulieu S, Lebecque S, Steinman RM, Muller WA: Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science* 282:480–483, 1998

Randolph GJ, Inaba K, Robbiani DF, Steinman RM, Muller WA: Differentiation of phagocytic monocytes into lymph node dendritic cells *in vivo*. *Immunity* 11:753–761, 1999

Rescigno M, Urbano M, Valzasina B, *et al:* Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria. *Nat Immunol* 2:361–367, 2001

Roake JA, Rao AS, Morris PJ, Larsen CP, Hankins DF, Austyn JM: Dendritic cell loss from nonlymphoid tissues after systemic administration of lipopolysaccharide, tumor necrosis factor, and interleukin 1. *J Exp Med* 181:2237–2248, 1995

Romani N, Stingl G, Tschachler E, Witmer MD, Steinman RM, Shevach EM, Schuler G: The Thy-1 bearing cell of murine epidermis. A distinctive leukocyte perhaps related to natural killer cells. *J Exp Med* 161:1368–1383, 1985

Romani N, Bhardwaj N, Pope M, *et al:* Dendritic cells. In: Herzenberg LA, Weir DM, Herzenberg L, Blackwell C, eds. *Weir's Handbook of Experimental Immunology*. Oxford: Blackwell Science, 1997:pp 156.1–156.14

Romani N, Ratzinger G, Pfaller K, Salvenmoser W, Stössel H, Koch F, Stoitzner P: Migration of dendritic cells into lymphatics – the Langerhans cell example: routes, regulation, and relevance. *Int Rev Cytol* 207:237–270, 2001

Saeki H, Moore AM, Brown MJ, Hwang ST: Cutting edge: secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in

the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. *J Immunol* 162:2472–2475, 1999

Sallusto F, Cella M, Danieli C, Lanzavecchia A: Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med* 182:389–400, 1995

Schuler G, Romani N, Stössel H, Wolff K: Structural organization and biological properties of Langerhans cells. In: Schuler G, ed. *Epidermal Langerhans Cells*. Boca Raton, FL: CRC Press Inc, 1991:pp 87–137

Schuler-Thurner B, Dieckmann D, Keikavoussi P, *et al:* Mage-3 and influenza-matrix peptide-specific cytotoxic T cells are inducible in terminal stage HLA-A2.1^{+} melanoma patients by mature monocyte-derived dendritic cells. *J Immunol* 165:3492–3496, 2000

Schwarzenberger K, Udey MC: Contact allergens and epidermal proinflammatory cytokines modulate Langerhans cell E-cadherin expression *in situ*. *J Invest Dermatol* 106:553–558, 1996

Silberberg-Sinakin I, Thorbecke GJ, Baer RL, Rosenthal SA, Berezowsky V: Antigen-bearing Langerhans cells in skin, dermal lymphatics and in lymph nodes. *Cell Immunol* 25:137–151, 1976

Steinman RM, Pack M, Inaba K: Dendritic cells in the T-cell areas of lymphoid organs. *Immunol Rev* 156:25–37, 1997

Steinman RM, Dhodapkar M: Active immunization against cancer with dendritic cells: The near future. *Int J Cancer* 94:459–473, 2001

Stoitzner P, Ratzinger G, Koch F, *et al:* Interleukin-16 supports the migration of Langerhans cells, partly in a CD4-independent way. *J Invest Dermatol* 116:641–649, 2001

Stössel H, Koch F, Kämpgen E, *et al:* Disappearance of certain acidic organelles (endosomes and Langerhans cell granules) accompanies loss of antigen processing capacity upon culture of epidermal Langerhans cells. *J Exp Med* 172:1471–1482, 1990

Thurner B, Haendle I, Röder C, *et al:* Vaccination with Mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. *J Exp Med* 190:1669–1678, 1999

Valladeau J, Ravel O, Dezutter-Dambuyant C, *et al:* Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 12:71–81, 2000

Weinlich G, Heine M, Stössel H, *et al:* Entry into afferent lymphatics and maturation *in situ* of migrating cutaneous dendritic cells. *J Invest Dermatol* 110:441–448, 1998

Zinkernagel RM: Immunology taught by viruses. *Science* 271:173–178, 1996