Matrix Metalloproteinases 9 and 2 Are Necessary for the Migration of Langerhans Cells and Dermal Dendritic Cells from Human and Murine Skin


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Matrix Metalloproteinases 9 and 2 Are Necessary for the Migration of Langerhans Cells and Dermal Dendritic Cells from Human and Murine Skin

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Dendritic cells migrate from the skin to the draining lymph nodes. They transport immunogenic MHC-peptide complexes, present them to Ag-specific T cells in the T areas, and thus generate immunity. Migrating dendritic cells encounter physical obstacles, such as basement membranes and collagen meshwork. Prior work has revealed that matrix metalloproteinase-9 (MMP-9) contributes to mouse Langerhans cell migration. In this study, we use mouse and human skin explant culture models to further study the role of MMPs in the migration and maturation of skin dendritic cells. We found that MMP-2 and MMP-9 are expressed on the surface of dendritic cells from the skin, but not from other sources. They are also expressed in migrating Langerhans cells in situ. The migration of both Langerhans cells and dermal dendritic cells is inhibited by a broad spectrum inhibitor of MMPs (BB-3103), by Abs to MMP-9 and -2, and by the natural tissue inhibitors of metalloproteinases (TIMP), TIMP-1 and TIMP-2. Inhibition by anti-MMP-2 and TIMP-2 define a functional role for MMP-2 in addition to the previously described function of MMP-9. The importance of MMP-9 was emphasized using MMP-9-deficient mice in which Langerhans cell migration from skin explants was strikingly reduced. However, MMP-9 was only required for Langerhans cell migration and not maturation, since nonmigrating Langerhans cells isolated from the epidermis matured normally with regard to morphology, phenotype, and T cell stimulatory function. These data underscore the importance of MMPs, and they may be of relevance for therapeutically regulating dendritic cell migration in clinical vaccination approaches. The Journal of Immunology, 2002, 168: 4361–4371.

Dendritic cells migrate from the skin and other tissues and organs to the draining lymphoid organs. They transport antigenic proteins (1), process them into immunogenic MHC-peptide complexes, present them to Ag-specific T cells in the T areas, and thus efficiently elicit immune responses (2). Besides their highly developed capacities to process Ags and to sensitize naïve T cells, dendritic cells are also capable of migrating, more so than other cell types (3). Migration occurs through the dermal lymphatic vessels (4–7).

Dendritic cell migration is regulated in a complex manner. Clearly, inflammatory cytokines such as TNF-α and IL-1β are critical initiation cytokines (8–11). Mechanistically, TNF-α down-regulates E-cadherin on epidermal Langerhans cells (12), thereby loosening these cells within the epidermis and rendering them responsive to chemotactic stimuli. Such stimuli were first found to be made by fibroblasts (13). Recently, chemokines were defined as potent chemoattractants for cutaneous dendritic cells: macrophage inflammatory protein-3β/CCL19 (14) and secondary lymphoid tissue chemokine/CCL21 (15) were shown to enhance the emigration of Langerhans cells and dermal dendritic cells from the skin.

On their way from the epidermis, Langerhans cells have to cross the basement membrane and move through connective tissue until they reach a lymph vessel, which they enter to travel further to the draining lymph node. The pathway through the collagenous connective tissue is the same for dermal dendritic cells. Interaction with the extracellular matrix is therefore important (16). α1 integrins (17) and CD44 (18) were shown to be critically involved in the migration of dendritic cells from the skin. Proteinases are probably also relevant, particularly when dendritic cells have to pass such relatively solid obstacles such as basement membranes. Matrix metalloproteinases (MMPs) constitute a family of proteinases (including collagenases) that participate in cell migration (19). MMPs may be expressed on the surface of cells, thus allowing for precise, localized proteolysis (20, 21). This would create a path (22) for migrating cells.

Indeed, MMP-9 could be detected in epidermal Langerhans cells by both immunohistochemistry (23) and enzymographic assays (24) that suggested the presence of a functional enzyme. Inflammatory cytokines (TNF-α, IL-1β) induce the expression of MMPs in human macrophages (25). Epicutaneous application of contact

1 Abbreviations used in this paper: MMP, matrix metalloproteinase; MMP1, inhibitor of MMP; TIMP, tissue inhibitor of metalloproteinase.
Materials and Methods

Mice

Mice of inbred strains C57BL/6 and BALB/c were purchased from Charles River Breeding Laboratories (Sulzfeld, Germany) and used at 8–12 wk of age. Gelatinase B/MMP-9-deficient mice were generated by targeted mutagenesis and maintained on a strain 129SvEv background (31).

Media and reagents

The culture medium was RPMI 1640 supplemented with 10% FCS, gentamicin (all from Biological Industries, Kibbutz Beit Haemek, Israel) and 2-ME (Sigma-Aldrich, St. Louis, MO). The following mAbs were used for immunohistochemistry of mouse skin: anti–I-Ab,d (clone B21-2/TIB229, rat IgG2b, from R. M. Steinman, The Rockefeller University, New York, NY), anti–I-A\(\alpha\) (clone MK-62/HB3, mouse IgG2a; American Type Culture Collection, Manassas, VA), anti–I-A\(\beta\) (clone 2G9, mouse IgG1, provided by Dr. K. Yoneda, Kyoto University, Kyoto, Japan) (34) were used. Abs were visualized by immunofluorescence using biotinylated anti-rat or anti-mouse Igs and streptavidin-Texas Red (both from Amersham, Amersham, U.K.), as previously described (5).

The culture medium was RPMI 1640 supplemented with 10% FCS, gentamicin, and tetracycline (all from Biological Industries, Kibbutz Beit Haemek, Israel) and used at 8–12 wk of age. Gelatinase B/MMP-9-deficient mice were generated by targeted mutagenesis and maintained on a strain 129SvEv background (31).

Preparation of epidermal cell suspensions

Preparation of epidermal cell suspensions was performed by standard trypsinization of epidermal tissue as previously described (29, 38). Cell suspensions were cultured in culture medium supplemented with 500 U/ml recombinant murine GM-CSF. On day 3 of culture, the percentage of Langerhans cells was determined in the hemocytometer. At this point mature Langerhans cells can be readily recognized by their pronounced veiled or hairy shape that clearly sets them apart from the other cells in the suspension (mainly keratinocytes).

Determination of T cell stimulatory capacity

The oxidative mitogenesis assay and the allogeneic MLR were used for this purpose as previously described (39). Resting T cells from spleen and lymph nodes were purified by means of nylon wool columns, followed by a discontinuous Percoll gradient as described previously (40). Varying numbers of Langerhans cells were cocultured with 5 \(\times\) 10^3 purified T cells that were either periodated (for the oxidative mitogenesis) or left untreated (for the MLR). After 24 h (oxidative mitogenesis) or 72 h (MLR) tritiated thymidine was added for another 6–18 h. Incorporated radioactivity was determined in a liquid scintillation counter.

FIGURE 1. A broad spectrum matrix metalloproteinase inhibitor restraints the migration of mouse dendritic cells. Murine skin explants were cultured for 48 h in the absence (□) or the presence of MMP-inhibitor BB-3103. a, and b, Representative experiments; absolute numbers of dendritic cells emigrated per one skin explant (i.e., one ear half) are indicated on the y-axis. c, All experiments (including those shown in a and b) are summed up. Spontaneous emigration of dendritic cells in plain culture medium was set equal to 100%. Differences were highly significant: medium only vs 10 \(\mu\)M BB-3103, \(p < 0.0001\) (range, 25–49%; \(n = 7\)); medium only vs 50 \(\mu\)M BB-3103, \(p > 0.0001\) (range, 11–46%; \(n = 9\)). □, emigration in the presence of DMSO only. Error bars indicate SDs.
Preparation of epidermal and dermal sheets for immunohistochemistry

Skin was floated dermal side down on 0.5 M ammonium thiocyanate for 20–30 min at 37°C. The epidermis was peeled off the dermis, and both parts were cut into 5 × 5-mm pieces and fixed in aceton for 20 min at ambient temperature. Sheets were immunostained as previously described (5).

Intradermal administration of reagents

Cytokines and the MMPI were diluted in PBS/10% FCS and injected with 1-ml tuberculin syringes equipped with a 30-gauge needle. Mice received 30 μl cytokine dilution intradermally into the pinna of one ear and the contralateral ear. After different time points epidermal sheets were prepared, and immunohistochemistry was performed as previously described (5).

Evaluation

The main read-out was the number of dendritic cells that had emigrated from the skin into the culture medium in the course of the 48-h culture. Dendritic cells could be readily identified by their hairy and veiled appearance under the hemocytometer. The mean ± SD are given; Student’s t test for paired samples was applied to test for the significance of differences. The phenotype of emigrated dendritic cells was determined by staining cytocentrifuge smears or flow cytometry. In addition, epidermal and dermal sheets were prepared by means of ammonium thiocyanate (41) and stained with mAbs against MHC class II to identify Langerhans cells in the epidermis and dermal dendritic cells. The density of Langerhans cells in epidermal sheets was counted under the microscope using ×40 objective lenses and a calibrated grid. Areas to be counted were selected for inter-follicular regions of even staining and regular distribution of positive cells. There, 16–50 grids were randomly chosen. Fields containing hair follicles were excluded from the analyses. Langerhans cells were counted, and basic statistical parameters (means and SD) were calculated.

Results

Emigration of dendritic cells from murine skin into the culture medium is strongly inhibited by a broad spectrum inhibitor of MMPs

Dendritic cells emigrate spontaneously from murine whole skin explants into the culture medium over a period of 1–3 days (6, 42). Between 5,000 and 20,000 dendritic cells can be retrieved from one dorsal ear half after 48 h. When compound BB-3101, an MMPI, was added to the cultures, a dose-dependent reduction in the number of dendritic cells retrieved from the culture medium was observed (Fig. 1). Vehicle controls (DMSO) had no effect on migration (Fig. 1). To rule out a cytotoxic effect of the MMPI, skin explants from cultures that had received the highest concentration of MMPI (50 μM) from days 0 to 3 were transferred to fresh, MMPI-free culture medium and cultured for another 1–3 days. During this period many dendritic cells emigrated from these explants (Table 1). By phase contrast and in the hemocytometer, MMPI-treated dendritic cells looked normal and viable (as assessed by trypan blue exclusion). They also had the hairy morphology typical of fully mature skin dendritic cells.

To correlate the numbers of emigrant dendritic cells with the events occurring within the tissue, we performed immunohistochemistry of epidermal and dermal sheets derived from cultures that had been treated or not with the MMPI. Compared with fresh, uncultured epidermis, a pronounced reduction in the density of Langerhans cells was observed when skin explants were cultured in the absence of MMPI (Fig. 2). This has been described previously (5, 6, 11). The presence of an MMPI during the culture period attenuated the decline in Langerhans cells; more Langerhans cells were observed (Fig. 2). Vehicle controls (DMSO) had no effect on migration (Fig. 1). To rule out a cytotoxic effect of the MMPI, skin explants from cultures that had received the highest concentration of MMPI (50 μM) from days 0 to 3 were transferred to fresh, MMPI-free culture medium and cultured for another 1–3 days. During this period many dendritic cells emigrated from these explants (Table 1). By phase contrast and in the hemocytometer, MMPI-treated dendritic cells looked normal and viable (as assessed by trypan blue exclusion). They also had the hairy morphology typical of fully mature skin dendritic cells.

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![Image](http://www.jimmunol.org/)

**FIGURE 2.** Immunohistochemistry of migrating murine and human dendritic cells. Epidermal and dermal sheets were prepared from skin explants that were freshly excised (a, d, and g) or were cultured for 48 h in the absence (b, e, and h) or the presence (c, f, and i) of 50 μM inhibitor BB-3103. Explants from mouse epidermis (top row) and dermis (bottom row) and from human epidermis (middle row) are shown. Photographs in each row were taken with identical exposure times to allow for a semiquantitative comparison of fluorescence intensities. Sheets were stained with mAbs against MHC class II. Langerhans cells become larger during culture (a vs b and d vs e). Note that the addition of the MMPI prevents many Langerhans cells from leaving the epidermis, but it does not prevent maturation as indicated by increased MHC II expression (c and f). In the dermis, lymphatics (cords) appear less densely filled with dendritic cells (h vs i). Magnification, ×430. Bar, 30 μm.
MMPs AND MIGRATION OF SKIN DENDRITIC CELLS

FIGURE 3. A broad spectrum MMP inhibitor restrains the migration of human dendritic cells. Human skin explants were cultured for 48 h in the absence (□) or the presence (■) of graded concentrations of inhibitor BB-3103. a and b, Representative experiments; absolute numbers of dendritic cells emigrated per one skin explant are indicated on the y-axis. c, All independent experiments with the high concentrations of MMPI (including those shown in a and b) are summed up. Spontaneous emigration of dendritic cells in plain culture medium was set equal to 100%. Differences were highly significant: q < 0.0001 (range, 4–40%; n = 10). Error bars indicate SDs.

expression and their enlarged size. Some specimens were embedded for conventional transmission electron microscopy. The light microscopic analysis on semithin sections of long stretches of basement membrane zone revealed clearly more basal Langerhans cells (i.e., branched cells with clear cytoplasm) in MMPI-treated cultures compared with untreated cultures (not shown). Immunohistochemical inspection of the dermis revealed the typical cords, i.e., lymphatic vessels filled with strongly MHC class II-expressing, migrating dendritic cells (5). The cords were present, but reduced in number, and they contained fewer dendritic cells in MMPI-treated cultures, indicating that fewer dendritic cells were mobilized when the action of MMPs was inhibited.

Emigration of dendritic cells from human skin into the culture medium is strongly inhibited by a broad spectrum inhibitor of MMPs

To obtain reproducible and reliable cell counts, it was necessary to standardize the human skin explant cultures as much as possible. This was achieved by using only skin that had been dermatomed with a thickness of 0.2–0.3 mm and by culturing 8-mm punches (0.5 cm²). It was important to culture each explant in a separate 24-well plate. For reasons not further explored, cultures in petri dishes yielded inconsistent results. This is exactly as we had previously described for murine skin explants (42).

When 72-h cultures of human skin explants were treated with graded doses of the MMPI (compound BB-3103), we observed the same dose-dependent inhibition of dendritic cell emigration as in the mouse (Fig. 3). At the highest concentration of MMPI, emigration was almost abrogated; compared with untreated cultures, fewer than 5% dendritic cells could be retrieved from the culture medium. Vehicle controls (DMSO) had no effect on migration (data not shown). Transfer of MMPI-treated explants onto fresh MMPI-free culture medium and further culture for 1–2 days yielded high numbers of emigrant dendritic cells, strongly suggesting that the MMPI was not toxic and apparently acted in a reversible manner (Table I). Lack of toxicity at concentrations of BB-3103 up to 100 μM was recently also shown for human keratinocytes in vitro (43).

Immunohistochemistry showed that more Langerhans cells stayed behind in epidermal sheets from explants that had been cultured in the presence of the MMPI; the remaining Langerhans cells appeared activated, as indicated by their increased size and their enhanced expression of MHC class II (Fig. 2, d–f).

A broad spectrum inhibitor of MMPs inhibits emigration of dendritic cells from both epidermis and dermis

Dendritic cells that emigrate into the culture medium from whole skin explants consist of both epidermal dendritic cells (Langerhans cells) and dermal dendritic cells. The ratio of these two types of dendritic cells is unknown in the mouse, since there is no commercially available reliable marker that would distinguish dendritic cells of epidermal vs dermal derivation; the mAb Lag does not cross-react with mouse Birbeck granules. In human explant cultures the majority of emigrated dendritic cells were Langerhans cells, as defined by their expression of the Birbeck-granule-associated Lag Ag (data not shown). Due to these uncertainties we decided to separate epidermis from dermis before the start of the culture and to assess the effects of MMPI on the two compartments separately. In murine and human cultures we found an effect on both epidermis and dermis. Although generally fewer dendritic cells emigrated from dermal sheets than from the corresponding epidermal sheets, we noted a similar degree of inhibition in both compartments (Fig. 4). The inhibitory effect also became obvious when comparing absolute numbers of emigrated cells in individual experiments (Table II).
Migration of dendritic cells is inhibited by a broad spectrum inhibitor of MMPs in vivo

Intradermal injection of an inflammatory cytokine such as TNF-α leads to the emigration of a substantial number of Langerhans cells from the epidermis (44). In three separate experiments the TNF-α-induced decrease in Langerhans cell density, as assessed by counting Langerhans cells in immunohistochemically labeled epidermal sheets, was between 15 and 30%. When the broad spectrum inhibitor of MMPs, BB-3103 (100 μM), was between 15 and 30%. When the broad spectrum inhibitor of MMPs, BB-3103 (100 μM), was added to whole skin explant cultures (at concentrations ranging from 12.5–200 ng/ml), a strong inhibition became evident with either TIMP (Fig. 6). Both TIMPs inhibited to a similar degree at the highest concentration of 200 ng/ml. The number of dendritic cells that could be retrieved from TIMP-treated cultures were about a quarter of those from untreated control cultures. Similar observations were made using neutralizing mAbs against MMP-2 (clones 45-5D11 and CA-4001) as well as MMP-9 (clone 6-6B).

When whole skin explants were cultured in the presence of either Abs against MMP-2 and MMP-9 were combined, the inhibitory effect was more pronounced, indicating an additive mode of action (Table V). Dendritic cell emigration from both cultured epidermal sheets and, to a lesser degree, cultured dermal sheets was also inhibited by the Abs (Table IV).

MMP-9 is a critical MMP: additional evidence obtained by the use of MMP-9 gene-deficient mice

Initially, the density of Langerhans cells in untreated epidermis of MMP-9-deficient mice was determined and found not to be different from that in control mice (data not shown). Differences in

### Table II. Migration of Langerhans cells from epidermal sheets and of dermal dendritic cells from dermal sheets is inhibited by a broad-spectrum MMPi

<table>
<thead>
<tr>
<th>Epidermal Explants&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Culture medium only</th>
<th>BB-3103 (50 μM)</th>
<th>Dermal Explants&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Culture medium only</th>
<th>BB-3103 (50 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Expt. 1</td>
<td>31,700&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8,700</td>
<td>7,100</td>
<td>3,500</td>
<td></td>
</tr>
<tr>
<td>Expt. 2</td>
<td>14,300</td>
<td>6,300</td>
<td>3,300</td>
<td>2,000</td>
<td></td>
</tr>
<tr>
<td>Expt. 3</td>
<td>37,900</td>
<td>5,000</td>
<td>6,600</td>
<td>2,300</td>
<td></td>
</tr>
<tr>
<td>Expt. 4</td>
<td>18,100</td>
<td>5,700</td>
<td>9,400</td>
<td>1,600</td>
<td></td>
</tr>
<tr>
<td>Expt. 5</td>
<td>35,000</td>
<td>9,200</td>
<td>11,900</td>
<td>3,100</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>27,400 ± 10,540</td>
<td>6,980 ± 1,860</td>
<td>7,700 ± 3,200</td>
<td>2,500 ± 780</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Murine or human skin was treated with dispase, epidermis and dermis were separated from each other, and both tissues were cultured separately for 48 h in the presence or absence of BB-3103 at a concentration of 50 μM.

<sup>b</sup> Absolute numbers of dendritic cells emigrated per one explant are indicated.

### Table III. Effects of a broad-spectrum inhibitor of MMPs on the migration of Langerhans cells in vivo<sup>c</sup>

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Carrier protein only</th>
<th>TNF-α</th>
<th>p &lt;</th>
<th>Carrier protein only</th>
<th>TNF-α + MMP</th>
<th>p &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1275 ± 179 (100%)</td>
<td>975 ± 152 (76%)</td>
<td>0.001</td>
<td>1145 ± 177 (100%)</td>
<td>1411 ± 181 (123%)</td>
<td>0.002</td>
</tr>
<tr>
<td>2</td>
<td>1194 ± 152 (100%)</td>
<td>844 ± 124 (71%)</td>
<td>0.0001</td>
<td>1185 ± 139 (100%)</td>
<td>1434 ± 179 (121%)</td>
<td>0.001</td>
</tr>
<tr>
<td>3</td>
<td>891 ± 105 (100%)</td>
<td>766 ± 133 (86%)</td>
<td>0.05</td>
<td>943 ± 154 (100%)</td>
<td>1427 ± 120 (151%)</td>
<td>0.0001</td>
</tr>
<tr>
<td>4</td>
<td>1070 ± 152 (100%)</td>
<td>781 ± 139 (73%)</td>
<td>0.0001</td>
<td>981 ± 109 (100%)</td>
<td>1619 ± 143 (165%)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

<sup>c</sup> Mice were injected intradermally in the pinnae of their ears with the reagents indicated. Twenty-four hours thereafter epidermal sheets were prepared, immunohistochemically stained for MHC class II molecules that identify Langerhans cells. Langerhans cells per mm² ± SDs are indicated here. As a control, carrier protein was injected into the respective contralateral ears. Each individual experiment comprises two mice, one used for TNF-α injection and control injection (columns A and B), and one used for TNF-α plus MMPi (BB-3103) injection and control injection (columns D and E). Both the reduction in the densities of Langerhans cells in response to the injection of TNF-α (columns A vs B) and the increased densities of Langerhans cells in TNF-α plus MMPi-treated ear epidermis (columns D vs E) were statistically significant (p values in columns C and F).
the degree of emigration of Langerhans cells could therefore not be attributed to a smaller starting population of Langerhans cells within the epidermis. Whole skin explant cultures from MMP-9-deficient mice revealed a striking impairment of emigration of cutaneous dendritic cells. In three independent experiments, the numbers of dendritic cells that could be retrieved from the culture medium were 13, 39, and 34%, respectively, of the numbers recovered from cultures of age- and sex-matched littermate controls (Fig. 7).

**MMP-9 and MMP-2 are expressed in Langerhans cells and dermal dendritic cells in situ and in culture**

We attempted to visualize the MMPs in situ in fresh and cultured skin. This was only performed with human specimens. In epidermal sheets obtained from fresh skin and from skin that had been cultured for 72 h, we noted the expression of both types of MMPs on MHC class II-positive cells, i.e., Langerhans cells. Expression was up-regulated upon culture of skin explants (Fig. 8). Staining of Langerhans cells in acetone-fixed epidermal sheets does not allow an unequivocal distinction between surface and intracellular localization of Ab reactivities. Therefore, this point was tested with dendritic cells that had “crawled out” from untreated human skin explants during the 48-h culture period. They were analyzed by flow cytometry for the expression of MMPs. Both MMP-2 and MMP-9 were detected on the surface of these cells (Fig. 9). In contrast, neither immature nor mature human monocyte-derived dendritic cells nor mature human CD34+ cord blood stem cell-derived dendritic cells expressed either of the two MMPs on the cell surface. Intracellular expression of MMPs in the latter cell types was not investigated.

**Maturation of Langerhans cells is not dependent on MMP-9**

Kobayashi et al. (27) noted an effect of purified MMP-9 on the expression of MHC class II on Langerhans cells, suggesting a role for MMP-9 in the maturation process. We have addressed this question in the MMP-9-deficient model. Epidermal cell suspensions were obtained by trypsinization and were cultured in bulk until day 3 (39). The numbers of mature Langerhans cells that could be recovered at the end of the culture period were comparable. Their morphology under phase contrast (veils) and under the hemocytometer (hairy) was identical. In both the allogeneic mixed leukocyte reaction and the oxidative mitogenesis assay cultured Langerhans cells from MMP-9−/− and MMP-9+/+ mice showed equal stimulatory capacity for resting T cells (Fig. 10). Finally, the
expression of the 2A1 Ag, a selective maturation marker (32), did not differ between MMP-9-deficient mice and controls. Moreover, MHC class II was strongly expressed, and the MHC class II-associated invariant chain (mAb In1) was largely absent on cultured Langerhans cells of both MMP-9-deficient mice and controls, as determined in cytospin preparations (data not shown).

### Discussion

We show here a critical involvement of MMPs in the migration of cutaneous dendritic cells. It has previously been reported that murine (16) and human (24) Langerhans cells express MMP-9. A functional role for MMPs was also described in a mouse model (27) and with human skin dendritic cells (45). The induction of contact hypersensitivity, which requires migration of dendritic cells from skin to lymph nodes, was recently shown to be dependent on the presence of MMP-3 (28). In this report we extend these data in several important and novel ways. 1) The effects of MMPs are dissected with regard to epidermal Langerhans cells vs dermal dendritic cells. The migration of both types of dendritic cells, human as well as murine, is dependent on MMPs. 2) MMPs are critical both in vitro (as shown in skin explant cultures) and in vivo (as shown by intradermal injection experiments). 3) A functional role for MMP-2 is defined in addition to the previously described role for MMP-9 in the migration of skin dendritic cells is confirmed and strengthened in the MMP-9-deficient mouse, in which migration was drastically reduced. 7) However, MMP-9 does not appear to be essential for the maturation of Langerhans cells, since nonmigrating Langerhans cells mature normally in MMP-9-deficient mice.

![Figure 7](image)

**Figure 7.** Emigration of cutaneous dendritic cells from skin explants of MMP-9-deficient mice is markedly impaired. Skin explants from sex- and age-matched normal littermates (■) and from MMP-9-deficient mice (▲) were cultured for 72 h, and the numbers of dendritic cells that had emigrated into the culture medium were counted in the hemocytometer. The results of three independent experiments are shown. The absolute numbers of dendritic cells (per one explant) that had migrated out of the explants are indicated on the y-axis. Combined data from these three experiments are demonstrated in the bottom right panel. Emigration of control mice was set equal to 100% in each individual experiment. On the average, 29 ± 14% of dendritic cells emigrate from explants of MMP-9-deficient mice. This is statistically significant by Student’s t test for paired samples (p < 0.02). Error bars indicate SDs.
Relevance of MMPs for the migration of epidermal Langerhans cells across the basement membrane

When a Langerhans cell leaves the epidermis it must first cross the basement membrane (7). We found that both MMP-2 and MMP-9 are essential for Langerhans cells to leave the epidermis. This was shown 1) by a synthetic broad spectrum inhibitor (BB-3103), 2) by inhibition experiments using anti-MMP-2 and -MMP-9 mAbs, and 3) most strikingly by the use of MMP-9-deficient mice. Given the known substrate specificity of MMP-2 and -9, namely, collagen type IV, this is not surprising. Despite their similar specificities, experiments using Abs against both MMPs suggest that the effects of the two MMPs may not be identical, but, rather, additive. Kobayashi et al. (27) have recently demonstrated an MMP effect in a murine contact hypersensitivity model in which the injection of a neutralizing anti-MMP-9 mAb into the skin prevented the contact sensitizer-induced emigration of Langerhans cells from the epidermis and the accumulation of dendritic cells in the regional lymph nodes. Similarly, in a human skin explant model Lebre et al. (45) noted that inhibitors of MMPs prevented the emigration of Langerhans cells from the epidermis in response to the epicutaneous...
application of a sensitizer (nickel). The critical role for MMP-9 in the transmigration through basement membranes has previously been shown for other cell types, e.g., T cells (46). It appears not to be an absolute necessity for all cell types, though, because granulocytes can migrate normally in the MMP-9-deficient mice (47). Interestingly, when the MMP inhibitor was injected into the ear skin together with TNF-α, we noted an increased density of Langerhans cells 24 h thereafter (Table III). Apparently, the compound had inhibited egress of Langerhans cells from the epidermis, but not the influx of Langerhans cell progenitors, leading to some degree of Langerhans cell accumulation in the epidermis. This may reflect different MMP-related requirements of emigrating Langerhans cells and immigrating precursors.

Relevance of MMPs for the migration of Langerhans cells and dermal dendritic cells through the dermal connective tissue

After traversing the basement membrane, migrating dendritic cells need to work their way through the dermal meshwork of collagen and elastin fibers until they finally enter lymphatic vessels (7). Clearly, MMPs are involved in dendritic cell migration through the dermis. This can be concluded from the marked inhibition of emigration from dermal explant cultures by a broad spectrum inhibitory compound and by mAbs against MMP-2 and MMP-9. Thus, dendritic cells use MMP-2 and -9 for "creating their path" (22) through the dermal extracellular matrix.

Relevance of MMPs in the contact hypersensitivity model

These data also bear on the recent observations that the development of contact hypersensitivity is not impaired in MMP-9-deficient mice (28). In light of the dramatic inhibition of Langerhans cell migration in these gene-deficient mice, three possible, not mutually exclusive, explanations might account for this surprising discrepancy. 1) Perhaps migration of Langerhans cells in response to a contact allergen such as 2,4-dinitro-1-fluorobenzene, which was used in the above-mentioned study, is not inhibited in MMP-9-deficient mice, as opposed to the data shown here in the skin explant model. This is unlikely, however, since the mechanisms of migration appear to be the same in contact hypersensitivity and explant cultures (5, 10, 11). 2) In addition, contact hypersensitivity might be brought about by the free diffusion of the contact allergen into the lymph nodes where it would bind to (i.e., haptenize) local dendritic cells. It has recently been emphasized that this route of haptenization is important and should not be forgotten (48). Yet, the fact that in MMP-3-deficient mice, where such free diffusion most likely occurs to the same extent as in MMP-9-deficient mice, contact hypersensitivity is suppressed (28) would argue against this explanation. 3) If migration of Langerhans cells is blocked or strongly inhibited, this would imply that dermal dendritic cells are responsible for transport of the hapten to the lymph nodes and thus for sensitization of the mice. We have not directly investigated the migration of dermal dendritic cells in MMP-9-deficient mice. Experiments with dermal explants from normal mice using neutralizing anti-MMP-9 mAbs revealed that dermal dendritic cells are also dependent on MMP-9, albeit not completely. In the presence of the blocking Ab substantial numbers of dendritic cells still emigrated from dermal explants. This may be similar in the MMP-9−/− mouse. Given the extraordinary T cell stimulatory capacity of dendritic cells, these few dendritic cells together with the few Langerhans cells that still migrate in the absence of MMP-9 might successfully induce contact hypersensitivity.

Dendritic cell maturation and MMPs

Maturation and migration of skin dendritic cells are tightly linked processes (5). Maturation can apparently not occur without concomitant maturation. Dendritic cells in skin explant cultures have presumably received their initial maturation and migration stimulus by the stress exerted when excising and handling the skin for the culture. Therefore, they migrate spontaneously. This initial stimulus could only partially be neutralized by the MMP inhibitor. Migration, however, was greatly reduced. Experimentally added inflammatory cytokines accelerate migration; anti-inflammatory treatments slow it down (11). Phenotypical features of dendritic cell maturation, however, such as augmented MHC class II expression, translocation of MHC class II from intracellular pools to the surface membrane (49), and enlargement of the cells, were not influenced by the inhibitor. Importantly, in MMP-9−/− mice the expression of maturation markers and the T cell stimulatory capacity of mature, i.e., cultured Langerhans cells were found to be normal. From this it appears that once an initial inflammatory stimulus has been delivered to dendritic cells, the neutralization of MMPs blocks migration, but not phenotypical, morphological, and functional maturation. This may be different when the MMPs are blocked before the initial inflammatory stimulus is given, such as in Kobayashi’s experiments in which an anti-MMP-9 mAb was injected into the skin before a contact sensitizer was applied epicutaneously (27). In that setting maturation, as measured by increased MHC class II expression on Langerhans cells, was indeed inhibited.

Relevance in vivo

Dendritic cells are increasingly used for immunotherapeutic approaches, predominantly in oncology (50). Tumor Ag-charged autologous dendritic cells are administered intracutaneously (s.c. or intradermally). They are expected to migrate to the draining lymph
nodes and to induce immunity there. This has been shown to happen, albeit at a low efficiency. A vast majority of dendritic cells remain at the injection site in the skin (51–53). Ways are being sought to improve the migration rate of these cells. Treatment of dendritic cells with TNF-related activation-induced cytokine has recently been shown to increase the numbers of injected dendritic cells that arrive in the lymph nodes in a mouse model (54). Two aspects of the data presented here may be of relevance to dendritic cell vaccinations in the skin. First, we showed that MMPs are critical for the migration of dendritic cells within the dermal meshwork and not only for penetration of basement membranes. This is the very situation of a vaccination where the injected cells are placed directly into the dermis or subcutis. It is therefore tempting to speculate that the concomitant administration of reagents that activate MMP function might be of benefit in dendritic cell vaccinations (24). Secondly, we found that cutaneous emigrant dendritic cells express MMP-2 and MMP-9 on their surfaces, whereas monocyte-derived dendritic cells do not have cell surface expression of these MMPs, although intracellular MMP-9 and MMP-9 secretion into the culture medium has recently been shown (55).

This discrepancy may be biologically relevant. The data emphasize the need to thoroughly investigate and compare the different types of dendritic cells with regard to their migratory behaviors.

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References


