Up-Regulation of α4 Integrin on Activated Langerhans Cells: Analysis of Adhesion Molecules on Langerhans Cells Relating to Their Migration from Skin to Draining Lymph Nodes

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After hapten application, epidermal Langerhans cells migrate into the regional lymph nodes through dermal lymphatics. Recently, we have demonstrated that some of them take the phenotypic and functional characteristics similar to those of in vitro cultured Langerhans cells, before disappearing from the epidermis. To analyze the mechanisms underlying the migration of Langerhans cells, we studied the expression of several adhesion molecules on freshly isolated LC and cultured LC. Pgp-1 (CD44), intercellular adhesion molecule 1, and α4 integrin were strongly expressed on cultured Langerhans cells. Among them, only α4 integrin was strongly up-regulated by cultured Langerhans cells, because its expression by freshly isolated Langerhans cells was very weak. This up-regulation of α4 integrin was also observed on in vivo activated Langerhans cells in the epidermis and draining lymph nodes after hapten application. These data suggest a possible role played by VLA-4 in the migration of Langerhans cells from the epidermis into the regional lymph nodes after hapten application. J Invest Dermatol 100:143–147, 1993

Langerhans cells (LC) are potent antigen-presenting cells in the skin. It has been reported by several authors that LC can take two different phenotypic and functional characteristics [1–5]. Namely, freshly isolated ones, probably those in the unstimulated epidermis, express much less class II major histocompatibility complex (MHC) antigen, and show weaker syngeneic and allogeneic T-cell stimulatory function, and potent antigen-processing capacity. In contrast, short-term cultured LC express more class II MHC antigen, and have potent syngeneic and allogeneic T-cell–stimulatory function and decreased antigen-processing function. Recently, we have demonstrated that the activated LC that have phenotypic and functional characteristics similar to cultured LC appear in the epidermis after hapten application [6]. Interestingly, even after high-dose hapten application, all LC do not up-regulate class II MHC antigen. Only one half or one third of LC in the epidermis up-regulate class II MHC antigen, and disappear from the epidermis 12 h after hapten application. Furthermore, Macatonia et al [7] have demonstrated that 12 h after hapten application, hapten-modified dendritic cells appear in the regional lymph nodes. These data suggest that in vivo activated LC, taking the phenotypic and functional characteristics similar to those of cultured LC (cLC), migrate form the epidermis into the regional lymph nodes.

There has been accumulated evidence indicating that adhesion molecules play a crucial role in the migration of leukocytes, as reviewed by several authors [8–10]. Therefore, it is reasonable to speculate that adhesion molecules also mediate the migration of LC from the epidermis to the regional lymph nodes. To identify such an adhesion molecule, we have compared the expression of several adhesion molecules among freshly isolated LC (fLC), in vivo activated LC in the epidermis and draining lymph nodes after hapten application, and cLC. We demonstrate here up-regulation of α4 integrin on LC activated in vivo by hapten application as well as on cLC.

MATERIALS AND METHODS

Mice Specific pathogen-free female C3H/HeN mice were obtained from the Institute of Experimental Animals, Tohoku University School of Medicine. They were 8 to 10 weeks of age.

Monoclonal Antibodies (MoAbs) We used the following monoclonal antibodies: anti–Pgp-1 (CD44) (KM81 [11]), anti–α4 integrin (PS/2 [12]), anti–vitronectin receptor (VNR) (α) (RMV-7 [13]), anti–Mac-1 (M1/70 from the American Type Culture Collection [ATCC]), anti–intercellular adhesion molecule (ICAM-1) 1 (YN/1.74 from ATCC), anti–I-A (b,c,d,h,l haplotypes) and anti–I-E (d,k,j haplotypes) (M5/114.5.2 from ATCC), and anti–I-A* (10.2.16 from ATCC) antibodies. Fluorescein isothiocyanate (FITC)–
Freshly-prepared Langerhans Cells

Cultured Langerhans Cells

Pgp-1

α4 Integrin

VNRα

ICAM-1

MAC-1

Rat Ig Control

Antibody Plus PE-labeled Rat Control MoAb with FITC-conjugated 10.2.16 (x-axis) and anti-α4 integrin (j), anti-Pgp-1 antibody (k), MAC-1 (l), or rat control MoAb (l) plus PE-labeled F(ab')2 fraction of anti-rat Ig (y-axis).

Figure 1. Two-color immunofluorescence analysis of freshly-prepared Langerhans cells and cultured Langerhans cells. The x-axis shows log10 green fluorescence intensity obtained with FITC-conjugated anti-1-Aκ antibody (10.2.16). The y-axis represents log10 red fluorescence intensity with rat MoAbs to various adhesion molecules or control rat monoclonal antibody plus PE-labeled F(ab')2 fraction of anti-rat Ig. ILC were stained with FITC-conjugated 10.2.16 (x-axis) and anti-Pgp-1 antibody (a), anti-α4 integrin (d), anti-ICAM-1 (g), MAC-1 (i), or rat control MoAb (k) plus PE-labeled F(ab')2 fraction of anti-rat Ig (y-axis). Cultured LC were stained with FITC-conjugated 10.2.16 (x-axis) and anti-Pgp-1 antibody (k), anti-α4 integrin (d), anti-vitronectin receptor (f), anti-ICAM-1 (h), MAC-1 (j), or rat control MoAb (l) plus PE-labeled F(ab')2 fraction of anti-rat Ig (y-axis).

Chemical Treatment and Cell Suspension

Mice were painted on the ears either with 50 μl of 3% 2,4,6-trinitrochlorobenzene (TNCB) (Tokyo Kasei Laboratories, Tokyo, Japan) in 1:4 acetone/olive oil or with 1:4 acetone/olive oil as a control. Twenty-four or forty eight hours after TNCB or vehicle treatment, epidermal cell suspensions were prepared. To examine the surface phenotype of lymph node cells migrating from the skin, mice were painted on the shaved thorax and abdomen with 0.4 ml of 5 mg/ml of FITC (isomer 1; Sigma Chemical Co., St. Louis, MO) in a 50:50 (vol/vol) acetone/dibutylphthalate (Wako Pure Chemical Inc., Tokyo, Japan) mixture.

Culture Medium

RPMI 1640 medium supplemented with 10% FCS, 2 mM glutamine ( Gibco Laboratories, Chagrin Falls, OH), penicillin, streptomycin, and fungizone antibiotic solution ( Gibco), non-essential amino acid solution ( Gibco), sodium pyruvate solution ( Gibco), 10 mM HEPES buffer solution ( Gibco), 5 × 10−5 M 2-ME, and 1 μg/ml indomethacin (Sigma Chemical Co., St. Louis, MO) (complete medium) was used for the culture of epidermal cells.

Preparation of fLC Enriched Populations

Single cell suspensions of EC from the ears of non-treated and chemically treated C3H mice were prepared by using standard techniques [6]. To obtain enriched fLC populations, these epidermal cell suspensions were applied onto Lymphohite M gradients (Cedarlane Laboratories Limited, Canada) and centrifuged at 1400 rpm for 10 min at room temperature. The interface cells were recovered, washed three times, and suspended in complete medium. In some experiments, single-cell suspensions of EC were obtained by treating the split skin sheets of ears with 1000 U/ml dispase (Sanko Pure Chemical Inc., Tokyo, Japan) in RPMI 1640 supplemented with 1% fetal calf serum (FCS) for 2 h as described previously [14,15].

Preparation of Enriched cLC Populations

Single-cell suspension of EC (1 × 107) were cultured in complete medium, 37°C in 5% CO2 using 25-ml T flasks (Falcon, Falcon plastics, Oxnard, CA). After 72 h, the cells were harvested by vigorous pipetting. These EC were also applied onto Lymphohite M gradients and centrifuged at 2500 rpm for 10 min at room temperature. The interface cells were washed three times and suspended in complete medium.

Preparation of Lymph Node Cells

Inguinal and axillary lymph node cells were taken from normal mice or those that had been painted with FITC 48 h before, and single cell suspensions were prepared by pressing the nodes through nylon mesh.

Flow Cytometry

For flow cytometry of adhesion molecules on fLC and cLC, enriched fLC populations and cLC populations were first incubated with various MoAbs against mouse adhesion molecules or isotype-matched rat control antibodies for 30 min on ice, washed with phosphate-buffered saline (PBS) supplemented with 1% FCS and 0.02% NaN3 (washing buffer), and then incubated with phycoerythrin-conjugated F(ab')2 fraction of anti-rat-Ig (Tago, Burlingame, CA) for 30 min on ice. After washing 3 times with washing buffer, cells were then serially treated with 10% normal mouse serum in PBS for 30 min and with FITC-conjugated 10.2.16 or FITC-conjugated mouse control antibody for 30 min on ice. After washing 3 times with washing buffer, they were analyzed on a FACScan (Becton Dickinson). For flow cytometry of lymph node cells, lymph node cell suspensions were incubated with PS/2, M5/114.5.2, and isotype-matched rat MoAb for 30 min on ice, washed with washing buffer, and then incubated with PE-conjugated F(ab')2 fraction of anti-rat-Ig (Tago, Burlingame, CA) for 30 min on ice. After washing 3 times with washing buffer, they were analyzed on a FACScan.
In after di s p a se tr ea tm e nt, we iso lated Up-Regulation of cLC ba sed on the retaining of antigenic de termina nt of resist ant to di s pa se treatment.

Figure 2. Trypsin and dispase sensitivity of antigenic determinant on α4 integrin and Pgp-1. cLC were treated with either 0.5% trypsin in PBS for 40 min or 1000 U/ml dispase for 2 h at 37°C. As a control, cLC were kept on ice. After incubation, these cLC were washed with PBS with 1% FCS, stained for α4 integrin or Pgp-1, and examined on a FACScan. cLC kept on ice were also stained with non-reactive isotype-matched rat MoAb. Anti-α4 integrin antibody ( — ), anti-Pgp-1 antibody ( --- ), and control antibody ( . . . .).

Trypsin and Dispase Sensitivity of α4 Integrin and Pgp-1 cLC were treated with either 0.5% trypsin in PBS for 40 min or 1000 U/ml dispase for 2 h at 37°C. As a control, cLC were just incubated in PBS for 2 h at 37°C. After incubation, these cLC were washed with PBS with 1% FCS, stained for α4 integrin and Pgp-1, and examined on a FACScan.

RESULTS

Expression of Adhesion Molecules on fLC and cLC fLC, which were isolated with the standard procedure using trypsin, expressed several adhesion molecules (Fig 1). Namely, in addition to the previously reported expression of MAC-1 and ICAM-1 [16,17], they expressed Pgp-1. On the other hand, cLC expressed MAC-1, ICAM-1, Pgp-1, and α4 integrin. When the expression of these adhesion molecules was compared for fLC and cLC, the latter exhibited a decreased MAC-1 expression, and an increased ICAM-1 and Pgp-1 expression. Furthermore, cLC showed a strong α4 integrin expression, making a striking contrast to its null expression on fLC. Other integrin such as the α chain of the vitronectin receptor was not expressed either on fLC or cLC.

Trypsin and Dispase Sensitivity Although we recognized up-regulation of Pgp-1 and α4 integrin on cLC, we could not exclude the possibility that the treatment required for the isolation of fLC might have caused a destruction of antigenic determinant of Pgp-1 and α4 integrin on fLC. Therefore we treated cLC with two standard enzymes used for isolating fLC, i.e., trypsin and dispase. The resultant data (Fig 2) showed that, in contrast to antigenic determinant of Pgp-1, which was totally destroyed by these enzymes, antigenic determinant of α4 integrin was sensitive only to trypsin, but resistant to dispase treatment.

Up-Regulation of α4 Integrin in In Vivo Activated LC and cLC Based on the retaining of antigenic determinant of α4 integrin after dispase treatment, we isolated fLC by using dispase, and compared the expression of α4 integrin among non-treated fLC, vehicle-treated fLC, hapten-treated fLC 24 h and 48 h after TNCB painting, and cLC (Fig 3). When compared with isotype-matched control antibody (Fig 3a), α4 integrin was expressed very weakly on fLC (Fig 3b). After hapten application, α4 integrin-bearing cells appeared among I-A<sup>k</sup> fLC 24 h after TNCB painting (Fig 3d), and almost half of them came to express α4 integrin 48 h after TNCB painting (Fig 3e). Again all the cLC strongly expressed α4 integrin (Fig 3f).

Fluorescent Lymph Node Cells After Skin Painting with FITC Express α4 Integrin We examined the surface phenotype of the draining lymph node cells after skin painting with FITC. Forty-eight hours after skin FITC painting, approximately 1--2% of lymph node cells were brightly labeled with FITC (Fig 4b,c,h) when compared with non-treated control cells (Fig 4a,d,g). To analyze the surface phenotype of FITC-labeled cells in lymph node cells, we gated only FITC-labeled cells using a C30 software of FACScan. More than 90% of FITC-labeled cells expressed Ia antigen as well as α4 integrin.
DISCUSSION

LC migrate from the epidermis into the regional lymph nodes after hapten application through dermal lymphatics [6,7,18]. There is accumulating evidence indicating that several adhesion molecules play a crucial role in the migration of various leukocytes. Therefore we speculated that adhesion molecule might also mediate the migration of LC, and that when LC migrate from the epidermis to lymph nodes, they should change the expression of those adhesion molecule(s). We at first compared the expression of several adhesion molecules between fLC and cLC, because we have previously reported that in vivo activated LC take the phenotypic and functional characteristics similar to those of in vitro cultured LC [6]. Among the adhesion molecules we have examined, Pgp-1, ICAM-1, and α4 integrin were more strongly expressed on cLC than fLC. However, because we used trypsin for preparing freshly prepared LC, we could not exclude the possibility that trypsin might have destroyed the antigenic determinants of these molecules, being accompanied by their seemingly lesser expression by fLC. Indeed, that was the case for Pgp-1 and ICAM-1 (unpublished data), and we could not demonstrate the up-regulation of these two molecules by cLC. However, at least we can interpret from our results that both fLC and cLC express Pgp-1 and ICAM-1. Tang and Uede [17] also reported that both fLC and cLC expressed ICAM-1. Binding of lymphocytes to high endothelial venules, activated endothelial cells, or some cultured endothelial cells has been demonstrated to be mediated by Pgp-1 (CD44) [19–21]. Although both fLC and cLC expressed Pgp-1, it is also possible that Pgp-1 on LC mediate their migration from the epidermis to lymph nodes. In contrast to these two molecules, we showed that cLC expressed much more α4 integrin than fLC isolated by disperse, which does not destroy the antigenic determinant of α4 integrin expression. Furthermore, we also showed that LC obtained from the epidermis painted with TNCB up-regulated α4 integrin. Macatonia et al. [7] reported that after FITC painting on the skin, FITC-labeled dendritic cells appeared in the draining lymph nodes, indicating the migration of LC after hapten painting. Our present analysis using this experimental system demonstrated that such FITC-labeled cells in the draining lymph nodes expressed Ig antigen and α4 integrin, suggesting that LC migrating into the lymph nodes after hapten painting express α4 integrin. Although we could not exclude totally the possibility of contamination by cells other than dendritic cells, these data clearly demonstrate the up-regulation of α4 integrin on in vivo activated LC as shown on cLC.

According to the extensive review by Hynes [22], integrins are a widely expressed family of cell surface adhesion receptors. They appear to be the major receptors by which cells attach to extracellular matrices, and some integrins also mediate important cell-cell adhesive events. All integrins are αβ heterodimers. There are eight known β subunits and 14 known α subunits, and it is well known that LC express Mac-1, αMβ2 integrin [23]. Recently, Varlet et al. [24] and Zambruno et al. [25] have demonstrated some β1 integrins on human LC by the electron microscopic observation using immunogold, although there remain some controversial points between these reports. Furthermore Le et al. [26] have demonstrated the crucial role of VLA-3, VLA-5, and VLA-6 on human LC in in vitro adhesion to fibronectin or laminin. In regard to the expression of VLA-4, these authors demonstrated VLA-4 on LC in the epidermis, although no clear data have been provided about the difference in the expression of integrins between fLC and cLC. Most of the study on β1 integrins on LC have been done on human LC. As far as we know, there are no definitive reports on β1 integrins of mouse LC.

Recently, it becomes clear that the migration of T cells into the
lymph nodes are mediated by several adhesion molecules, such as L-selectin and integrins. Although it is also involved in both neutrophil and lymphocyte adhesion to activated endothelial cells [27,28], L-selectin, which is also designated LECAM-1, MEL-14, and LAM-1, is a lymphocyte molecule mediating its homing to peripheral lymph nodes, although it is also involved in both neutrophil and lymphocyte adhesion to activated endothelial cells [27,28]. In the mouse, two integrin molecules, lymphocyte Peyer’s patch high endothelial venules adhesion molecule 1 (LPAM-1) and LPAM-2, each of which is composed of α4 integrin but with distinct β chains, have been implicated as receptors mediating lymphocyte migration to mucosal lymphoid organs, such as Peyer’s patches [29,31]. In the present study, we demonstrated up-regulation of α4 integrin on both in vivo activated LC after hapten application and cLC. α4 integrin can associate with β1 and β7. Because of the limited numbers of MoAbs to mouse integrin molecules we can use, we could not examine whether LC express β1 or β7 [29,31]. Therefore it is still not clear whether LC express VLA-4 (α4β1) or LPAM-1 (α4β7), although both fibronectin and VCAM-1 have been reported to be the ligands for VLA-4 and LPAM-1 [31]. In contrast to accumulating data about the expression of adhesion molecules on vascular endothelial cells, there are no definite reports on the adhesion molecules of lymphatic vessels. Therefore, it is still unknown how VLA-4 plays a role in the migration of LC from epidermis to lymph nodes through the lymphatic endothelial cells.

This work was supported in part by funds from the Lydia O’Leary Memorial Foundation (SA), and by grant 04857100 from the Ministry of Education, Science and Culture of Japan.

REFERENCES


