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The Actin-Bundling Protein Fascin Is Involved in the Formation of Dendritic Processes in Maturing Epidermal Langerhans Cells

Ralf Ross, Xiao-Lan Ross, Jens Schwing, Tina Längin, and Angelika B. Reske-Kunz

Dendritic cells (DC) are characterized by their unique potential to prime naïve T cells. Epidermal Langerhans cells (LC), the DC resident in the epidermis, gain this immunostimulatory capacity following Ag contact in vivo or during in vitro culture of epidermal cell suspensions. To analyze differential gene expression in maturing LC, we constructed a highly representative cDNA library of cultivated LC (cLC) in λ ZAP II containing 18 × 10⁶ independent clones. This library was screened with freshly isolated Langerhans cell (fLC) and cLC-derived probes for cLC-specific cDNAs. The cDNAs identified were sequenced and analyzed by database searches. Two cDNA fragments were identified as fragments of fascin, indicating that fascin is differentially expressed in LC. By competitive RT-PCR, we confirmed that fascin is highly expressed in cLC cultivated for 1, 2, and 3 days, while no signals were obtained with fLC. Western blot and immunofluorescence analysis revealed cLC-specific expression of fascin on the protein level as well. Fascin is known to be involved in the organization of the actin cytoskeleton in cytoplasmatic extensions of nerve growth cones. Its differential expression in maturing LC coincides with the formation of numerous dendritic projections in LC. Their formation was inhibited by incubation of LC with fascin antisense oligonucleotides during cultivation. Therefore, we conclude that fascin is necessary for the formation of the dendritic processes of maturing Langerhans cells and may thus influence T cell-LC interaction. The Journal of Immunology, 1998, 160: 3776–3782.

B one marrow-derived dendritic cells (DC) are pivotal for the induction of an acquired immune response, since among APC they are uniquely able to prime naïve T cells. Epidermal Langerhans cells (LC) are characterized as a uniformly immature subpopulation of DC not yet capable of primary T cell activation (1). Following contact with Ag, LC capture the Ag and migrate via lymph vessels to the draining lymph nodes, thereby maturing into potent APC (2, 3). Now referred to as interdigitating DC, they present the processed Ag peptides bound to MHC class II molecules to naïve T cells resulting in T cell activation. It is well established that LC maturation can be simulated by cultivating freshly isolated LC (fLC) for 3 days (cLC) in the presence of keratinocytes (4, 5).

The structural and functional changes that LC undergo during this process are numerous, and some of them pose special demands on the cytoskeleton: before activation, LC reside within the epidermis in contact with surrounding keratinocytes; once activated, LC leave their position and migrate actively to the draining lymph nodes. During movement they retract the few, but very long dendritic processes. Finally, within the lymph nodes they elaborate multiple thin projections. A network of actin filaments associated with actin-binding proteins is important for each of these processes, namely for movement and cell shape as well as for cell attachment (6). The attachment of LC to surrounding cells within the epidermis is mediated by homodimerization of E-cadherin molecules (7). E-cadherin is anchored within the cell to the actin cytoskeleton via actin-binding proteins (8). Activation and emigration of LC is associated with down-regulation of E-cadherin surface expression (9).

The three-dimensional network of actin filaments is structured by interaction of actin filaments with capping proteins and by cross-linking of the filaments via actin-binding proteins. A growing number of actin-binding proteins such as actinin, gelsolin, and villin has been identified, and differential expression of subsets of actin-binding proteins is necessary for specialized functions and structures (10, 11).

Ample evidence exists that the 55-kDa actin-bundling protein fascin organizes actin filaments into bundles (12, 13). It is, furthermore, likely to be involved in formation of dendritic projections, as it was detected in cells with dendritic processes such as neuronal growth cone filopodia and human blood-derived DC (14, 15). Here, we report that fascin is differentially expressed during maturation of murine LC. The formation of pronounced and numerous dendritic projections coincided with strong fascin expression and was impaired by fascin antisense oligonucleotides.

Materials and Methods

Animals

BALB/c Ann mice were bred in our animal facilities from breeding pairs originally obtained from the Zentralinstitut für Versuchstierkunde (Hannover, Germany). The mice were used at 2 to 5 mo of age.

Monoclonal Abs

Clone 2G9 (anti-I-A¹,b, I-E¹, rat IgG2a) (16) was a kind gift from Dr. M. Mohamadzadeh, Department of Dermatology, University of Mainz, Germany. 55K-2 mAb (anti-human fascin, mouse ascites) (17) was a kind

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3 Abbreviations used in this paper: DC, dendritic cell(s); fLC, freshly isolated Langerhans cells(s); cLC, cultured Langerhans cell(s); DTAF, dichlorotriazinyl aminofluorescein; HPRT, hypoxanthine-guanine phosphoribosyltransferase.
donation by Dr. F. Matsumura, Department of Molecular Biology and Biochemistry, Rutgers University, Piscataway, NJ. Isotype control mAb were obtained from Pharmingen, Hamburg, Germany.

Cell lines

The Th line BK-OVA-1R (18) recognizes OVA in the context of Aeq^Bb MHC class II molecules. The murine B cell lymphoma A20/21 (19), the macrophage cell line P388D1 (20), the fibroblast line WEHI-164 (21), and the melanocyte line B7/8 (22) were cultured as described before. Cells were harvested and used directly for mRNA preparation or Western blots, or alternatively, cell pellets were shock frozen in liquid nitrogen and stored at −70°C for later analysis.

Preparation of fLC and cLC

Epidermal cell suspensions were prepared from pelts as described previously (23). These were obtained either directly or from rollof mRNA in vitro cultivation for 1 to 3 days in Iscove’s modified Dulbecco’s medium (IMDM, Life Technologies, Eggenstein, Germany) supplemented with 2 mM l-glutamine, 5 × 10⁻⁵ M 2-ME, 100 IU penicillin, 100 µg/ml streptomycin, 12.5 µg/ml amphotericin B, 500 µg/ml gentamicin, and 10% FCS in 175 cm² tissue culture flasks (Techno Plastic-Products, Trasadingen, Switzerland). LC were enriched from epidermal cells by immunomagnetic separation with Dynabeads M-450 (Dynal, Hamburg, Germany) loaded with anti-MHC class II mAb 2G9 as described (24). MHC class II-positive, bead-coupled LC and cells without attached beads were counted using a Neubauer chamber. LC purity of ~92 to 95% was obtained as determined by the ratio of bead-rosetted to nonrosetted cells. LC viability, determined by trypan blue exclusion, was >95%.

Mixed epidermal cell lymphocyte reaction

The mixed epidermal cell lymphocyte reaction was conducted as described (24). Briefly, nylon wool-enriched (25) splenic C57BL/6 J cells were stimulated with titrated numbers of allogeneic fLC or cLC of BALB/c origin. T cell proliferation was measured by incorporation of 28 kBq [3 H]thymidine into cellular DNA during the last 12 h of a 96-h incubation period.

cDNA library construction

A cDNA library derived from cLC was constructed in λ ZAP II (26). mRNA was isolated from cLC (purity, 95% rosetted cells) using the Quick-Prep mRNA Purification Kit (Pharmacia, Freiburg, Germany). Double-stranded cDNA was obtained from 5 µg mRNA using the Time Saver cDNA synthesis kit (Pharmacia) and ligated with EcoRI/NdI adaptors (Pharmacia) according to the recommendations of the manufacturer. The adaptor-flanked cDNA fragments were ligated with dephosphorylated, EcoRI-digested λ ZAP II vector arms (Stratagene, Heidelberg, Germany) and were in vitro packaged using the in vitro packaging kit Gigapack II Gold (Stratagene).

Preparation of screening probes from fLC and cLC

The cDNA probes were prepared as we described recently (27). Briefly, double-stranded cDNA was obtained from approximately 100 ng mRNA derived from 10^⁷ fLC or cLC using the Time Saver cDNA synthesis kit (Pharmacia), ligated with Uni-Amp adaptors (Clontech Laboratories, Palo Alto, CA) and amplified by PCR in 25 cycles with 1 min denaturation at 94°C, 1 min annealing at 60°C, and 2 min extension at 72°C. The radioactive labeling of the double-stranded reamplified cDNAs was performed by random-primed oligo labeling using the Multiprime DNA labeling system (Amersham Buchler, Braunschweig, Germany).

Differential plaque filter screening

Bacteria and recombinant phages were plated on 24.5 × 24.5 cm culture dishes (Nunc, Roskilde, Denmark) in concentrations allowing the distinguishing of single plaques following cultivation overnight. The plaques were transferred to nitrocellulose filters, and replicate filters from each plate were treated and hybridized according to Benton and Davis (28). A single replicate filter was hybridized with a fLC-derived probe as outlined above, while the corresponding filter was hybridized with a cLC-derived probe. Following autoradiography, the hybridization patterns were compared, and phages yielding cLC-specific signals were picked and replated. Replicate filters were rescreened with cLC- and cLC-derived probes.

Southern hybridization and sequencing

The internal pBluescript SK(−) plasmid containing the cLC-specific cDNA was recovered from pure, cLC-specific, recombinant λ ZAP II phages by in vivo excision (26). The plasmid DNA, purified using the Plasmid Kit Midi (Qiagen, Hilden, Germany), was restricted, subjected to Southern hybridization (29), and sequenced using the T7 Sequencing Kit (Pharmacia) with vector-specific primers (T3A: AAT AAC CCT CAC TAA AGG G; and T7A: AAT ACG ACT CAC TAT AGG G) according to the recommendations of the manufacturer.

Competitive RT-PCR

mRNA was isolated using the Quick-Prep Micro mRNA Purification Kit (Pharmacia). The RNA concentration and quality were assessed by RT-PCR with primers specific for hypoxanthine-guanine phosphoribosyltransferase (HPRT-1: GGT TGG ATG CAC GCC AGA CTG TTG TG and HPRT-2: GAA GGG TAG GTC GGC CTA TAG GCT). The primers FAS-1 (CCC GCC TCA CTC TGG GAG TA) and FAS-2 (CTC CTA GCC CAC TTC CTC GG) were chosen to amplify a 329 bp fragment of fascin cDNA. For competitor construction, the hybrid primers FASST-1 (CCC GCC TCA CTC TGG GAC TAC CGC TCT AGG CAC CA) and FASST-2 (CTC CTA GCC CAC TTC CTC GG) were used. All primers were generated by MWG-Biotech (Ebersberg, Germany).

RT-PCR was performed as described previously (30) using a modul 480 DNA Thermal Cycler (Perkin-Elmer, Uerberg, Germany). The competitor fragment (365 bp) was constructed as we described earlier (30). It is flanked by the primer-binding sites for FAS-1 and FAS-2, but has no further sequence homology to fascin. For quantitative analysis of the PCR reactions, equal amounts of the competitor fragments were added to each PCR reaction (30).

Western blotting

Tissues were shock frozen, pulverized under liquid nitrogen in a mortar, and resuspended in SDS sample buffer. Following ultrasonication, protein concentration was determined by Bradford assay (Bio-Rad, München, Germany). Aliquots equivalent to 10^3 cells or 5 µg protein, respectively, per lane were separated on 15% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). Bound primary Ab (anti-human fascin mAb 55K-2) was visualized by horseradish peroxidase-labeled anti-mouse Ig Ab using the BM Chemiluminescence Western Blotting Kit (Boehringer Mannheim, Mannheim, Germany) following the recommendations of the manufacturer.

Immunofluorescence

For fluorescence staining, epidermal cells were grown on coverslides or were transferred onto glass slides by centrifugation (cytospin) for 1 min. Epidermal cells for cytospins were either used directly or following three days in vitro cultivation and Ficolldensity centrifugation (Histopaque, density 1.077; Sigma, Deisenhofen, Germany). All preparations were fixed for 10 min in 90% acetone at −20°C, washed three times in PBS, and incubated overnight at 4°C or 3 h at 37°C with the primary mAb (55K-2 and 2G9). Following three washes in PBS, cells were incubated for 1 h at 37°C with dichlorotriazinyl aminofluorescein (DTAF)-conjugated goat anti-mouse IgG (0.75 µg) and with phycocyanin-labeled goat anti-rat IgG (0.5 µg) secondary Abs (both from Dianova, Hamburg, Germany).

Cultivation of LC with antisense oligonucleotides

Epidermal cell suspensions were prepared from pelts (23) and cultivated as described on 8-well or 16-well chamber slides (Nunc). Cells were cultivated either without addition of oligonucleotides or with fluorescein-labeled (0.5 µM) or unlabeled (2 µM) phosphorothioate oligonucleotides. An antisense oligonucleotide (FAS), the reverse complement to the murine and human fascin mRNA, was used as well as a matched control oligonucleotide of the same length (15 bp) and base content, which had similar hybridization characteristics and showed no cross-homology to any known gene. The HPLC-purified oligonucleotides were purchased from Biognostik, Göttingen, Germany. Oligonucleotides were added to epidermal cell suspensions at the beginning of the 3-day cultivation period.

Results

fLC and cLC prepared as described met the criteria reported in the literature. Thus, cLC, but not fLC, derived from BALB/c mice (H-2b) were efficient stimulator cells for nylon wool-enriched T cells from C57BL/6 mice (H-2d) (data not shown). On the other hand, fLC were capable of processing OVA and presenting OVA-peptides to the T cell hybridoma 3D0-54.8, that recognizes OVA in the context of I-A^d (data not shown). Furthermore, we detected...
enhanced surface expression of the costimulatory molecules B7.1, B7.2, ICAM-1, and LFA-3 on cLC, as compared with fLC, and up-regulated mRNA expression of ICAM-1 in cLC, while mRNA expression of MHC class II was down-regulated (data not shown).

Construction, validation, and differential screening of a cLC-derived cDNA library in λ ZAP II

cLC (5 × 10^7, purity > 95% rosetted cells) were subjected to mRNA isolation. The integrity of the mRNA was analyzed by Northern blotting and hybridization with β-actin cDNA probes. A clear, distinct signal was obtained (data not shown). Five micrograms of mRNA was used for generation of double-stranded cDNA, subsequent ligation into λ ZAP II, and in vitro packaging. Determination of the phage titer of the cDNA library indicated that the library contained 1.8 × 10^7 independent clones. Based on blue/white-screening, we estimated that 90% of the clones were recombinant. Five randomly picked clones contained cDNA fragments of 300 to 1900 bp. By plaque filter hybridization of 3000 clones with β-actin cDNA probes, we obtained eight β-actin cDNA clones (not shown).

Approximately 20,000 clones of the cLC-derived cDNA library were plated onto 24.5 × 24.5-cm culture dishes; replicate filters were taken and hybridized with fLC- or cLC-derived probes, respectively, that had been validated before, as described earlier (27). The hybridization patterns of the replicate filters with fLC- and cLC-derived probes were compared. Fifty clones yielding strong signals with cLC-derived probes and weak or no signals with fLC-derived probes were selected, replated, and rescreened (Fig. 1). Thirty-eight clones yielded cLC-derived signals.

Analysis of the isolated cLC-specific cDNA clones

The pBluescript phagemids containing the cLC-specific cDNAs were recovered by in vivo excision from all 38 isolated λ clones. Plasmid DNA was isolated and digested with EcoRI. The restriction pattern revealed similar patterns for a number of clones. Therefore, one of them was radioactively labeled and subjected to Southern hybridization with blots of all 38 clones. Twenty-two clones yielded strong signals indicating that homologous or identical cDNAs were cloned. Two of the hybridizing clones and the 16 remaining nonhybridizing cDNAs were sequenced from both ends with the vector-specific primers T3A and T7A.

Comparison of the sequences obtained with the Geall databases (Deutsches Krebs-Forschungszentrum, Heidelberg, Germany) revealed that the cross-hybridizing clones contained identical cDNAs of a so far unknown gene. Two of the remaining cDNAs were identical with the murine fascin cDNA (accession No. L33726). The clone pcLC12 contained a nearly complete cDNA of fascin (Fig. 4). The cDNA clone pcLC5 contained 1221 bp of the 3′ end (Fig. 2A). Fascin was selected for further investigation, as a mAb was available (17), its ability to organize actin into bundles was known (12, 13), and its functional role in organizing the actin cytoskeleton promised to be of importance.

Analysis of fascin expression in LC by RT-PCR

To analyze at what time point during maturation of LC fascin expression is induced, we performed competitive RT-PCR using mRNA isolated from equal numbers of fLC and cLC cultured for 1, 2, and 3 days. Equal amounts of mRNA were subjected to reverse transcription, and aliquots were used for PCR with specific primers for the housekeeping gene, HPRT. Signals of equal intensity were obtained indicating comparable amounts of mRNA (Fig. 2C). Aliquots of the same RT reaction were used for PCR with fascin-specific primers. Serial dilutions of the RT reaction were mixed with a constant amount of competitor molecules. The competitor molecules were generated as we described recently (27). They are flanked by the primer-binding sites for the fascin primers but have no further homology to fascin to minimize cross-hybridizations. Following gel electrophoresis, competitor-derived upper bands (365 bp) and cDNA-derived lower bands (329 bp) fragments were distinguishable by size. As shown in Figure 2B, no fascin signals were obtained with fLC, while strong signals were obtained with cLC cultivated for 1, 2, and 3 days. The signal intensities on days 1 through 3 were similar.

Detection of fascin protein by immunofluorescence analysis in cLC, but not in fLC

Freshly isolated or 3-day-cultured epidermal cells were transferred onto glass slides by cytopin. Double fluorescence labeling for MHC class II (red) and fascin (green) revealed that fLC, identified by bright MHC class II staining, were negative for fascin staining, while cLC were clearly positive (Fig. 3). MHC class II-negative cells, either freshly isolated or cultivated, were negative for fascin staining. With isotype-matched control Ab, no staining was observed.

To analyze the distribution of fascin within the cell, we grew epidermal cells on cover slides in 6-well culture dishes for 3 days and double stained with anti-MHC class II and anti-fascin mAb. cLC showed elaborate dendritic processes, stained clearly positive for fascin (Fig. 4A).
Analysis of fascin expression by Western blotting

Extracts of equal numbers of fLC and cLC separated by paramagnetic beads coupled with anti-MHC class II mAb, as well as equal amounts of protein derived from brain, liver, spleen, and kidney, were subjected to Western blotting. Fascin was detected by mAb 55K-2 only in extracts of cLC, but not of fLC (Fig. 5). The estimated molecular mass (approximately 55 kDa) of the corresponding protein is identical with that of fascin. In addition, a prominent fascin signal was obtained with brain extract (Fig. 5), which is compatible with the notion that fascin is highly expressed in neurons and glial cells (31). A strong signal was obtained with splenic cells as well. Weak signals were obtained with kidney and liver cells. Among a panel of cell lines including B cells, T cells, macrophages, fibroblasts, and melanocytes, only the fibroblast cell line yielded a fascin signal (Fig. 5). No signals were obtained with isotype control Abs (not shown).

**FIGURE 2.** Differential expression of fascin mRNA by competitive RT-PCR. A, Schematic view of the fascin mRNA (2634 bp). The coding region is boxed, and the locations of the primer sequences and the fragment amplified by PCR are marked. The isolated cDNA clones pCLC 5 and pCLC 12 are shown below. B, Competitive RT-PCR with fascin-specific primers. Equal amounts of competitor fragments (upper bands) were added to serial dilutions of RT reactions of fLC (d0) and cLC cultivated for 1 (d1), 2 (d2), or 3 (d3) days (lower bands). The RT reactions were diluted twofold per lane from left to right. st, Indicates amplification of competitor fragments without RT reaction. Marker: φX174 × HaeIII. C, The concentration and quality of the mRNA used in B, derived from fLC (d0) and cLC cultivated for 1 (d1), 2 (d2), or 3 (d3) days, was assessed by RT-PCR. Aliquots of the same RT reaction as described in B were amplified with specific primers for the housekeeping gene, HPRT. c, Indicates negative control without mRNA; m, molecular weight marker φX174 × HaeIII.
Dendritic morphology of cLC is inhibited by fascin antisense oligonucleotides

Epidermal cells were cultivated in 8- or 16-well chamber slides, with or without addition of phosphorothioate oligonucleotides, for 3 days. The class II-positive cLC were then immunostained with mAb 2G9. Incubation with 2 μM FAS oligonucleotides (reverse complement to murine and human fascin mRNA) clearly inhibited the formation of dendritic projections (Fig. 6c) compared with untreated cells (Fig. 6a). In parallel experiments, 2 μM control oligonucleotides were added to rule out cytotoxic or nonspecific effects of the antisense oligonucleotides (Fig. 6b). Cellular uptake was monitored by addition of 0.5 μM fluorescein-labeled oligonucleotides (Fig. 6d).

FIGURE 3. Detection of fascin in cLC, but not in fLC, by immunofluorescence. Immunofluorescence staining of fLC and cLC transferred onto glass slides by cytopsin was performed. a, d, and g. Phase contrast control. The cells were analyzed with epifluorescence filter sets detecting phycoerythrin labeling (red, anti-MHC class II staining; b, e, and h) and DTAF labeling (green, anti-fascin staining, c, f, and i). a–c, fLC; d–f, cLC; g–i, cLC with isotype-matched control mAbs. Scale bar represents 30 μm.

Dendritic morphology of cLC is inhibited by fascin antisense oligonucleotides

Epidermal cells were cultivated in 8- or 16-well chamber slides, with or without addition of phosphorothioate oligonucleotides, for 3 days. The class II-positive cLC were then immunostained with mAb 2G9. Incubation with 2 μM FAS oligonucleotides (reverse complement to murine and human fascin mRNA) clearly inhibited the formation of dendritic projections (Fig. 6c) compared with untreated cells (Fig. 6a). In parallel experiments, 2 μM control oligonucleotides were added to rule out cytotoxic or nonspecific effects of the antisense oligonucleotides (Fig. 6b). Cellular uptake was monitored by addition of 0.5 μM fluorescein-labeled oligonucleotides (Fig. 6d).

FIGURE 4. Expression and distribution of fascin on cLC. Immunofluorescence staining of cLC grown on cover slides was performed. The cells were analyzed with epifluorescence filter sets detecting phycoerythrin-labeled anti-MHC class II staining (a) and DTAF-labeled anti-fascin staining (b). Scale bar represents 20 μm.

FIGURE 5. Differential expression of fascin in murine cell types and tissues. Extracts of equal numbers of fLC (d0), cLC (d3), the murine Th line BK-OVA-1R (BK-OVA), the B cell lymphoma A20/2J, the fibroblast line WEHI-164 (WEHI), the macrophage line P388D1, and the melanocyte line B78 as well as equal amounts of total brain, liver, spleen, and kidney protein from BALB/c mice were subjected to SDS-PAGE and Western blotting. Equal protein loading was ensured by using the Bradford assay and Coomassie staining of aliquots loaded on neighboring lanes of the same gel. Fascin expression was detected by chemiluminescence using mAb 55K-2. With isotype-matched control mAb, no signals were obtained (not shown).
Dendritic morphology of cLC is impaired by fascin antisense oligonucleotides. freshly isolated epidermal cells from BALB/c mice were cultivated without addition of oligonucleotides (a), with 2 μM control oligonucleotides (b), or with 2 μM fascin antisense oligonucleotides (c) for 3 days. MHC class II-positive cLC were phycoerythrin labeled using mAb 2G9. Cellular uptake of 0.5 μM fluorescein-labeled oligonucleotides is shown in d. Scale bar represents 20 μm.

FIGURE 6. Dendritic morphology of cLC is impaired by fascin antisense oligonucleotides. Freshly isolated epidermal cells from BALB/c mice were cultivated without addition of oligonucleotides (a), with 2 μM control oligonucleotides (b), or with 2 μM fascin antisense oligonucleotides (c) for 3 days. MHC class II-positive cLC were phycoerythrin labeled using mAb 2G9. Cellular uptake of 0.5 μM fluorescein-labeled oligonucleotides is shown in d. Scale bar represents 20 μm.

Class II-positive cLC within wells containing untreated cells or cells treated with control oligonucleotides (control) or FAS antisense oligonucleotides (FAS) were microscopically examined by the experimenter and grouped into either of two categories according to the following criteria: category 1 cLC were large cells of irregular shape with or without dendritic projections (part of the cLC lost their dendritic protrusions during the immunostaining procedure); category 2 cLC were small, round cells without dendritic projections. As depicted in Figure 7, category 1 cLC outnumbered category 2 cLC by a factor of 4, when the epidermal cells had been cultured without addition of oligonucleotides. In cultures to which control oligonucleotides had been added, a factor of 3.2 resulted. In contrast, when FAS antisense oligonucleotides were present during cultivation of epidermal cells, category 1 cLC were outnumbered by category 2 cLC (factor of 0.2).

Discussion

Using the strategy of differential screening of a cLC-derived cDNA library with ILC- and cLC-derived probes, we identified differential gene expression of the fascin gene in maturing epidermal LC.

The results obtained by differential screening were confirmed by investigations on the mRNA and protein level, indicating that the screening procedure and the probes generated for screening were reliable. While fascin was not detectable on the mRNA level in ILC, we detected significant expression of mRNA by quantitative RT-PCR already on day 1 and comparable transcript levels on day 2 and 3 of culture. For detection of fascin expression on the protein level, mAb 55K-2 was used (17). Although generated against human fascin, this mAb cross-reacts with the homologous proteins of mouse and Xenopus, as the gene is highly conserved (32). Differential expression of fascin mRNA was paralleled by differential expression of fascin protein. The data indicate that fascin expression is regulated on the mRNA level and that fascin may be required early after activation as well as at later time points. Expression of fascin in mouse cLC is compatible with results published recently by Mosialos et al. (15) and Pinkus et al. (33) showing that fascin is highly expressed in human DC. Furthermore, we observed high levels of fascin in spleen, known to harbor elevated numbers of mature DC. Weak fascin signals with cells derived from kidney and liver may be due to DC resident in these organs. Whether DC contribute to fascin expression in these organs has to be shown in further studies.

Many blood-derived or skin-derived cell types do not express fascin. Thus, fascin was not expressed in ILC and in freshly isolated and in cultured MHC class II-negative epidermal cells, as indicated by immunofluorescence, and was not detectable by Western blotting in several murine cell lines including B cells, T cells, macrophages, and melanocytes.

Fascin expression is, however, not restricted to DC. It was reported to be expressed in fibroblasts, neurons, glial cells, and in EBV-transfected B cells, but not in untransfected T cells and B cells (17, 31). Accordingly, we obtained strong fascin signals with murine brain cells and with the murine fibroblast cell line WEHI-164. Nevertheless, lacking deal markers for DC, fascin may serve as a marker molecule for DC, taking into account its strong expression in cLC and its absence in many other blood- and skin-derived cell types. Furthermore, fascin may serve as a marker for maturing LC and possibly other DC.

The actin cytoskeleton is essential for a number of important cellular functions including motility (34, 35), exocytosis (36), phagocytosis (37), formation of stress fibers (34), activity of ion channels and transport proteins (38), cell surface receptor perturbation (39), cell polarity, and contact to extracellular matrix components (40). It is not yet clear whether fascin is involved in these processes. Fascin organizes actin into bundles in vitro, and Drosophila singed mutants deficient in the Drosophila fascin homologue show that fascin is required for proper bristle formation as well as formation of actin filaments in nurse cells during oogenesis.
Differential expression of fascin in Langerhans cells

(12). The limited expression of fascin and the differential expression in LC eliminate the possibility that it might be of crucial importance for most of the processes listed above, e.g., LC show phagocytotic activity despite lack of detectable fascin expression. Fascin may be involved in formation and/or motility of the newly shaped dendritic processes. It might not be merely coincidence that the cells with the most elaborated projections, namely DC and neurons, express fascin. In neurons, fascin protein is not detectable in axons; it is restricted to the cytoplasm of the cell body and expressed particularly in the dendritic processes of the nerve growth cone (14). The latter strikingly resemble dendritic projections of cLC and other DC in shape and are mobile as well. In neurons, this movement might be necessary in the course of the pathfinding and growth of the axon to its destination. In DC, this active movement allows DC to attach to T cells and to interact with them closely. Fascin is expressed within the dendritic projections of cLC. As these cover the entire surface of the cell, there are no regions that might be devoid of fascin.

The fact that anti-fascin oligonucleotides impair the dendritic morphology of cLC strongly supports the view that fascin is pivotal for the formation of numerous dendritic projections of maturing LC. It is unlikely that the impaired dendritic morphology is due to unspecific effects of the antisense oligonucleotides, as the number of cells with dendritic morphology is only slightly reduced by the dimethis and E-cadherin expression in murine Langerhans cells following in vivo or in vitro administration of contact allergens. J. Invest. Dermatol. 90:545.


