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Maturation-Dependent Expression and Function of the CD49d Integrin on Monocyte-Derived Human Dendritic Cells

Amaya Puig-Kröger,* Francisco Sanz-Rodríguez,* Natividad Longo,† Paloma Sánchez-Mateos,† Luisa Botella,* Joaquín Teixidó,* Carmelo Bernabéu,* and Angel L. Corbi‡

Dendritic cells (DC) are highly specialized APC that are critical for the initiation of T cell-dependent immune responses. DC exert a sentinel function while immature and, after activation by inflammatory stimuli or infectious agents, mature and migrate into lymphoid organs to prime T cells. We have analyzed integrin expression on monocyte-derived DC (MDDC) and found that expression of CD49d integrins (CD49d/CD29 and CD49d/β7) was induced/up-regulated during TNF-α- or LPS-initiated MDDC maturation, reflecting the induction/up-regulation of CD49d and β7 mRNA. CD49d mRNA steady-state level increased more than 10 times during maturation, with the highest levels observed 24 h after TNF-α treatment. CD49d integrin expression conferred mature MDDC with an elevated capacity to adhere to the CS-1 fragment of fibronectin, and also mediated transendothelial migration of mature MDDC. Up-regulation of CD49d integrin expression closely paralleled that of the mature DC marker CD83. CD49d integrin expression was dependent on cell maturation, as its induction was abrogated by N-acetylcysteine, which inhibits NF-κB activation and the functional and phenotypic maturation of MDDC. Moreover, CD49d integrin up-regulation and MDDC maturation were prevented by SB203580, a specific inhibitor of p38 mitogen-activated protein kinase, but were almost unaffected by the mitogen-activated protein/extracellular signal-related kinase kinase 1/2 inhibitor PD98059. Our results support the existence of a link between functional and phenotypic maturation of MDDC and CD49d integrin expression, thus establishing CD49d as a maturation marker for MDDC. The differential expression of CD49d on immature and mature MDDC might contribute to their distinct motility capabilities and mediate mature DC migration into lymphoid organs. The Journal of Immunology, 2000, 165: 4338–4345.

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†Abbreviations used in this paper: DC, dendritic cell; BCECF, 2′,7′-bis(2-carboxyethyl)-5′(and -6)-carboxyfluorescein acetoxymethyl ester; ERK, extracellular signal-related kinase; FN, fibronectin; MAPK, mitogen-activated protein kinase; MDDC, monocyte-derived DC; MFI, mean fluorescence intensity; NAC, N-acetylcysteine; SDF, stromal cell-derived factor.

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increase in CD49d and β mRNA steady-state levels from immature to mature MDDC, and conveying mature MDDC with an elevated capacity to adhere to the FN CS-1 fragment. The regulated expression of CD49d integrins from immature to mature MDDC might contribute to the distinct motility properties of both cell populations, including the maturation-dependent migration of DC into lymphoid organs.

Materials and Methods
Cytokines and reagents
GM-CSF (Leucomax) was purchased from Schering-Plough (Madison, NJ) and used at 1000 U/ml. TNF-α and IL-4 were obtained from PeproTech (Rocky Hill, NJ) and used at 100 and 1000 U/ml, respectively. Stromal cell-derived factor 1 (SDF-1α) was obtained from PeproTech and used at 100 ng/ml. LPS from Escherichia coli 055:B5 was obtained from Sigma (St. Louis, MO) and used at 10 ng/ml. N-acetylcysteine (NAC) was obtained from Sigma and dissolved in RPMI. SB203580 and PD98059 were obtained from Calbiochem (La Jolla, CA) and used at 13 and 40 μM in DMSO, respectively.

Cells
Human PBMC were isolated from buffy coats from normal donors over a Lymphoprep (Nycomed, Oslo, Norway) gradient according to standard procedures. Monocytes were purified by PBMC by a 1-h adherence step at 37°C in complete medium. Nonadherent cells were washed off by extension washing with PBS, and the remaining adherent cells were >90% monocytes, as determined by flow cytometric analysis of forward scatter, side scatter, CD14, and CD11c staining. Monocytes were immediately subjected to the DC differentiation protocol, as previously described (5, 6). Briefly, monocytes were resuspended to 0.5–1×10^6 cells/ml and cultured in RPMI supplemented with 10% FCS, 100 U/ml Penicillin, 100 μg/ml streptomycin, 250 μg/ml fungizone, 50 μg/ml gentamycin, 2 mM HEPES, and 2 mM glucose in RPMI supplemented with 10% FCS and 25 mM HEPES. Nonadherent cells were washed off by extension washing with PBS, and the remaining adherent cells were >90% monocytes, as determined by flow cytometric analysis of forward scatter, side scatter, CD14, and CD11c staining. Monocytes were immediately subjected to the DC differentiation protocol, as previously described (5, 6).

Human endothelial cells (HUVEC) were obtained from umbilical veins (Bio-Whittaker, Verviers, Belgium) supplemented with 20% FCS (Life Technologies, Gaithersburg, MD), 50 U/I unit penicillin, 50 μg/ml streptomycin, 250 μg/ml fungizone, 50 μg/ml endothelial cell growth supplement (prepared from bovine brain), and 100 μg/ml heparin, and kept up to the third passage. The human cell line U937 (histiocytic lymphoma) was cultured in complete medium, at 37°C in a humidified atmosphere with 5% CO₂.

Determination of the level of CD49d mRNA along the MDDC differentiation was conducted overnight at 42°C in 50% formamide, 5× SSC, 5× Denhardt’s, 50 mM sodium phosphate, pH 6.5, and 250 μg/ml denatured salmon sperm DNA, and membranes were hybridized for 16 h at 42°C in the same solution containing 10^6 cpm/ml of oligo-labeled probe. Blots were subsequently washed in 0.5× SSC, 0.5% SDS at room temperature, in 0.3× SSC, 0.5% SDS at 65°C, and exposed to x-ray film at −70°C. Detection of CD49d mRNA was accomplished with a 1.8-kbp EcoRI fragment of the CD49d cDNA (18), while the 1.8-kbp insert from the β2 cDNA clone P9 (19) was used to detect β2 mRNA. For comparative purposes, all filters were subsequently hybridized with a 417-bp fragment from the GAPDH cDNA (20).

Polymerase chain reaction
Determination of the level of CD49d mRNA along the MDDC differentiation/maturation pathway was accomplished by relative PCR. To that end, 2 μg of RNA from either immature or mature MDDC was reverse transcribed in a total volume of 20 μl of the amplification buffer (50 mM Tris-HCl, pH 8.2, 5 μM MgCl₂, 10 mM DTT, 50 mM KCl, 1 mM of each deoxynucleotide, 0.5 μM random hexamers) including RNAsin and AMV reverse transcriptase at 1 U/μl. The mixture was incubated at 42°C for 60 min, followed by a 30-min incubation at 52°C, and the final volume was taken to 100 μl with water. Amplification of the CD49d mRNA was conducted on 5 μl of cDNA synthesis reaction in 50 μl of a solution containing 0.2 mM of each deoxynucleotide, 1 μM of each oligonucleotide primer, and 2.5 U of Pfu DNA polymerase (Stratagene, La Jolla, CA). Preliminary experiments indicated that the CD49d mRNA was optimally amplified after 35 cycles of denaturation at 95°C, 1 min, followed by a 10-min extension step at 72°C. Oligonucleotides used for CD49d mRNA amplification (VLA4-I-3, 5′-GCTGGATTTACACGTTTCTGC-3′; VLA4-II-5′-AATTCTTGACGTTAGTACAGGAGAC-3′) flanked 286-bp fragment from the CD49d mRNA (between nucleotides 1946 and 1660), and their sequences are derived from separate exons in the CD49d gene (21). As an internal control, each PCR also included oligonucleotides 5′-GGTGGAGAGCGGAACTGGTTGTA-3′ and 5′-CGGCC ATCCGCGCAGATCTTG-3′ (1 μM), which together amplify a 417-bp fragment from the GAPDH mRNA (20). Analysis of the relative levels of
CD49d mRNA in immature and mature MDDC was done by removing 15-μl aliquots of the PCR during the log phase of the amplification (after 22, 24, 27, and 30 cycles), and the amplified fragments were detected by agarose gel electrophoresis.

Western blot
Total cell lysates were obtained in 50 mM HEPES, pH 7.5, 250 mM NaCl, 1 mM EDTA, 0.5% Triton X-100, 0.5 mM DTT, 10 mM NaF, 1 mM Na2VO4, 20 mM Pefabloc, and 2 μg/ml of aprotinin, antipain, leupeptin, and pepstatin. A total of 10 μg of each lysate was subjected to SDS-PAGE under reducing conditions and transferred onto an Immobilon polyvinyldene difluoride membrane (Millipore, Bedford, MA). After blocking of the unoccupied sites with 5% BSA in 50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.1% Tween-20, protein detection was performed using the Supersignal West Pico Chemiluminescent system (Pierce, Rockford, IL), according to the manufacturer’s instructions. For reprobing, membranes were incubated in stripping buffer (62.5 mM Tris-HCl, pH 6.7, 100 mM 2-ME, 2% SDS) for 30 min at 50°C with occasional agitation. Detection of extracellular signal-related kinase (ERK)1/2, p38, phospho-ERK1/2 (pERK1/2), and phospho-p38 (pp38) was conducted using specific polyclonal Abs from New England Biolabs (Beverly, MA).

Results
CD49d integrin expression upon activation of MDDC
The pattern of integrin expression changes during differentiation in numerous cell lineages (22, 23). Conversely, integrin molecules directly affect the differentiation program of hemopoietic cells (24, 25). Because the functional activity of DC is dependent on their adhesive and migratory capabilities, the levels of integrin expression were analyzed during DC differentiation/maturity from peripheral blood monocytes. Flow cytometry analysis revealed that the expression of several α subunit integrins greatly differed between immature and mature MDDC. Immature MDDC expressed high levels of CD49e/CD29, CD11a-c/CD18, moderate levels of CD51, and very low (or absent) levels of CD49d (Fig. 1, left). The low expression of CD49d on immature MDDC is in agreement with our previous results, showing that its expression on monocytes is down-regulated upon in vitro culture (26). TNF-α-induced MDDC maturation led to induction of CD83 expression, without affecting the expression of the CD11a-c/CD18 integrins (Fig. 1). By contrast, the expression of CD49d was up-regulated during MDDC maturation. Analysis of MDDC from more than 30 donors revealed that CD49d was either absent or extremely low on immature MDDC, while TNF-α maturation either induced or greatly up-regulated the expression of CD49d (Fig. 1, left). To exclude a conformational change as the basis for the differential CD49d expression, both cell types were analyzed with Abs recognizing distinct functional epitopes on the CD49d molecule (27). mAbs against epitopes A, B1, and B2 similarly detected the changes in CD49d expression between immature and mature MDDC (Fig. 1, right), thus ruling out an epitope-specific effect and confirming that CD49d expression is greatly increased by TNF-α on MDDC. Furthermore, LPS treatment of MDDC up-regulated CD49d (see below, Fig. 3), indicating that maturation-inducing agents other than TNF-α are also capable of increasing the expression of CD49d-containing integrins.

Kinetics of CD49d integrin acquisition during MDDC maturation
Maturation of MDDC by TNF-α increases the expression of co-stimulatory molecules (CD80, CD86) and induces the expression of the mature DC marker CD83 after 2–4 days of treatment (3, 11). To determine the kinetics of the TNF-α-mediated up-regulation of CD49d, integrin expression was measured at different time points during MDDC differentiation/maturation and compared with CD83. Forty-eight hours after TNF-α addition, a time point at which a CD83-expressing subset had already appeared, CD49d expression had also started to increase (Fig. 2, A and B). The proportion of cells with high CD49d expression steadily increased during MDDC maturation, and the whole cell population had acquired CD83 expression and exhibited high levels of CD49d 96 h after TNF-α addition (Fig. 2, A and B). Therefore, expression of the CD49d integrins is up-regulated concomitantly with CD83 induction, suggesting that a high expression of CD49d can be considered as a maturation marker on MDDC. To determine whether CD49d expression was associated with specific cell subsets in mature MDDC, double-labeling experiments were performed with anti-CD83 and anti-CD49d Abs on TNF-α-treated MDDC. Two days after initiation of the maturation program, all CD83-positive cells exhibited high expression of CD49d (Fig. 2B), and a similar result was observed 72 h after TNF-α addition (Fig. 2B). Therefore, CD83 and CD49d expression are almost simultaneously acquired by maturing MDDC, indicating that CD49d integrin expression can be considered a marker for mature MDDC.

MDDC maturation can be initiated by either proinflammatory cytokines or bacterial agents like LPS. To determine whether CD49d induction represents a general phenomenon upon DC maturation, CD49d expression was analyzed on MDDC treated with LPS. As shown in Fig. 3, CD49d integrin expression was induced upon LPS-triggered maturation, emphasizing the importance of the CD49d expression along MDDC maturation. Moreover, because CD49d integrins include the heterodimers CD49d/CD29 and

**FIGURE 1.** Phenotypic changes during TNF-α-induced MDDC maturation. Left, Cell surface expression of CD11c, CD49d, CD49e, CD29 integrins and the maturation marker CD83, as determined by indirect immunofluorescence. Immature MDDC were obtained as described in Materials and Methods, and either treated with TNF-α (mature DC) or left untreated (immature DC) for 4 days. Two independent sets of experiments were performed, of which one is shown. Right, Cell surface expression of CD49d on mature (TNF-α-treated) and immature (untreated) DC, as determined by indirect immunofluorescence with mAbs recognizing the three distinct functional epitopes on CD49d. The percentage of positive cells and MFI (between parenthesis) are shown in the upper right corner. Gray profiles indicate the fluorescence produced by an irrelevant Ab (X63).
CD49d/β7, flow cytometry experiments were performed to determine the identity of the induced molecules. CD49d/β7 cell surface expression was not detected on immature MDDC, as analyzed with the CD49d/β7-specific mAb Act-1 (Fig. 3). However, TNF-α or LPS induced the expression of CD49d/β7 on mature MDDC (Fig. 3). The Act-1 epitope appeared at lower levels (Fig. 3) and with slower kinetics (data not shown) than the CD49d-specific epitopes. Therefore, while TNF-α- or LPS-triggered MDDC maturation induces expression of both CD49d integrins, CD49d/CD29 appears to be the predominant CD49d integrin on mature MDDC.

Agents blocking MDDC maturation inhibit CD49d integrin up-regulation

DC constitutively express NF-κB (2), and DC maturation is dependent on the functional activity of the NF-κB family of transcription factors (2, 28). In fact, NAC, an antioxidant agent that inhibits activation of the transcription factor NF-κB (29), inhibits the phenotypic and functional maturation of DC (28). To analyze whether CD49d integrin up-regulation requires NF-κB-mediates MDDC maturation, immature MDDC were treated with NAC before exposure to maturation-inducing agents. As shown in Fig. 4, NAC abrogated the TNF-α-dependent up-regulation of CD49d expression and, in agreement with previous data (28), prevented CD83 induction and CD54 up-regulation. By contrast, CD11c expression was unaffected by NAC treatment (data not shown). The inhibition could be observed 24 and 48 h after TNF-α addition, and similar results were observed upon LPS-induced MDDC maturation (data not shown). Therefore, CD49d induction appears to be dependent on the NF-κB activation initiated by MDDC maturation-inducing agents.

TNF-α stimulation of immature MDDC also initiates activation of several MAPK, including ERK2, stress-activated protein kinase/c-Jun N-terminal kinase, and p38 MAPK (30). To determine the influence of these signaling pathways on the maturation-dependent CD49d integrin up-regulation, immature MDDC were treated with TNF-α in the presence of either PD98059 (31), an inhibitor of mitogen-activated protein/ERK kinase 1/2 in the ERK2 activation pathway, or SB203580 (32), which specifically inhibits p38 MAPK. Inhibition of the ERK signaling pathway slightly increased the TNF-α-triggered up-regulation of either CD83 or CD49d (Fig. 5A). By contrast, the p38 MAPK inhibitor SB203580 reduced to one-third the percentage of cells that had acquired CD83 after treatment with TNF-α (Fig. 5A). While 85 ± 5% of TNF-α-treated cells were CD83+ after 48 h (average mean fluorescence intensity (MFI) 4.53), SB203580 pretreatment reduced CD83-expressing cells to 26 ± 6% (average MFI 0.6). Interestingly, analysis of CD49d integrin expression revealed a similar inhibitory effect: in the presence of SB203580, the percentage of CD49d+ cells and the level of CD49d expression were comparable with those observed on immature MDDC after 48 h (Fig. 5A). As a control, Western blot analysis was performed to verify that SB203580 and PD98059 were actually inhibiting MAPK activation. In agreement with previous results (30), TNF-α induced ERK1/2 and p38 activation after 10 min (Fig. 5B). SB203580 reduced the phosphorylation of p38 in response to TNF-α, while PD98059 completely blocked the activation of ERK1/2 (Fig. 5B), thus demonstrating the activity and specificity of both inhibitors. Therefore, inhibition of p38 MAPK activity prevents the acquisition of the CD83 and CD49d maturation markers on TNF-α-treated MDDC, suggesting that activation of the p38 MAPK pathway is required for MDDC to complete their phenotypic maturation after stimulation by inflammatory agents.

Functional activity of CD49d integrins on MDDC

The CD49d/CD29 integrin mediates cell-extracellular matrix and cell-cell interactions via recognition of the CS-1 fragment of FN and the cell surface molecule VCAM-1, respectively (33, 34). To find out whether the increased expression of CD49d conveyed a better phenotype to mature MDDC with an increased adhesive capacity, adhesion assays...
were performed with immature and mature MDDC on the fibronectin FN-H89 fragment, which includes the CS-1 region and does not contain the RGD motif recognized by the CD49e/CD29 integrin (34). Immature MDDC exhibited a very low adhesion to FN-H89, while mature MDDC exhibited adhesion levels that were 10-fold higher than immature MDDC (Fig. 6A). MDDC adhesion to FN-H89 was reduced to 30–40% in the presence of Abs against CD49d or function-blocking anti-CD29 Lia1/2, indicating that the interaction is specifically mediated by CD49d/CD29 and that mature MDDC express functionally active CD49d/CD29 molecules on the cell surface (Fig. 6A). Accordingly, treatment of mature MDDC with the activating anti-CD29 TS2/16 mAb did not increase adhesion significantly (Fig. 6A), further indicating that CD49d integrins are constitutively active on mature MDDC. In accordance with flow cytometry data, additional experiments revealed that an anti-CD49d/β3 Ab (Act-1) inhibited the adhesion of mature MDDC to FN-H89 (Fig. 6B). The involvement of CD49d/β3 in MDDC adhesion to FN-H89 might explain the distinct inhibitory effect of CD29- and CD49d-specific Abs (Fig. 6, A and B), and indicates that MDDC attachment to the FN-H89 is mediated by both CD49d-containing integrins. When MDDC were assayed for adhesion to intact FN, it became evident that both immature and mature MDDC bound to FN in a CD49e- and CD29-dependent manner (Fig. 6, C and D). In fact, comparison of immature and mature MDDC binding to either FN or FN-H89 revealed that immature MDDC exhibited higher binding ability to FN than mature cells (Fig. 6, C and D), while only mature MDDC bound significantly to FN-H89 (Fig. 6C). Therefore, the increase in binding to FN-H89 is not due to an overall augmented adhesive-ness of mature MDDC and appears to reflect the augmented expression of CD49d integrins.

In addition to the adhesive function, the involvement of the newly synthesized CD49d integrins in the MDDC migratory capability was evaluated in a transendothelial migration assay. As shown in Fig. 6E, mature MDDC seeded onto TNF-α-stimulated endothelial cells significantly migrated into the SDF-1α-containing lower chamber. More importantly, MDDC transendothelial migration was greatly reduced in the presence of blocking anti-CD49d mAbs (Fig. 6E), thus demonstrating that CD49d integrins on mature MDDC mediate both FN attachment and transendothelial migration.

**Steady-state levels of CD49d and β7 integrins during MDDC maturation**

Changes in the level of expression of CD49d integrins have been previously described in differentiating cells of distinct lineages (22, 23). In differentiating myeloid leukemic cells, CD49d/CD29 is dramatically down-regulated as a consequence of a considerable decrease in the steady-state levels of CD49d mRNA (22). In the case of DC, and because immature cells have extraordinarily high levels of endocytic activity (35, 36), changes in integrin expression could be due to molecule recycling and/or internalization. To analyze the molecular basis for the differential expression of CD49d integrins in immature vs mature MDDC, the steady-state levels of CD49d and β7 mRNA were determined by Northern blot. As shown in Fig. 7A, no hybridization could be detected in RNA from immature cells, while CD49d mRNA species were readily detected in mature MDDC. Similarly, β7 mRNA was only detected in mature MDDC, while GAPDH mRNA levels were similar in both cell populations (Fig. 7A). As a control, and in agreement with our previous results (23), β7 mRNA was induced and CD49d mRNA was down-regulated during U937 cell differentiation. To evaluate

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**FIGURE 4.** CD49d integrin acquisition requires NF-κB-dependent MDDC maturation. CD49d, CD83, and CD54 expression was determined by indirect immunofluorescence on MDDC either untreated (control) or treated with TNF-α for 48 h in the absence (TNF-α) or in the presence of the NF-κB inhibitor NAC (NAC + TNF-α). The percentage of positive cells and MFI (between parenthesis) are indicated. A representative experiment, of three, is shown.

**FIGURE 5.** Effect of MAPK inhibitors on the acquisition of CD49d integrins and CD83 along MDDC maturation. A, CD49d and CD83 expression was determined by indirect immunofluorescence on MDDC either untreated (control) or treated with TNF-α for 48 h in the absence (TNF-α) or in the presence of the mitogen-activated protein/ERK kinase 1/2 inhibitor PD98059 (PD98059) or the p38 MAPK inhibitor SB203580 (SB203580 + TNF-α). The percentage of positive cells and the MFI (between parenthesis) are indicated. A representative one is shown. B, Determination of the levels of phosphorylated (activated) forms of p38 MAPK and ERK1/2 by Western blot. Immature MDDC were incubated in the presence of the indicated inhibitors (SB203580, upper panel; PD98059, lower panel) for 1 h and either left untreated or treated with TNF-α for 10 min. Levels of activated (pp38, upper panel; pERK1/2, lower panel) were determined by Western blot using specific polyclonal Abs, and sample loading was controlled using Abs recognizing total p38 (upper panel) or ERK1/2 (lower panel).
the difference between the CD49d mRNA levels in immature and mature MDDC, relative RT-PCR was performed. Amplification of CD49d mRNA, using oligonucleotides encoded by distinct exons, and control amplification (GAPDH) were accomplished simultaneously on the same sample and analyzed after 24, 27, and 30 cycles. After normalizing according to the GAPDH amplification, mature cells exhibited CD49d mRNA levels, which were at least 10 times higher than those detected in immature MDDC (Fig. 7B).

The dramatic up-regulation of the CD49d mRNA was further analyzed by determining its steady-state levels along TNF-α-initiated MDDC maturation. As shown in Fig. 7C, kinetic analysis indicated that CD49d mRNA increase becomes evident 12 h after TNF-α treatment, reaches a maximum after 24 h, and remains at high levels up to 48 h after cytokine addition. Altogether, these results demonstrate that CD49d integrin membrane expression is strongly up-regulated as a consequence of a dramatic increase in the steady-state levels of CD49d and β7 mRNA during MDDC maturation.

Discussion

Once exposed to inflammatory mediators or bacterial products, DC undergo functional maturation and reenter the circulatory system to home to the T cell areas of the draining lymphoid organs. As a consequence, DC maturation and migration take place concomitantly and influence each other. In the present manuscript, we describe the induction of functional CD49d integrins upon MDDC maturation, which reflects an increase in CD49d and β7 mRNA steady-state levels, and enables mature MDDC to interact with specific domains within the FN molecule. Thus, CD49d integrins can be considered as maturation markers on MDDC. In fact, and like CD83, CD49d induction is dependent on MDDC maturation and can be prevented with agents inhibiting either NF-κB activation or the p38 MAPK signaling pathway. The relevance of the phenotypic changes that we have noted during MDDC maturation is supported by previous results indicating that CD49d integrins are up-regulated on murine Langerhans cells upon in vivo activation (37).

The expression of the CD49d integrin subunit is subjected to a tight control in leukocytes and other cell types. In muscle cells, CD49d/CD29 integrin is induced during myotube formation, and it is exclusively expressed at sites of secondary myogenesis in vivo (22). CD49d is down-regulated upon T cell alloantigen or superantigen recognition, after CD43-induced T lymphocyte homotypic aggregation (38), during monocyte activation (26), myeloid cell differentiation (23), and upon cytokine treatment of bone marrow cells (39). In the last two cases, a specific down-regulation of CD49d mRNA was reported, indicating that the loss of CD49d/CD29 cell surface expression reflected the steady-state level of the CD49d mRNA. By contrast, our findings demonstrate that CD49d mRNA is greatly up-regulated during MDDC maturation, with CD49d mRNA levels being at least 10 times higher in mature MDDC than in their immature counterparts. Although leukocyte CD49d expression appears to be mainly regulated at the transcriptional level (40), the possibility that CD49d mRNA levels are also posttranscriptionally regulated during MDDC maturation cannot be ruled out because 1) the 3’ untranslated region of the CD49d mRNA contains several AU-rich elements (23, 41) that might be targets of signal-induced mRNA stabilization; and 2) p38 MAPK, whose activity is involved in MDDC maturation and CD49d up-regulation, is known to contribute to mRNA stabilization through an AU-rich element-targeted mechanism (42).
mature MDDC binding to fibronectin FN-H89 (Fig. 6), CD49d/b stimulatory (CD80, CD40) molecules. In this sense, recent reports response to TNF-α/β factors, and especially RelB (2, 44). In this regard, two well-established NF-κB activators such as TNF-α and LPS (45) trigger CD49d expression. The influence of NF-κB activation on CD49d expression on DC can also be inferred from the transcriptional mechanisms is currently under investigation, as is the search of additional MDDC markers and functional activities that might be regulated by p38 MAPK activation.

In summary, based on the induction of CD49d by TNF-α and LPS, and the inhibitory effect of maturation-interfering agents (NAC, SB203580) on CD49d up-regulated expression, our results support the existence of a link between the phenotypic maturation of MDDC and the acquisition of CD49d integrin expression, thus establishing CD49d integrin expression as a maturation marker for MDDC.

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References


FIGURE 7. Changes in CD49d and β7 mRNA steady-state levels along MDDC maturation. A, Determination of the steady-state levels of CD49d, β7, and GAPDH mRNA in immature MDDC (I), mature MDDC (M), untreated U937 cells (U), and PMA-differentiated U937 cells (U + PMA). B, Relative levels of CD49d and GAPDH mRNA in immature (I) and mature (M) MDDC by relative PCR. Analysis was performed after the indicated PCR cycles, and the ratio CD49d/GAPDH was determined after densitometric measurement of the PCR results. The experiment was performed on two independent donors with identical results, and one of them is shown. C, Immature MDDC were treated with TNF-α for the indicated periods of time, cells were lysed, total RNA was extracted, and CD49d mRNA levels were determined by Northern blot.


