CD38 orchestrates migration, survival, and Th1 immune response of human mature dendritic cells

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CD38, an ectoenzyme and a signaling receptor, is a novel marker of human mature monocyte-derived dendritic cells (MDDCs). The working hypothesis is that CD38 is not only a marker but also contributes to functions specifically gained by MDDCs with maturation. This was tested by assessing the role(s) of CD38 after signaling with agonistic anti-CD38 monoclonal antibodies or by blocking the interactions taking place between CD38 and CD31, its counterreceptor. The results indicate the following: (1) CD38 engagement in MDDCs ensures efficient chemotaxis and transendothelial migration driven by CC chemokine ligand 21 (CCL21); (2) CD38 is laterally associated with the CCL21-specific CC chemokine receptor 7 and with CD83 and CD11b; (3) CD38 localizes in membrane lipid domains; (4) CD38 signaling contributes to support longevity of lipopolysaccharide (LPS)–matured MDDCs after growth factor withdrawal; and (5) IFN-γ is produced by cocultured T lymphocytes, thus affecting T-helper 1 (Th1) polarization. These data suggest that the localization of CD38 in lipid rafts and its multiple interactions with signaling receptors rule innate and adaptive immune responses by tuning DC migration, survival, and Th1-polarization ability. These findings may lay out the basis to assess the functional role(s) of human CD38 in infections, autoimmune diseases, and neoplastic disorders. (Blood. 2006;107:2392-2399)

Introduction

CD38 is a pleiotropic cell surface molecule acting as an ectoenzyme and a receptor. The enzymatic activity ends in the synthesis of Ca2+-mobilizing metabolites (ie, ADP-ribose [ADPR], nicotinic acid adenine dinucleotide phosphate [NAADP], and cyclic ADP-ribose [cADPR]) involved in the regulation of calcium-dependent calcium release.1,2 CD38 has a unique pattern of surface expression among cells of the human immune system, being present on lymphoid and myeloid progenitors, lost during differentiation, and re-expressed at high density in activated T, B, and natural killer (NK) cells.3,4 The molecule shows a broad distribution in different tissues.5 CD38 ligation in immune cells delivers activation signals and induces cytokine production and secretion by T,6 B,7 and NK cells8 and monocytes.9 CD38 also regulates cell viability by preventing human germinal center B cells from undergoing apoptosis10 and contributing to increased survival of B-cell chronic lymphocytic leukemia (B-CLL) cells.11 This panoply of different functional roles may be explained considering some nonconventional features of CD38 as a receptor. The intrinsic ineptitude of CD38 to transduce signals is overcome by working in physical and functional associations with specialized signaling molecules, such as T-cell receptor on T cells,12-14 B-cell receptor on B cells,15,16 and CD16 on NK cells.17 CD38 can sustain adhesion and rolling of CD38+ lymphocytes on endothelial cells through interaction with CD31 (identified as a specific counterreceptor18), suggesting its possible role in lymphocyte trafficking.19 All the signals mediated by monoclonal antibody (mAb) ligation of CD38 can be reproduced by its interaction with CD31.

In murine models, CD38 is involved in chemotaxis and transendothelial migration of both polymorphonuclear leukocytes (PMNs) and dendritic cells (DCs) and this function requires its enzymatic activities.20,21 Consequently, CD38 knockout mice have impaired capacity to respond to infections.20 The evaluation of the expression of the molecule also has applications in clinical diagnosis, such as in AIDS (where CD38 is one of the earliest indicators of infection22) and B-CLL (where the expression is generally associated with poor prognosis23). Autoantibodies specific for CD38 are found in diabetes and thyroid disorders.23-26 The agonistic properties of these autoantibodies envisage pathogenic role(s) in these diseases.

We recently reported a pulsatile pattern of surface CD38 expression in human monocyte-derived dendritic cells (MDDCs).27 The molecule is highly expressed by monocytes while down-regulated during differentiation into immature MDDCs (iMDDCs). Its re-expression by mature MDDCs (mMDDCs) attributes to CD38 the role of a genuine maturation marker. We also showed that disruption of CD38/CD31 interactions during MDDC maturation...
induces down-regulation of the maturation marker CD83, inhibition of interleukin-12 (IL-12) production, and mMDDC capacity to present alloungtems.27

The working hypothesis of this study is that CD38 may drive (or significantly contribute to) the functions that are specifically gained by MDDCs during maturation. The strategy adopted included analysis of the involvement of CD38 in MDDC migratory capacity in response to constitutively expressed lymphoid organ chemokines.28 The consequent approach was the identification of MDDC surface receptor potentially helping in transducing activation signals in association with CD38. The last approach was the exploration of the regulatory role(s) exerted by CD38 in mMDDC signals in association with CD38. The last approach was the exploration of the regulatory role(s) exerted by CD38 in mMDDC signals in association with CD38. The last approach was the exploration of the regulatory role(s) exerted by CD38 in mMDDC signals in association with CD38. The last approach was the exploration of the regulatory role(s) exerted by CD38 in mMDDC signals in association with CD38.

Materials and methods
Reagents and mAbs
Lipopolysaccharide (LPS) from Escherichia coli, carbonyl cyanide p-(trifluoro-methoxy) phenylhydrazone (FCCP), fluorescein isothiocyanate (FITC)-labeled cholera toxin (CTX), phorbol myristate acetate (PMA), ionomycin, and brefeldin A were from Sigma Chemicals (St Louis, MO). Human recombinant (r) granulocyte-macrophage colony-stimulating factor (GM-CSF) and rIL-4 were from Novartis Pharma (Basel, Switzerland). Human recombinant soluble CD38 (sCD38), kindly provided by H. C. Lee (University of Minnesota, Minneapolis), was obtained as reported35 and used at a concentration of 1 µg/mL.

Agonistic anti-CD38 mAbs (IB4 [IgG2a],36 AT2 [IgG1]); antagonistic anti-CD38 mAbs (IB4 F(ab')2 fragment;36 AT13/5 [IgG1]), kindly provided by Prof G. Stevenson [General Hospital, Southampton, United Kingdom];4) agonistic anti-CD31 mAb (Moon-1 [IgG2a]; and irrelevant control mAbs (X63 [IgG1] and anti-CD8 [OKT8, IgG 2a] from ATCC [Manassas, VA]) were purified in-house and detoxified.38 Affinity-purified F(ab')2, was from ICN Cappel (Aurora, OH); anti–CC chemokine receptor goat F(ab')2 fragment to murine IgG F(ab')2 (G Meier-Chen, South San Francisco, CA). Tetrarhodamine isothiocyanate (TRITC)–conjugated anti–dendritic cell–specific intercellular adhesion molecule 1 (ICAM-1), phycoerythrin (PE)–conjugated anti-CD83, purified anti-CD11b, FITC–conjugated anti–dendritic cell–specific intercellular adhesion molecule 3–tetrahydroxyphenylpiperazin-N'-2-ethansulfonic acid), 100 U/mL penicillin, 100 µg/mL streptomycin (HyClone Laboratories, Logan, UT).

Purification and culture of MDDCs
Monocytes were purified by positive sorting using CD14–mAb–conjugated magnetic Miltenyi microbeads (Bergisch Gladbach, Germany) from peripheral blood mononuclear cells obtained after Ficoll gradient (Lymphocyte-H; Cedarlane, Hornby, ON, Canada).34 CD14+ cells were cultured at 5 × 10^6/mL in RPMI 1640 (Gibco) and supplemented with heat-inactivated 10% LPS-screened FCS (LAL < 1 ng/mL), 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 25 mM HEPES (N-(N-2-hydroxyethylpiperazin-N'-2-ethansulfonic acid), 100 U/mL penicillin, 100 µg/mL streptomycin (HyClone Laboratories), and 0.05 mM carbonyl cyanide m-chlorophenylhydrazone (Sigma; hereafter defined as complete medium), at 37°C in 5% CO2 in the presence of 100 U/mL rIL-4 and 50 ng/mL rGM-CSF.34 At day 7, mMDDCs were analyzed for CD1a, CD14, and CD38 expression by use of a FACSscan flow cytometer (BD Biosciences) using the CellQuest software (BD Biosciences). mMDDCs were further cultured (1 × 10^6 cells) in the presence of LPS (100 ng/mL) for either 24 or 48 hours to induce maturation. mMDDCs were analyzed for expression of CD80, CD83, CD86, HLA-DR, CCR7, and CD38 by use of a FACSscan.

Cocapping experiments
mMDDCs (0.5 × 10^6) were incubated with the indicated purified mAbs (30 min on ice), washed, and reacted with TRITC–labeled GoMlg (20 min on ice).35 Samples were resuspended in RPMI 1640 10% FCS and moved to 37°C (30 minutes) to induce capping before adding PBS 0.5% BSA and 0.1% NaN3 at 4°C. Counterstaining was performed with direct FITC–labeled mAbs. After washing, cells were fixed (4% paraformaldehyde in PBS with 2% sucrose, pH 7.6), settled on coverslips coated with poly-l-lysine, and analyzed using an Olympus IX71 confocal microscope (Olympus, Melville, NY) with a 60 oil immersion objective and no. 2 confocal aperture. Images were captured using a FV5-ZM camera and Fluoview software (both from Olympus). Images were processed using Adobe Photoshop CS software (San Jose, CA). Percentages of capping and cocapping were calculated by considering at least 90 to 100 cells in each sample.

Chemokine-driven chemotaxis and transendothelial migration
Chemotaxis experiments were performed by using polycarbonate filters of 5 µm pore size in 24-transwell chambers (Corning Costar, Cambridge, MA). Six hundred microliters of complete medium was added to the lower chamber containing 10 ng/mL of CCL21. LPS-matured MDDCs (1.25 × 10^6; cell input) were added to the upper chamber in 100 µL of complete medium in the presence of the indicated reagents and incubated for 3 hours. Migrated cells were counted by flow cytometry in a FACSscan, acquiring events for 60 seconds using CellQuest software.21,40

In transendothelial migration assays, 4 × 10^5 cells of the HPMEC–ST1.6R line were grown to confluence on the polycarbonate filter in Medium 199 10% FCS. After 24 hours, medium was removed and 600 µL of complete medium containing 10 ng/mL of CCL21 was added to the lower chamber. mMDDCs (1.25 × 10^6; cell input) were added to the upper chamber in 100 µL of complete medium in the presence of the indicated reagents and incubated for 18 hours. Cells migrated to the bottom chamber were counted as in chemotaxis experiments. Scores for chemotaxis and transendothelial migration experiments are calculated as percentage of migrated cells versus control (input: 1.25 × 10^6 mMDDCs). Where indicated, mMDDCs used for chemotaxis and transendothelial migration experiments were first treated for 15 minutes with 8-Br-cADPR (100 µM)41 and then added to the upper chamber of the transwell in the presence of the compound (also added to the lower chamber).

Detection of apoptosis
MDDC apoptosis was detected by using APOPTEST–FITC (Dako Cytomation). Briefly, mMDDCs were treated with stimuli for either 24 or 48 hours, as indicated, harvested, and double-stained with FITC–conjugated annexin V and iodide propidium (PI), according to the manufacturer’s protocol.41 Cells were analyzed by flow cytometry in a FACSscan using CellQuest
software. Apoptosis was measured in parallel with Bcl-2 and Bax intracellular staining and DePsipher (Trevigen, Gaithersburg, MD) staining.

Detection of Bcl-2 and Bax in MDDCs

iMDDCs were treated with stimuli for 24 hours. Cells were then fixed and permeabilized as described in the Cytofix-Cytoperm and Perm/Wash protocols (BD Biosciences) and separately stained with predetermined optimal concentrations of anti–Bcl-2 or anti-Bax mAbs or appropriate isotype control mAb, followed by staining with the FITC-conjugated anti–rabbit IgG mAb. Samples were washed and analyzed by flow cytometry in a FACScan using the CellQuest software.

Detection of \( \Delta \Psi_m \)

Mitochondrial transmembrane electric potential (\( \Delta \Psi_m \)) in MDDCs was measured by using the DePsipher kit. Briefly, MDDCs were treated with the stimuli for either 24 or 48 hours, harvested, and incubated at 37°C with DePsipher for 20 minutes. As positive control, iMDDCs were treated with FCCP (250 nM), a protonophore that dissipates the H⁺ gradient across the inner membrane of mitochondria and induces apoptosis. Cells were analyzed by flow cytometry in a FACScan using the CellQuest software. Living cells stained with DePsipher gave a high red labeling (FL-2 fluorescence intensity), whereas cells with a disrupted \( \Delta \Psi_m \) feature a low FL-2 fluorescence.

Cytokine measurement by enzymatic or cytometric bead array (CBA) assay

MDDCs (1 \( \times \) 10⁶/mL) were cultured in the presence of the indicated stimuli in 0.5 mL complete medium in 48-well plates (Costar) or in 5-mL tubes (Falcon, Lincoln Park, NJ) at 37°C in a 5% CO² atmosphere. Stimulation with human T-helper 1 (Th1)/Th2 CBA (BD Biosciences) was initiated by using cytokine capture beads and stained with phycoerythrin detection reagent. After 3-hour incubation at room temperature, samples were washed and analyzed by FACScan, using the appropriate software provided by BD Biosciences.

Th1/Th2 polarization experiments

T-lymphocyte polarization capacity of MDDCs was measured using cord blood–derived naïve T cells as responders. T cells (0.5 \( \times \) 10⁶) were cultured with MDDCs (0.5 \( \times \) 10⁵) in complete medium in 24-well plates (Costar) in a final volume of 1 mL. rIL-2 (50 U/mL) was added on day 5, and supernatants were harvested on day 12 and analyzed by CBA assay. Recovered T cells were treated with PMA (40 ng/mL) and ionomycin (1 μg/mL) for 5 hours in the presence of brefeldin A, a compound that blocks proteins in the endoplasmic reticulum, thus inhibiting cellular secretion and avoiding binding of secreted cytokines to the cell membrane. Cytokine production was then measured by intracellular staining.

Intracellular staining

T lymphocytes from polarization assays were stained using pretitrated fluorochrome-conjugated anti-CD3 mAb (BD Biosciences). Cells were then fixed and permeabilized using Cytofix-Cytoperm and Perm/Wash protocols (BD Biosciences) and stained with a predetermined optimal concentration of fluorochrome-conjugated anticytokine antibody (IFN-γ, IL-4) or appropriate isotype controls. After 30-minute incubation at 4°C, cells were washed and analyzed by flow cytometry in a FACScan using the CellQuest software.

Statistical analysis

Statistical descriptive analyses were carried out using the SPSS statistical software package (Chicago, IL). Differences between mean values were assessed by Student t test. The statistical significance was set at \( P < .05 \).

Results

Inhibition of CD38 engagement reduces CCL21-driven chemotaxis and transendothelial migration

CD38 expressed by human lymphocytes supports rolling and adherence to endothelial cells through interaction with the counterreceptor CD31. In this scenario, CD38 engagement by CD31 (both molecules are expressed by MDDCs) could be instrumental to favor adhesion or induce CD38 signaling that may activate the migratory machinery. This was tested by assessing the individual or contributory role(s) of CD38 after signaling with agonistic anti-CD38 mAbs, which are believed to mimic the interactions with the CD31 ligand, and by blocking the interactions taking place between CD38 and CD31 by anti-CD31 mAb. The latter interaction was also inhibited with blocking anti-CD38 mAbs or sCD38.

We first performed chemotaxis experiments in response to CCL21, results of which, reported in Figure 1A, indicate a significant inhibition (\( P = .001 \)) of MDDC migration in the presence of AT13/5, a blocking anti-CD38 mAb. Significant inhibition is also observed in the presence of Moon-1 anti-CD31 mAb (\( P = .003 \)) and sCD38 (70.63% ± 1.3% of migrated cells, \( P = .002 \)). The addition of IB4 and AT2 (agonistic anti-CD38 mAbs) is not followed by apparent inhibition of the migration as is the case when using appropriate isotype-matched irrelevant mAbs. A blocking mAb specific for CCR7, the chemokine receptor that binds CCL21, used as positive control in these experiments, provided significant inhibition (81.3% ± 3.0% of migrated cells, \( P = .003 \)).

The results obtained in chemotaxis experiments support the view of CD38 as a receptor transducing signals implemented by the interactions taking place between CD38 and CD31 by anti-CD31 mAb.

Figure 1. CD38 is involved in mMDDC chemotaxis and migration. Lymphoid organ–derived CCL21-driven chemotaxis (A) and transendothelial migration (B) of mMDDCs. (A) LPS-matured MDDCs (1.25 × 10⁵; LPS) were added to the transwell upper chamber in the presence of the indicated stimuli: blocking anti-CD38 mAb (AT13/5), agonistic anti-CD38 mAbs (IB4 and AT2), anti-CD31 mAb (Moon-1), 8-Br-cADPR, or irrelevant control mAb (Ctr mAb). Results are expressed as percentage of migrated cells with respect to migration of LPS-matured MDDCs (28.7% ± 5.3% of the cell input) and are the mean values ± SE of 5 independent experiments. Statistically significant differences (\( P < .05 \)) with respect to LPS-induced migration are indicated. (B) HPMEC-ST1.6R cells (4 × 10⁵) were grown to confluence on the polycarbonate filter. LPS-matured MDDCs (1.25 × 10⁵; LPS) were added to the top chamber in the presence of the indicated stimuli: blocking anti-CD38 mAb (IB4 F(ab′)₂), agonistic anti-CD38 mAb (IB4), anti-CD31 mAb (Moon-1), 8-Br-cADPR, or irrelevant control mAb (Ctr mAb). Results are the mean values ± SE of 5 independent experiments. The transendothelial migration of LPS-matured MDDCs represented 32.5% ± 4.5% of the cell input. *Statistically significant differences (\( P < .05 \)) with respect to LPS-induced migration.

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interactions among mMDDCs, which express CD31 at consistent levels.27 Switching to a physiologic setting, CD38 binding to CD31 expressed by endothelial cells lining the blood vessels or lymphoid endothelium (both CD31+) could represent a starting point of a cascade of events favoring CD38-driven signals necessary for or involved in transendothelial migration processes. To mimic the interactions taking place in vivo with endothelial cells during their transit to lymph nodes, mMDDCs were allowed to migrate in response to CCL21 across a monolayer of HPMEC-ST1.6R, a human cell line of endothelial origin.39

In keeping with the results of chemotaxis experiments, transendothelial migration, driven by CCL21, of LPS-matured MDDCs is significantly inhibited (P = .003) by using blocking F(ab')2 preparations of the anti-CD38 IB4 mAb. The agonistic signal is not observed when the mAb is used as F(ab')2 fragment, likely reflecting a significant reduction of the clustering features of the antibody. A previous study performed on human monocytes demonstrated that removal of the Fc portion of the anti-CD38 IB4 mAb significantly limited its agonistic properties.9

A significant inhibition (P = .015) of transendothelial migration is also detected in the presence of anti-CD31 (Moon-1) mAb. Addition of either isotype-matched irrelevant mAb or F(ab')2 preparation of appropriate isotype-matched irrelevant mAb has no effects, supporting the specificity of the inhibition observed.

Role of CD38 ADPR cyclase activity in the migration of mMDDCs

CD38 enzymatic activity is required for chemotaxis and transendothelial migration of DCs in the mouse system.21 Thus, an important issue to clear up concerns the role of CD38 as an ectoenzyme in human MDDC migration. The question was partially answered by performing the chemotaxis and transendothelial migration experiments in the presence of 8-Br-cADPR, an inhibitor of cADPR, a key metabolite produced by CD38 enzymatic activities.1,2,21,44 The results indicate that 8-Br-cADPR does not induce a recordable influence on chemotaxis (Figure 1A) and transendothelial migration of mMDDCs (Figure 1B).

CD38 is laterally associated with CD83, CD11b, and CCR7 in mMDDCs

MDDCs express cell surface molecules implicated in T-cell stimulatory activity and/or migration, some of which (ie, CD80, CD86, CD83, CCR7) are up-regulated upon treatment with maturation stimuli. Starting from the involvement of CD38 receptorial activity in mMDDC migration in response to CCR7 ligand and T-cell stimulatory activity,27 the next step was the identification of molecules sustaining the role of CD38 in the observed phenomena. This goal was pursued by performing cocapping experiments in LPS-matured MDDCs by using mAbs specific for surface receptors crucial for DC signaling (Figure 2).

Capping indicates the redistribution of a surface molecule to a single cell pole upon cross-linking with a specific antibody or ligand. Only the molecule bound by the antibody will redistribute to the cap area, but if it has an association with or is in close vicinity to a second molecule, the latter is induced to cocap to the same area. The results indicate that CD83 and CD11b are constantly present in the CD38 caps. The lateral associations observed are bidirectional, as inferred after reversing the order of the capping molecule (Figure 2A-D). Cocapping is also observed with the chemokine receptor CCR7 (Figure 2E). No association is found with other signaling receptors, such as CD86, CD80, DC-SIGN, and CD31 (Figure 2F-G; Table 1). The results obtained from a large number of cells acquired in 3 independent experiments are reported in Table 1.

CD38 colocalizes with lipid domains in mMDDCs

A significant fraction of CD38 is reported as constitutively present within specific membrane microdomains (or lipid rafts) in T lymphocytes.13,14 Lipid rafts are critical for initiating and sustaining signaling events in the cells.45 The presence of CD38 in rafts in mMDDCs was studied by inducing a polar aggregation of CD38 and then staining with FITC-labeled CTX, which binds ganglioside GM1, a constitutive marker of membrane microdomains.45 Figure 2H shows that cross-linking of a specific anti-CD38 mAb induces
Table 1. CD38 cocapping with mDDC membrane receptors and lipid domain-associated molecules

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<tr>
<th>Cap and cocap</th>
<th>Caps, %</th>
<th>Cocaps, %</th>
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<tr>
<td><strong>Associated molecules</strong></td>
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<tr>
<td>CD38 and CD11b</td>
<td>86</td>
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<td>CD11b and CD38</td>
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<td>CD83 and CD38</td>
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<tr>
<td>CCR7 and CD38</td>
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**Nonassociated molecules**

| CD38 and CD31 | 90 | 15 |
| CD38 and CD80 | 88 | 9  |
| CD83 and DC-SIGN | 87 | 7  |
| CD38 and CD86 | 75 | 9  |

**Lipid domain-associated molecules**

| CD38 and CTX | 69 | 62 |
| CD38 and CD81 | 75 | 54 |
| CD81 and CD38 | 78 | 68 |

Cumulative data from 3 independent experiments are shown. The number of caps and cocaps are presented as a percentage of the cells analyzed. Cells exhibiting partial redistribution of the surface molecule detected by the primary capping antibody were excluded from the analysis.

CD38 to colocalize with GM1+ domains. We also demonstrate a lateral association of CD38 with CD81, a tetraspanin family member described as constitutively associated with lipid rafts.46 The CD38/CD81 association is bidirectional, as evident after reversing the order of the capping molecule (Figure 2I-J). The percentages of colocalization observed in the experiments are reported in Table 1.

Inhibition of CD38 engagement during LPS-driven maturation increases the sensitivity of mDDCs to growth factor withdrawal apoptosis

Pathogen- or T-cell–derived maturation stimuli confer resistance to environmental and intrinsic death signals in murine as well as human DCs by up-regulating antiapoptotic factors.29-33,47 Among these, Bcl-2 was reported to be up-regulated by CD38 ligation in human germinal center B lymphocytes.10,48 With this in mind, we assessed the viability of mDDCs and their ability to up-regulate Bcl-2 expression when CD38 signaling was blocked. Expression of the proapoptotic factor Bax, which mediates mitochondrial damage,48 was also assessed in the same culture conditions.

The strategy adopted was to block signals mediated by CD38 by adding to the culture reagents (sCD38 or anti-CD31 mAb) specifically disrupting CD38/CD31 interactions at the time of LPS administration. To better compare the results of the different experiments, and also taking into account the high interdonor variability presented by the different MDDCs tested, the data are reported as percentage of apoptosis compared with spontaneous apoptosis of untreated MDDCs, arbitrarily set at 100%.

Figure 3A-B shows a representative annexin V/P staining obtained using MDDC preparations from 2 independent donors, whereas Figure 3C-D displays the results of cumulative experiments. The results obtained show that MDDCs treated with LPS are protected from spontaneous apoptosis induced by growth factor withdrawal compared with iMDDCs. The addition of sCD38 or anti-CD31 mAb (Moon-1) abolishes the protective effects of LPS. The addition of either sCD38 (Figure 3A,C) or Moon-1 mAb (Figure 3B,D) to untreated MDDCs does not significantly modify the percentage of apoptotic cells. LPS-induced survival is restored when the signals mediated by CD38, interrupted by sCD38 or anti-CD31 mAb, are induced by ligation using IB4, an agonistic anti-CD38 mAb (Figure 3C-D).

LPS treatment induces the expected up-regulation of Bcl-2 expression in MDDCs23,33 (Figure 3E). The up-regulation is inhibited by concomitant addition of sCD38 to the cultures. CD38 ligation by IB4 mAb restores LPS-induced Bcl-2 up-regulation in the presence of sCD38 (Figure 3E), whereas the addition of IB4 mAb to untreated or LPS-treated MDDCs does not affect Bcl-2 expression (data not shown). High Bcl-2/Bax ratio reflects an indication of protection from death. Maximal levels are scored following LPS treatment and after restoring CD38 signaling by the agonistic anti-CD38 mAb (Figure 3F). The values correlate well with the mean values of percent apoptosis measured in the experiments shown in Figure 3C.

The increased Bcl-2/Bax ratio after LPS treatment is primarily due to a significant up-regulation of Bcl-2 expression (LPS: 75.8% ± 8.6% versus none: 30.2% ± 9.8% positive cells, n = 13, P = .006). In contrast, expression of Bax in LPS-treated MDDCs shows only a modest and nonsignificant up-regulation (LPS: 8.6% versus none: 9.8% positive cells, n = 13, P = .13).

![Figure 3. CD38 is involved in mMDC protection from apoptosis induced after withdrawal of growth factors.](image_url)

(A) IMDDCs were either untreated (none) or treated with LPS alone, sCD38 alone, or LPS plus sCD38 for 24 hours. Shown is one representative experiment of 9 performed. (B) IMDDCs were either untreated (none) or treated with LPS alone, anti-CD31 mAb (Moon-1) alone, or LPS plus anti-CD31 mAb for 24 hours. One representative experiment of 4 performed is shown. (C-D) Cumulative apoptosis values (means ± SE of 4 to 9 independent experiments, according to the experimental conditions) are presented as percentage of apoptosis compared with untreated cells (100% spontaneous apoptosis; none). The percentage of spontaneous apoptosis (mean ± SE [range] of 9 donors) of untreated MDDCs was 27.5% ± 3.5% (8.7%-63.2%). Agonistic anti-CD38 mAb (IB4) was added as indicated. Statistically significant differences are indicated. (E) IMDDCs were either untreated (none) or treated with LPS alone or LPS plus sCD38 for 24 hours. Agonistic anti-CD38 mAb (IB4) was added as indicated. Cells were intracellularly stained for Bcl-2 expression. One representative experiment of 4 performed is shown. (F) Cumulative Bcl-2/Bax ratio values calculated after 24 hours of culture in the same conditions as in panel C. Results are expressed as mean ± SE of 4 to 9 independent experiments, according to the experimental conditions. (G) IMDDCs were either untreated (none) or treated with LPS alone or LPS plus sCD38 for 48 hours. iMDDCs were also treated with FCCP as control. Cells were stained with DePsipher. Percentages of gated cells displaying decreased ΔΨm are indicated. One representative experiment of 3 performed is shown. (A-B, E) Numbers in graphs are percentages of positive cells.
The ratio between death-inducing (Bax) and death-inhibitory (Bcl-2) members determines whether a cell will respond to an apoptotic signal mediating disruption of the mitochondrial transmembrane electric potential ($\Delta$Psi,m). The $\Delta$Psi,m modifications were evaluated by performing staining with DePsipher (Figure 3G), which confers a bright red color only to cells with intact $\Delta$Psi,m. Cytofluorimetric analysis shows that the iMDDC population is characterized by a percentage of cells with decreased $\Delta$Psi,m (ie, low FL-2 fluorescence intensity) significantly higher than that of the LPS-matured MDDC population (31.3% ± 3.3% versus 13.6% ± 0.5%, n = 3, P = .013). However, a block of CD38/CD31 interaction by sCD38 at the time of LPS administration is followed by a significant increase of the percentage of cells with low $\Delta$Psi,m (22.1% ± 1.6%, n = 3, P = .02).

### Discussion

The results of this study show that CD38 is not only a novel marker of human mMDDCs but also a receptor involved in the regulation of crucial DC functions acquired at the mature stage, such as CCL21-driven migration, survival, and Th1-polarizing activity.

The modality used by CD38 to affect migration could be at least 2-fold. Indeed, CD38 might work as a receptor transducing signals generated after engagement by counterreceptor CD31 (in homotypic [among DCs themselves] and heterotypic [with endothelial cells] fashions). Alternatively, CD38 might form molecular bridges promoting adherence to endothelial cells through CD31 and, in turn, favoring diapedesis across the endothelium.

The results obtained seem to support the first hypothesis for 2 main reasons: (1) mAbs and reagents able to interfere with CD38-mediated signals determined a powerful inhibition of migration; (2) agonistic anti-CD38 mAbs had no effects on chemotaxis and the transendothelial migration process. If the adhesive function mediated by CD38 binding to CD31+ endothelium was involved in transendothelial migration, one should have observed a significant inhibition of the migration also while using agonistic anti-CD38 mAbs.

CD38 is reported to promote chemotaxis and transendothelial migration of PMNs and DCs, both processes requiring its enzymatic activities. These findings were only partially confirmed in a human system, where CD38 plays a key role in chemotaxis and transendothelial migration of mMDDCs in response to CCL21. We failed to observe an impaired migration when the experiments were done in the presence 8-Br-cADPR, a c-ADPR antagonist. The receptorial nature of the molecule is supported by its lateral associations indicating close proximity with CCR7, CD11b, and CD83.

The peculiar localization is a feature of CD38 in mMDDCs, confirming the observations derived from T and B lymphocytes. Another set of results supporting the view of CD38 as a receptor comes from the analysis of its lateral associations indicating close proximity with CCR7, CD11b, and CD83.

CCR7 is the chemokine receptor that binds CCL21: the ligand/receptor interaction takes part in DC migration by finely tuning [Ca$^{2+}$]i levels. Indeed, preliminary data indicate that Ca$^{2+}$ mobilization is induced in mMDDCs upon CD38 cross-linking (S.D. and C.M.A., unpublished data, November 16, 2004).
The CD11b molecule, part of the complement receptor 3, is known to possess adhesive properties and favor migration of leukocytes. CD38, a marker of human mMDDCs, has been described as playing a pivotal role in mMDDC-mediated T-cell activation through an unknown ligand. Thus, association between CD38 and CD38 can be instrumental in mMDDC-mediated T-cell activation of naive T cells, particularly expressing CD31. The relevance of the cellular FLICE inhibitory protein. CD38 engagement may concur to these events and finely tune the expression of proapoptotic and antiapoptotic molecules, increasing DC longevity, at least for the time needed to reach the lymph nodes and prime T cells. Supporting this scenario, our results not only confirm that human DCs are protected from intrinsic pathways of apoptosis after LPS treatment but also clearly show the involvement of CD38 signals in this process.

Consequently, the observation that CD38 contributes to ensure longevity of DCs, which in turn affects the magnitude of T-cell activation, allows us to attribute to CD38 the role of a fine regulator of the adaptive immune response. This is in agreement with other results presented in this study, providing support to our previous hypothesis that CD38 plays a role in the regulation of responses requiring Th1 cells. Indeed, here we show that the decreased IL-12 release, observed after inhibition of CD38-mediated signaling, interferes negatively with the capacity of mMDDCs to polarize T-cell response toward a Th1 phenotype.

In conclusion, our study reinforces the notion that CD38 is not simply a maturation marker of human MDDCs but is involved in maturation-associated functions crucial for establishing and regulating the adaptive immune response. This latter relies on the capacity of mMDDCs to stay alive, migrate in sufficient numbers to opportune sites, and efficiently present the antigens in order to activate and polarize naive T cells. By residing in rafts, CD38 may work in association with key molecules warranting the achievement of the mMDDC functions. This model has recently found confirmation at a molecular level, as inferred by the availability of crystals of the extracellular portions of human CD38: indeed, Liu’s group confirmed all the inferences so far derived using mAbs as probes for drawing a map of the functional domains located on the CD38 molecule.

These results may also be of potential clinical relevance. The experience in clinical immunology indicates CD38 as a marker of HIV infection and immune response and is also implicated in autoimmune diseases, prevalently type 1 diabetes and thyroiditis, characterized by the presence of agnostic anti-CD38 autoantibodies. Another field where CD38 has gained applications is B-CLL, where CD38 engagement triggers a chain of events leading to increased growth of tumor cells and ultimately to a poor clinical prognosis. These disease models might be completed by considering the adjunctive role of DCs in the cellular interplay with T and B lymphocytes taking place in the bone marrow, lymph nodes, or circulation. The unifying trait for all these unrelated diseases is that an inappropriate CD38 signaling may contribute to disease by operating on the physiology of the immune cells and in particular of DCs, a crucial link between innate and adaptive immunity. Similar effects may be secondary to levels of agnostic anti-CD38 autoantibodies, which can alter homeostatic mechanisms governing DC trafficking and survival, along with secretion of proinflammatory cytokines exacerbating autoimmune phenomena.

A further issue that could also be considered is the role of CD157, the CD38 parologue expressed by monocytes and by DCs themselves (G.F. and L.F., unpublished data, January 18, 2005). CD157 is an ectoenzyme and is involved in adhesion and in chemotaxis, as confirmed by cells from patients with paroxysmal nocturnal hemoglobinuria, a natural CD157 knockout.

A final issue is the notion that the CD38 gene is polymorphic. Currently under test is the design of a genetic model that may adapt all the different tiles in a physiologically satisfying mosaic.

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References

15. Deaglio S, Capobianco A, Bergui L, et al. CD38 is...


CD38 orchestrates migration, survival, and Th1 immune response of human mature dendritic cells

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