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The Adaptor Protein Bam32 in Human Dendritic Cells Participates in the Regulation of MHC Class I-Induced CD8+ T Cell Activation

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The B lymphocyte adaptor molecule of 32 kDa (Bam32) is strongly induced during the maturation of dendritic cells (DC). Most known functions of Bam32 are related to the signaling of the B cell receptor for Ag. Because DC do not express receptors specific for Ags, we aim at characterizing the role of Bam32 in human monocyte-derived DC in this study. Our results show that binding of allogeneic T cells to mature DC causes accumulation of Bam32 on the contact sites and that this translocation is mimicked by Ab-mediated engagement of MHC class I. Silencing of Bam32 in immature monocyte-derived DC results in an enhanced proliferation of CD8+ T cells in an Ag-specific T cell proliferation assay. Further studies identify galectin-1 as an intracellular binding partner of Bam32. Regulating immune responses via regulatory T cell (Treg) modulation is one of the many immunological activities attributed to galectin-1. Therefore, we assayed mixed leukocyte reactions for Treg expansion and found fewer Treg in reactions stimulated with DC silenced for Bam32 compared to reactions stimulated with DC treated with a nontarget control. Based on our findings, we propose a role for Bam32 in the signaling of MHC class I molecules in professional Ag-presenting DC for the regulation of CD8+ T cell activation. It is distinct from that of MHC class I recognized by CD8+ T cells leading to T cell death. Thus, our data pinpoint a novel level of T cell regulation that may be of biological relevance. The Journal of Immunology, 2011, 187: 000–000.

Dendritic cells (DC) are professional APCs with a unique T cell stimulatory capability. They play a crucial role in orchestrating the initiation of diverse effector functions that are suitable for the elimination of pathogens. The adaptive immune response is shaped by innate immunity and depends on functions unique to DC and DC-derived effector molecules such as cytokines and chemokines (1, 2). Better understanding of the mechanisms that control the induction of different T cell effector functions will enable the development of strategies to manipulate the immune system in the context of vaccination, tumor immunotherapy, transplantation, and autoimmunity (3). Our approach to contribute to this is to isolate and characterize molecules induced or upregulated during the maturation of DC (4–7), assuming that many of them might be involved in mechanisms regulating the initiation of immune responses. We therefore compared the gene expression of immature versus mature monocyte-derived DC. Through a gene array approach to identify such differentially expressed molecules during the maturation of DC, we found the mRNA of the B lymphocyte adaptor molecule of 32 kDa (Bam32) to be substantially induced.

Adaptor proteins are often located in the cytoplasm of resting cells and are physically recruited to activated receptors or associated signaling complexes at the cell membrane after cell stimulation and account for the specific outcome of signaling pathways (8, 9). In B cells, Bam32 is recruited to the plasma membrane through interaction of its PH domain with the lipid products of PI3Ks (10). Bam32 was found to be expressed mainly in murine hematopoietic cells but also in human cells, including B and T lymphocytes. It has multiple functions in B cell activation and is required for biological responses including BCR-induced proliferation, Ab responses to type II T-independent Ags, germinal center progression, and formation of polarized conjugates with T cells (11–14). It functions by regulating F-actin formation, and this Bam32-induced cytoskeletal rearrangement has been shown to be important for receptor endocytosis and membrane ruffling (15, 16). Bam32−/− mice vaccinated with Streptococcus pneumoniae capsule Ag did not produce IgG3 Abs, and these mice were more susceptible to infection with live bacteria after vaccination. The authors therefore suggest that mutations of Bam32 might be responsible for some of the unexplained immunodeficiencies that predispose individuals to infection by bacterial pathogens with a polysaccharide capsule (11). Comparing CD8+ T cells from lymph nodes of wild-type and Bam32-deficient mice, Sommers et al. (17) could show that Bam32 was required for

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optimal TCR-induced ERK activation, cytokine production, and proliferation and actin-mediated spreading of CD4+ T cells. The induction or upregulation of Bam32 during the maturation of DC, however, is entirely unrelated to receptors specific for Ag because DC do not express such receptors at any point in their life span. We therefore aimed at characterizing the role of Bam32 in mature DC.

Materials and Methods

Generation of monocye-derived DC

Human DC were prepared from peripheral blood monocytes essentially as described (18, 19). Anonymous human blood components were obtained from the local blood bank according to the guidelines of the Institutional Review Board of the Innsbruck Medical University and the tenets of the Helsinki Protocol. Monocytes were obtained by isolating CD14+ cells by magnetic sorting (MACS; Miltenyi Biotec, Bergisch-Gladbach, Germany). Briefly, 1.5 × 10^7 cells/well were plated in 6-well tissue culture plates in 3 ml complete culture medium (RPMI 1640 [PAN]; Biotech GmbH, Aidenbach, Germany) supplemented with 50 μg/ml gentamicin, 2 mM l-glutamine (Sigma Chemicals, St. Louis, MO), 2% autologous human serum or 10% FCS (Biological Industries or Seromed Biochrom, Berlin, Germany), 800 U/ml GM-CSF (sp. act. 1.1 × 10^6 U/mg; Leukomax; Novartis, Basel, Switzerland), and 20 U/ml IL-4 (Strathmann, Hamburg, Germany). Culture medium was renewed every other day by removing 1 ml medium and substituting with 1.5 ml fresh medium containing 1600 U/ml GM-CSF and 20 U/ml IL-4. On day 6, cells were cultured for 2 additional days with or without a defined cytokine mixture consisting of TNF-α (10 ng/ml; kindly provided by Dr. G.R. Adolf, Bender, Vienna, Austria), IL-1β (2 ng/ml; PeproTech EC, London, U.K.), IL-6 (1000 U/ml; PeproTech), and PGE2 (1 μg/ml; Pharmacia & Upjohn SA, Buurs, Belgium) as maturation stimulus.

Whole-skin explants

To obtain Langerhans cells and dermal DC, we used an established method (20–22). Briefly, standardized pieces of normal skin were prepared by means of an 8-mm punch from split-thickness skin (0.3 mm). These explants were floated on 1.5 ml culture medium (RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM l-glutamine; Sigma Chemicals), 100 U/ml penicillin, 100 μg/ml streptomycin (Irvine Scientific) in 24-well plates (one explant per well) for 2 to 5 d at 37˚C. Cells that had emigrated into the culture medium during this time were harvested, counted, and further evaluated by flow cytometry.

Preparation of T cells

Bulk T cells were isolated from the rosettes that had formed with neuraminidase-treated sheep RBCs during the monocyte isolation procedure by lysing the sheep RBCs with ammonium chloride as described (20). CD8+ and CD4+ T cells were isolated from bulk T cells using a panning technique described previously (23, 24). Briefly, 300 × 10^6 to 400 × 10^6 T cells in 1 ml PBS/BSA were treated with mouse anti-human CD19,-CD16,-CD56,-CD14,-CD36,-CD235a,-CD123, and -CD4 or -CD8 Abs, respectively, for 30 min, washed, and allowed to adhere to a goat anti-mouse–coated Petri dish. Nonadherent cells were harvested, washed, and counted.

Preparation of B cells

B cells were isolated from PBMC using the B Cell Isolation Kit II (Miltenyi Biotec).

Generation of mAb against Bam32

A peptide comprising amino acids KMSTQDPSDLWSRSDG of Bam32 was synthesized and coupled to keyhole limpet hemocyanin and OVA. Rats were immunized s.c. and i.p. with a mixture of 50 μg peptide–keyhole limpet hemocyanin, 5 nmol CpG oligonucleotide (Tib Molbiol), 500 μl PBS, and 500 μl IFA. A booster without adjuvant was given 6 wk after the primary injection. Fusion was performed using standard procedures. Supernatants were tested by a differential ELISA with the Bam32 peptide coupled to OVA and an irrelevant peptide coupled to the same carrier. mAbs that reacted specifically with the peptide were further analyzed by Western blot. The Ab 3E10 of the rat IgG1 subclass reacted specifically without background in Western blotting with the recombinant Bam32 and was therefore used in this study.

Quantitation of Bam32 and FOXP3 transcripts

Total RNA was extracted using TRIzol (Life Technologies BRL) and reverse transcribed with random hexamers and Superscript II RNaH2O-negative reverse transcriptase (Life Technologies, Vienna, Austria). Quantitative PCR analysis was performed using the quantitative PCR Core Reagent Kit from Stratagene (Heidelberg, Germany) and the ABI PRISM 7700 sequence detector (Applied Biosystems, Vienna, Austria). Sequences for probes and primers (synthesized by Microsynth, Balgach, Switzerland) specific for human Bam32 mRNA molecules were selected using the Primer Express software (Applied Biosystems): forward primer 5′-ATC-TTTGAATTGTGCTAGACA-3′; reverse primer 5′-GTCCTGTTCTGTC-CTTCCAP-3′. Fam/Tamra-labeled probe: 5′-TTTGCAAATCAAGGCTT-3′. Probes and primers for FOXP3 transcripts were used as described (25).

Western blot

Cells were lysed in buffer containing 50 mM Tris HCl pH 7.5, 150 mM NaCl, 1% (v/v) Nonidet P-40, 1 μM PMSF, and 1 μM DTT supplemented with 2 μg/ml aprotinin, 10 μg/ml leukipin, and 1 μg/ml pepstatin A and subjected to SDS-PAGE. Bam32 was detected by standard Western blot using the Bam32-specific Ab 3E10 followed by Alexa Fluor 680 goat anti-rat IgG (Invitrogen, Eugene, OR). Galectin-1 was detected with the monoclonal anti–galectin-1 VP-G801 mouse IgG1 (Vector Laboratories, Burlingame, CA) followed by Alexa Fluor 680 goat anti-mouse IgG (Invitrogen). The membranes were scanned using an Odyssey Infrared Imaging System (LiCor Biosciences, Lincoln, NE).

Immunocytochemical analysis

Glass slides were coated with 200 μl of affine pure goat anti-mouse IgG (1:250 in PBS) (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h at 37˚C. After three washes with PBS, 5 ng/ml of the specific Ab was added, and the slides were kept at 4˚C overnight. Mature DC (100,000) were allowed to adhere for 45 min at 37˚C. Cells were fixed with acetone for 10 min. Immunostaining was performed with the Bam32-specific Ab 3E10 or with mouse anti-human galectin-1 Ab (Vector Laboratories) and visualized with Alexa Fluor 568 goat anti-rat Ab or Alexa Fluor 488 goat anti-mouse Ab (Invitrogen). Immunolabeled specimens were mounted with Vectashield (Vector Laboratories) and viewed with a micromols-enhanced Nipkow disk-based confocal system (PerkinElmer, Wellesley, MA) mounted on an Olympus IX-70 inverse microscope (Olympus, Nagano, Japan). Images were acquired with a 100× oil immersion objective with a numerical aperture of 1.4. Laser lines at 488 nm and 568 nm were used for excitation. Acquisition was performed with the UltraVIEW RS software (PerkinElmer). A series of 10 optical sections was taken at intervals of 0.2 μm. Densitometric analysis was performed with the UltraVIEW RS software (PerkinElmer), measuring the average fluorescence intensity of regions of interest in single focal-plane images.

FIGURE 1. Bam32 mRNA expression levels. Total RNA was prepared from immature monocyte-derived DC (iDC) or DC matured with a maturation mixture consisting of IL-1, IL-6, TNF-α, and PGE2 (mature monocyte-derived DC, mDC), cells emigrated from a whole-skin explant culture within 3 d consisting of a mixture of Langerhans cells and dermal dendritic cells (Lz/Dc), and from B and T cells. The RNA was subjected to Bam32-specific RT-PCR. Bam32 mRNA transcripts relative to TATA binding protein are shown as mean of three measurements ± SD. Bam32 mRNA levels increase during DC maturation to a higher concentration than that found in B and T cells.
Silencing of Bam32 expression

DC were cultured as described. On day 5, immature DC were harvested and counted. Then, \(1 \times 10^5\) cells/well were seeded in 24-well plates in 500 \(\mu\)l culture medium without antibiotics (RPMI 1640 supplemented with 2 mM l-glutamine and 800 U/ml GM-CSF (sp. act. \(1.1 \times 10^6\) U/mg; Leukomax; Novartis) and 20 U/ml IL-4 (Strathmann)). Cells were transfected with 100 pM Bam32-specific small interfering RNA (siRNA) or a nontarget siRNA (On-TARGETplus SMARTpool [Thermo Fisher Scientific, Lafayette, CO]) using Lipofectamine 2000 (Invitrogen, Life Technologies) according to the manufacturer’s protocol. Twelve hours after transfection, DC were stimulated for maturation. Forty-eight hours later, DC were harvested and used for further experiments. Transfection efficiencies were determined by Western blots.

Stimulation of Ag-specific CD8+ T cells

Expansion of EBV A2.1 peptide-specific CD8+ T cells was induced by stimulation of enriched CD8+ T cells from HLA A2.1+ donors with autologous DC silenced or not for Bam32 expression at a ratio of 1:10 for 5 d. DC were either pulsed or unpulsed with 10 \(\mu\)M HLA A2.1-restricted EBV peptide (GLCTLVAML; ProImmune, Oxford, U.K.) for 3 h at 37°C at \(1 \times 10^6\) DC/ml complete medium. Expansion of Ag-specific CTL was quantified by pentamer staining according to the manufacturer’s protocol (ProImmune).

Yeast two-hybrid screening

To identify binding partners of Bam32, the coding region was cloned into the bait vector and used to screen a mature monocyte-derived DC (mDC) derived cDNA library prepared using the Yeast Two-Hybrid Library Preparation Kit (Clontech, Palo Alto, CA) with the GAL4-based Matchmaker Two-Hybrid System 2 (Clontech) according to the manufacturer’s protocol.

Coimmunoprecipitation

Matured DC (\(5 \times 10^6\)) were lysed in 0.5 ml modified RIPA buffer (50 mM TrisCl pH 7.4, 1% IGEPAL, 1 mM EDTA, 0.25% Na deoxycholate; 150 mM NaCl, and protease inhibitor mixture). Immobilized protein A agarose were incubated with 8 \(\mu\)g of the polyclonal rabbit anti-human galectin-1 serum or a rabbit control serum and incubated overnight at 4°C. One hundred microliters of protein lysate were added to the protein A agarose by adding 30 \(\mu\)l modified RIPA buffer and 10 \(\mu\)l loading buffer and heating at 95°C for 5 min. After 15 min of centrifugation at 9000 \(\times\) g, the supernatant was collected to be analyzed for the presence of Bam32 by Western blots.

**FIGURE 2.** Localization of endogenous Bam32 to the membrane in mature monocyte-derived DC (mDC). Glass slides were coated with PLL and mDC cocultured with T cells for 30 min or mDC that were treated with a mouse anti-human MHC class II or mouse anti-human MHC class I mAb were allowed to adhere for 20 min. A control, mDC were allowed to adhere directly to the PLL for 20 min. Fixing was with acetone. Staining was with rat anti-human Bam32 mAb 3E10 visualized with Alexa 568-conjugated anti-rat Ab and a microens-enhanced Nipkow disk-based confocal system (PerkinElmer) mounted on an Olympus IX-70 inverse microscope (Olympus). A. Isotype controls are shown in the lower row. Images were acquired with a \(\times\)100 oil immersion objective with a numerical aperture of 1.4. Laser lines at 568 nm were used for excitation. Acquisition was performed with the UltraVIEW RS software (PerkinElmer); a series of 10 optical sections was taken at intervals of 0.2 \(\mu\)m. Densitometric analysis was performed with the UltraVIEW RS software (PerkinElmer), measuring the average fluorescence intensity of regions of interest in single focal-plane images. The measurements for 6 to 12 cells are shown with median and SDs. B. Paired, two-tailed Student \(t\) test was applied to the data for statistical analysis yielding significant differences in the staining intensity of Bam32 on the contact sites of DC–T cell cocultures (DC–T contact site) compared with that of the membrane staining outside the contact site (DC–T membrane) or that of the staining in the cytosol of DC in DC–T cell cocultures (DC–T cytosol). No differences were seen in DC cultures without T cells between membrane staining (DC membrane) and staining in the cytosol (DC cytosol). C. In addition, significant differences were obtained in the staining intensity of Bam32 on the membrane compared with that in the cytosol in MHC class I immobilized mature DC, but not in MHC class II immobilized mature DC.
FACS analysis of CD4<sup>+</sup>CD25<sup>+</sup>FOXP3<sup>+</sup> T cells

T cells from a large-scale MLR (1.5 × 10<sup>6</sup> DC and 1 × 10<sup>6</sup> allogeneic T cells per well were cocultured for 5 d) were collected and stained with a fixable dead cell stain kit (LIVE/DEAD; Invitrogen, Molecular Probes, Eugene, OR), followed by staining with Human Regulatory T Cell Staining Kit No. 2 (eBioscience, San Diego, CA). Cells were analyzed on a FACSCalibur (Becton Dickinson) and data handled with FlowJo software. T cells were gated for LIVE/DEAD negative and CD4 positive.

Results

**Bam32 is strongly induced during DC maturation in monocyte-derived DC and is expressed in ex vivo-cultured skin DC**

To verify the induction of Bam32 expression during the maturation of DC, RNA from immature and mature monocyte-derived DC, B cells and T cells prepared from the same donor, as well as from Langerhans cells and dermal DC collected from skin organ cultures were subjected to RT-PCR. mRNA levels coding for Bam32 relative to TATA binding protein show a clear increase of Bam32 mRNA in mature monocyte-derived DC and the presence of transcripts in DC derived from skin. When we compared expression levels of Bam32 in B cells, T cells, and immature and mature monocyte-derived DC from the same donor, we found the levels of Bam32 to be equal or higher in mature monocyte-derived DC than in B cells or T cells (Fig. 1).

**Bam32 localizes to DC–T cell contact sites and moves to the membrane upon Ab-mediated MHC class I engagement**

For a first glance at a possible involvement of Bam32 in DC function, we stained cells from a DC–T cell coculture with anti-Bam32 mAb. In some DC–T cell aggregates, accumulation of Bam32 near the contact site was visible (Fig. 2 A, left panel, and Supplemental Fig. 1). This accumulation was measured with grayscale quantification. Statistical analyses (paired, two-tailed Student t test) show significant accumulation of Bam32 at the contact sites (Fig. 2 B). To evaluate the interaction site between DC and T cells at the molecular level, we immobilized DC treated with mAbs to several different surface molecules on glass slides coated with poly-L-lysine (PLL). Of all Abs tested, only the anti-MHC class I Ab but not Abs directed against MHC class II or PLL alone (Fig. 2 A and Supplemental Fig. 2 C) or Abs directed against ICAM, CD11c, VCAM, CD43, or CD45 (Supplemental Fig. 2 A, 2B) induced the localization of Bam32 to the membrane. The accumulation of Bam32 to the membrane in response to MHC class I (HLA-ABC) Ab-mediated binding was measured by grayscale quantification and found to be significantly higher than in the cytosol, as opposed to membrane accumulation in response to MHC class II (HLA-DR) Ab (Fig. 2 C).

**Silencing of Bam32 expression boosts CD8<sup>+</sup> T cell stimulation**

For a functional analysis of Bam32, we silenced the expression of Bam32 in monocyte-derived DC prior to maturation induction. We controlled both DC populations for maturation marker expression but found no differences (Supplemental Fig. 3). We then compared the T cell stimulatory capacity of DC silenced for Bam32 expression with that of DC treated with a nontarget control. For this, we loaded DC with an MHC class I-restricted EBV-peptide to measure Ag-specific proliferation of CD8<sup>+</sup> T cells. Higher stimulation of CD8<sup>+</sup> T cells was achieved with DC silenced for Bam32 expression (Fig. 3), indicating an involvement of Bam32 in the limitation machinery of T cell stimulation.

**Bam32 binds to galectin-1 in mature DC**

To get a hint about how Bam32 might be involved in the signaling pathway for T cell stimulation in mature DC, we searched for a binding partner for Bam32 in a cDNA library prepared from mature DC using Bam32 as the bait. We isolated 16 clones, 14 of them coded for galectin-1. These 14 clones comprised three different sequence parts, overlapping in a 74-nucleotide region (Fig. 4A). We hypothesize that the binding site of Bam32 to galectin-1 lies within this region. To check for the presence of galectin-1 in DC, we first performed Western blot analysis of DC lysates. We could clearly detect galectin-1 in mature and immature monocyte-derived DC using an anti–galectin-1 mAb. To verify interaction of Bam32 and galectin-1, commmunoprecipitation in lysates prepared from mature and, as a control, from immature DC using anti–galectin-1 polyclonal Ab for immunoprecipitation and anti-Bam32 mAb for detection was performed. We clearly detected Bam32 in the precipitate isolated from mature DC lysates with anti–galectin-1 rabbit polyclonal Ab but not in lysates from immature DC or in

**FIGURE 3.** T cell proliferation in response to mature monocyte-derived DC silenced or not for Bam32. Expansion of Ag-specific CD8<sup>+</sup> T cells in response to EBV-peptide loaded DC silenced (siBam) or not (nontarget) for Bam32 are shown for five separate experiments. Data were statistically analyzed using paired, two-tailed Student t test. The proliferative response of Ag-specific CD8<sup>+</sup> T cells was higher when mature monocyte-derived DC silenced for Bam32 were used as stimulators.

**FIGURE 4.** Bam32 commmunoprecipitates with galectin-1. A, Fourteen clones of galectin-1 were isolated from a cDNA library derived from mature DC using Bam32 as bait. These 14 clones consisted of three different sequence parts, overlapping in a 75-bp-long region, which therefore we hypothesize to contain the binding site for Bam32. B, Forty micrograms of whole-cell lysates of immature monocyte-derived DC (iDC) and mature monocyte-derived DC (mDC) and the galectin-1 Ab-immunoprecipitate from 1 mg of mDC and iDC lysate were stained with anti-human Bam32 mAb and visualized with Alexa Fluor 680 goat anti-mouse Ab. As a negative control, preimmune rabbit serum was used for immunoprecipitation. Bam32 is expressed in mDC and coprecipitates with galectin-1 from mDC lysates but not from iDC lysates and cannot be commmunoprecipitated with the rabbit control serum.
the control immunoprecipitation using preimmune rabbit serum. This indicates an interaction of the two molecules in mature DC (Fig. 4B).

**Galectin-1 colocalizes to the plasma membrane with Bam32 upon binding of mature DC to anti-MHC class I Ab**

To evaluate whether the Bam32–galectin-1 interaction in DC has a functional consequence, we investigated the relocalization to the membrane of both molecules upon treatment of mature DC with anti-MHC class I Abs. We stained mature DC treated with anti-MHC class I Abs and immobilized on PLL-coated glass slides with both anti-Bam32 mAb and anti–galectin-1 mAb followed by secondary Abs conjugated to Alexa 488 and Alexa 647, respectively. In Fig. 5A, the colocalization of both molecules is indicated in the overlapping of red and green fluorescence on the rim of the cells. Fig. 5B shows staining of non-relocalized Bam32 and galectin-1 in the cytosol of untreated mature DC immobilized on PLL.

**Bam32 silencing reduces the induction of FOXP3+ regulatory T cells or their proliferation by mature DC**

To follow up the boosted T cell stimulatory capacity of DC silenced for Bam32 expression in light of a reported function of galectin-1 (26, 27), we investigated the role of Bam32 in the induction of regulatory T cell (Treg) proliferation. We measured the percentage of CD25+FOXP3+ T cells in the CD4+ population after 6 d of coculture with mature DC or mature DC silenced for Bam32 and found a small but consistent and significant reduction (see Fig. 6A for the summary of eight individual experiments and Fig. 6B for an exemplary result). To strengthen this result further, we also performed real-time RT-PCRs for FOXP3 transcripts (Fig. 6C).

**FIGURE 5.** Colocalization of endogenous Bam32 and galectin-1 at the cell membrane upon MHC class I engagement. A and B, Glass slides were coated with PLL before adding DC treated with a mouse anti-human MHC class I mAb (A) or not (B). Mature monocyte-derived DC were allowed to adhere for 20 min before fixing with acetone. Bam32 staining was with rat anti-human Bam32 mAb 3E10 visualized with Alexa Fluor 568 goat anti-rat Ab (red fluorescence). Galectin-1 staining was with mouse anti-human galectin-1 mAb visualized with Alexa Fluor 488 goat anti-mouse Ab (green fluorescence). Isotype controls remained negative (right panels). Images were acquired with a 100× oil immersion objective with a numerical aperture of 1.4. Laser lines at 488 nm and 568 nm were used for excitation. Only in DC treated with anti-MHC class I Ab do both Bam32 and galectin-1 localize at the membrane.

**FIGURE 6.** Induction of Treg by DC is reduced upon silencing of Bam32. Bam32-silenced mature monocyte-derived DC (siBam; 10^5) or mature monocyte-derived DC treated with a nontarget siRNA (10^5) were cocultured with 10^6 bulk T cells for 5 d. A, Individual cocultures were collected and stained for CD4, CD25, and FOXP3. Gating was on LIVE/DEAD-negative, CD4+ cells. Percentage of CD25+/FOXP3+ cells in the CD4+ population are shown. The results from seven individual experiments are shown. Data were statistically analyzed using paired, two-tailed Student t test. B, Individual FACS analyses of one of the experiments are shown for T cells cocultured with mature monocyte-derived DC treated with a nontarget siRNA (nt) or with a Bam32-specific siRNA (siBAM). C, Individual cocultures were collected and RNA was prepared and subjected to RT-PCR for FOXP3. Amounts of mRNA relative to 18S RNA are shown. The results from six individual experiments are shown. Data were statistically analyzed using paired, two-tailed Student t test. Silencing of Bam32 in mature monocyte-derived DC reduces the number of FOXP3-expressing T cells after 5 d of coculture.
Again, FOXP3 expression in T cells stimulated with mature DC silenced for Bam32 was significantly decreased.

Discussion

The strong induction of Bam32 mRNA during the maturation of monocyte-derived DC and the level of expression equivalent to or higher than that of B cells (Fig. 1) points to a prominent role for Bam32 in DC. When mature monocyte-derived DC are cocultured with allogeneic T cells in a part of the formed aggregates endogenous Bam32 accumulates near the contact site (Fig. 2A, left panel). The activation of T lymphocytes is mediated by the interaction of the TCR with its ligand, the MHC–peptide complex (28). The stabilization of these clusters is supported through different adhesion molecules. Considering our results on the relocation of Bam32 to the contact sites between DC and T cells, we assumed a role for this molecule during DC–T cell interaction. Therefore, we stimulated DC via some of the molecules forming the immunological synapse to induce the observed translocation of Bam32 to the membrane in a better-defined situation. The strongest localization to the membrane was achieved by Ab-mediated binding of MHC class I molecules (Fig. 2). MHC class I molecules are important signal-transducing molecules involved in the finely tuned regulation of immune responses. The downstream events of Ab-mediated engagement of MHC class I molecules have mainly been studied in the context of apoptosis induction and are still poorly understood (29). Based on our results, we hypothesized an involvement of the adapter molecule Bam32 in the downstream events of MHC class I engagement in mature DC. To find possible functional consequences of the involvement of Bam32 in MHC class I signaling, we measured the stimulation of EBV-peptide specific CD8+ T cells by DC silenced or not for Bam32 expression. After thorough controls with respect to possible side effects of the silencing procedure (Supplemental Fig. 2), we found higher proliferation of Ag-specific CD8+ T cells stimulated with DC silenced for Bam32 expression (Fig. 3). As an approach to characterize further the role of Bam32 in DC on the molecular level, we searched for an interaction partner of Bam32 and isolated 14 clones coding for β-galactoside–binding protein (GBP/galectin-1, consisting of three different partial sequences (Fig. 4A) overlapping in a 72-bp-long region. This overlapping region of the three cDNA species isolated probably contains the interaction site with Bam32, as the proteins produced by these clones are all able to bind Bam32. We verified this interaction with coimmunoprecipitation (Fig. 4B). Indirect proof for an interaction of the endogenous Bam32 with endogenous GBP/galectin-1 in mature DC comes from immunofluorescent microscopy data showing a colocalization of Bam32 and GBP/galectin-1 to the membrane in DC–T cell cocultures and in DC stimulated with anti-MHC class I Abs (Fig. 5A). Galectins have a broadly diverse array of activities inside and outside of cells (30). Different findings point to roles of galectins in cancer, immunity and inflammation, and development. They are found in a variety of cell types, including fibroblasts, thymic epithelial cells, endothelial cells, DC, macrophages, T and B cells, and bone marrow stromal cells (31–33). In DC, expression of transgenic galectin-1 has been shown to delay the onset of autoimmune diabetes (34). Galectin-1 is expressed in Treg, and the monomeric form of galectin-1, GBP, the monomeric form of galectin-1, has been shown to be used by human Treg to inhibit the proliferation of CD8+ T cells in response to self-antigen. This was proposed as a mechanism for the control of peripheral tolerance to self-antigens (40). This raises the possibility that DC use Bam32 as a mechanism to control the secretion of GBP/galectin-1 for regulating the activation of CD8+ T cells either directly or via Treg proliferation induction.

Our results on the functional characterization of Bam32 in mature DC implicate a role for this molecule in MHC class I-mediated signaling, probably via binding of GBP/galectin-1, leading to the regulation of the stimulatory capacity of mature DC for CD8+ T cells.

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Disclosures

The authors have no financial conflicts of interest.

References


Corrections


In the abstract, the phrase “T cell death” is incorrect in the next to the last sentence, which reads “It is distinct from that of MHC I recognized by CD8⁺ T cells leading to T cell death.” The sentence should read, “It is distinct from that of MHC I recognized by CD8⁺ T cells leading to target cell death.”

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