Engagement of SLAMF2/CD48 Prolongs the Time Frame of Effective T Cell Activation by Supporting Mature Dendritic Cell Survival

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Engagement of SLAMF2/CD48 Prolongs the Time Frame of Effective T Cell Activation by Supporting Mature Dendritic Cell Survival

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Signaling lymphocytic activation molecule family (SLAMF)2/CD48 is a coactivator and adhesion molecule on cells of hematopoietic origin. It ligates mainly SLAMF4 on effector/memory CD8+ T cells and NK cells, suggesting a potential role during viral infection, with SLAMF2 acting as a ligand to activate SLAMF4-bearing cells. The ability of SLAMF2 to signal on its own after it is engaged and the functional consequences are largely unknown. We found that cytosolic DNA-activated dendritic cells (DCs) upregulate the expression of SLAMF2 molecules. Using anti-SLAMF2 Ab and SLAMF4 recombinant protein, we found that SLAMF2 engagement activates immature DCs and, more interestingly, prolongs the survival of DNA-activated DCs by inhibiting IFN-β production and IFN-β–induced apoptosis and promotes the production of the granzyme B inhibitor protease inhibitor-9. Thus, SLAMF2 can serve as a survival molecule for DNA-activated DCs during their interaction with SLAMF4-expressing cytotoxic T cells. Based on our results, we propose that SLAMF2 engagement regulates adaptive immune responses by providing longer access of putative APCs to virus-specific effector T cells by prolonging the time frame of effective stimulation. The Journal of Immunology, 2014, 192: 4436–4442.

Antigen presentation by dendritic cells (DCs) is critical for the induction of immune responses and for the differentiation and expansion of effector and memory T cells (1). DCs are sensitive to signals originated from pathogens as well as from other immune cells. The balance of these signals ultimately determines DC activation, cytokine secretion, migration, survival, and the nature and magnitude of the immune response. DCs are essential in the CD8+ T cell immune response that developed to specifically eliminate infections and to provide lasting protection against reinfection (2). DCs prime naive CD8+ T cells to various pathogens (3–5) and play a significant role in the generation of an effective effector CD8+ T cell response and in the production of memory CD8+ T cells (6–8). DCs are also responsible for memory CD8+ T cell maintenance and reactivation (9–13). CD8+ T cell–dependent killing of Ag-presenting DCs has been described through perforin/granzyme B secretion, and it represents a negative feedback mechanism to attenuate immune responses (14). However, the phenomenon of memory CD8+ T cells protecting DCs from CTL killing has also been described (2, 15–17), and granzyme B inhibitor serine protease inhibitor-6 (SPI-6) (human ortholog protease inhibitor-9 [PI-9]) has been shown to protect DCs against cytotoxicity (18, 19). The question though how DCs are able to present viral Ags to CTLs without themselves being killed through contact-mediated cytotoxicity has remained largely elusive, especially in human cells.

Myeloid DCs produce large amounts of IFN-β in response to infection signals through TLR3 and TLR4 (20) or through cytosolic receptors activated by nucleic acids (21), especially by dsDNA, which can serve as a mimic of viral infection (22). Production of IFN-β is part of a defense mechanism to induce an antiviral state that prevents productive viral infection and to modulate cell viability and function (23, 24). IFN-β has been reported to induce apoptosis in certain cell types (25–28) and specifically in mouse DCs (29), where IFN-β activates the caspase-11/caspase-3 apoptotic pathway. How human DCs are able to escape from autocrine IFN-β–induced apoptosis during Ag presentation to CTLs remains an unanswered question.

Signaling lymphocytic activation molecule family (SLAMF)2/CD48 is a GPI-anchored protein found on the surface of several hematopoietic cells and serves as an adhesion and costimulatory protein (30). SLAMF2 is a ligand for the immunoreceptors CD2 and SLAMF4 (CD244/2B4). Despite the absence of a cytoplasmic domain, SLAMF2 initiates a potent signaling cascade comparable to that of other immune-regulating molecules (31). However, the SLAMF2–SLAMF4 interaction is bidirectional, and most studies have focused on SLAMF2 as a ligand of SLAMF4 on NK cells and CD8+ effector/memory T cells reporting either activator or inhibitory effects (26, 32–35).

In this study, we investigated the role of SLAMF2 in dsDNA-activated human DCs (DNA-DCs), a model of viral infection. We found that SLAMF2 engagement results in the activation of immature DCs (IDCs). More interestingly, to our knowledge, we showed for the first time that this engagement acts as a survival factor by protecting mature DNA-DCs from cell death first by inhibiting the autocrine production of apoptosis-inducing IFN-β, and second, by promoting the production of the granzyme B inhibitor PI-9, and protects against cytotoxicity by CTLs. Thus, SLAMF2 ligation in mature DCs may empower and prolong the potent APC-dependent T cell activation during viral infection.
Materials and Methods

Generation of monocyte-derived DCs, cell culture, and treatments

Human monocyte-derived DCs were generated from CD14+ blood monocytes isolated from PBMCs separated from buffy coats by Ficoll-Paque (Fisher, Pittsburgh, PA) gradient centrifugation (36), followed by positive selection with anti-CD14-coated magnetic beads (Miltenyi Biotec, Auburn, CA). Purified CD14+ monocytes (≥95%) were plated at 2 × 10^5 cells/ml concentration and cultured in RPMI 1640 medium (Sigma-Aldrich, St. Louis, MO) supplemented with 2 mM glutamine, 100 U/ml penicillin, and 100 g/ml streptomycin in the presence of 100 ng/ml IL-4 and 75 ng/ml GM-CSF (PeproTech, Rocky Hill, NJ) on days 0 and 2 and in a specific experiment on days 2 and 4 of extended culturing. Activation of IDCs was induced on day 5 by poly(α:dT) (2.5 μg/ml) complexed with LyoVec transfection reagent (Invivogen, San Diego, CA) for 24 h or for the entire period of culture. For the anti-SLAMF2 treatment, 5 μg/ml BJ40 Ab (BioLegend) was coated or added soluble to IDCs or together with poly(α:dT) on day 5 for the indicated time points. In some experiments, 5 μg/ml recombinant human SLAMF4-Fc fusion protein or control protein (Sino Biological Beijing) was added to the cells for 24 h.

In certain experiments, IDCs and DNA-DCs were treated with the IFN-β blocking Ab mixture anti–IFN-β and anti-IFNRA (PBL IFN Source, Piscataway, NJ) or purified mouse IgG1 and rabbit IgG isotype control Abs for the indicated time points. The Abs were left in the culture during the subsequent treatment.

The IFNs IFN-α, IFN-β, and IFN-γ were purchased from PeproTech and used in the indicated concentrations for 24 h.

Flow cytometry

The identification of DC and T cell activation was monitored by flow cytometric analysis using fluorochrome-conjugated anti-SLAMF2, anti-CD80, anti-CD83 and anti-CD86 (DCs), anti-CD3, anti-CD25, and anti-CD69 (T cells) Abs as compared with isotype-matched control Abs (BioLegend). To check cell viability, 7-amino-actinomycin D (7AAD) staining was used, and the positive cells were excluded as dead. Fluorescence intensities were measured and analyzed by FACS Calibur flow cytometer (BD Biosciences Immunometry Systems, Franklin Lakes, NJ). Data analysis was performed using the FlowJo Flow Cytometry Analysis software (Ashland, OR).

Real-time quantitative RT-PCR

Real-time PCR was performed, as described previously (37). Briefly, total RNA was isolated from DCs by RNeasy Mini Kit (Qiagen, Valencia, CA). Reverse transcription was performed at 37˚C for 120 min from 100 ng total RNA using the High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). Quantitative real-time PCR for SLAMF2, CCR7, matrix metalloproteinase (MMP)-9, MMP-12, tissue inhibitor of MMP (TIMP)-2, TRAIL, IFN-β, and PI-9 genes were performed (Light Cycler 480; Roche, Indianapolis, IN) with 40 cycles at 94˚C for 12 s and 60˚C for 60 s using TaqMan assays (Applied Biosystems). All PCRs were run in triplicates with a control reaction containing no reverse-transcriptase enzyme. The OD of the wells was determined in a final volume of 600 μl. DCs were added to the upper chamber in a final volume of 250 μl and chemotaxis assays were conducted for 4 h in 5% CO2 at 37˚C. At the end of the assay, the inserts were discarded and cells migrated to the lower chamber were collected. Migrated cell numbers were counted by using polystyrene standard beads (Sigma-Aldrich) by flow cytometry.

Migration

DCs were suspended in migration medium (0.5% BSA in RPMI 1640) at 10^6 cells/ml. Transmigration inserts (diameter 6.5 mm; pore size 5 μm) were obtained from Sigma-Aldrich. MMP-β chemokine (PeproTech) was diluted to 200 ng/ml in migration medium and added to the lower chambers in a final volume of 600 μl. DCs were added to the upper chamber in a final volume of 250 μl. The Transwell was incubated at 37˚C for 24 h. At the end of the assay, the inserts were discarded and cells migrated to the lower chamber were collected. Migrated cell numbers were counted by using polystyrene standard beads (Sigma-Aldrich) by flow cytometry.

Transfection of small interfering RNA

A mix of three different constructs of IFI16 small interfering RNAs (siRNAs) and control siRNAs (Applied Biosystems) was transfected into DCs on the third day of differentiation to a final concentration of 2.5 nM using the GenePulsar X Cell electroporator and 0.4-cm cuvettes (Bio-Rad, Hercules, CA). After 2 additional days, the knockdown of the IFI16 gene was tested by quantitative RT-PCR.

Mixed leukocyte reaction

DCs were differentiated from monocytes, as mentioned previously, and labeled with CFSE. IDCs and DNA-activated DCs were cocultured with blood purified, and sorted SLAMF4+ naïve or SLAMF4+ effector/memory CD8+ T cells in 1:4 ratio for 4 d and the viability of the CFSE-labeled DCs were measured by flow cytometry using 7AAD staining to exclude necrotic cells.

Statistical analysis

Paired Student one- and two-tailed t tests were used (*p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005). The statistical calculations compared experimental samples with the IDC or nontreated samples, unless noted otherwise. For all experiments, the mean and the SD are reported for at least n = 3 independent experiments.

Results

SLAMF2 expression is upregulated by cytosolic DNA activation in DCs

To study the role of SLAMF2 on DCs, first we investigated the expression levels of SLAMF2 on monocytes, IDCs, and DNA-stimulated DCs. SLAMF2 expression was found to be high in monocytes (0-h time point in Supplemental Fig. 1A), but it was rapidly downregulated following incubation with IL-4 and GM-CSF (monocyte-derived IDCs) (Supplemental Fig. 1A). Yet, stimulation with DNA resulted in a significant upregulation of SLAMF2 mRNA (Fig. 1A) and a moderate upregulation of the cell surface expression of SLAMF2 protein (Fig. 1B). Higher SLAMF2 expression was recorded on the surface of proinflammatory-prone CD1a+ subpopulation of DCs compared with the rather inhibitory CD1a- cells (Fig. 1D) (38). Because cytosolic DNA-activated DCs produce high amount of IFN-β (22), we tested whether IFN-β itself can induce SLAMF2 expression. Treatment of cells with type I (IFN-α or IFN-β) but not with type II IFN-γ induced SLAMF2 gene expression (Supplemental Fig. 1B). Previously, we had identified IFI16 as a key intracellular DNA receptor responsible for the activation of DCs by cytosolic dsDNA (22). Silencing of IFI16 in DNA-DCs (IDC: 79 ± 13%, DNA-DC: 82 ± 9% knockdown efficiency compared with control siRNA) inhibited the upregulation of SLAMF2 expression (Fig. 1D), demonstrating the role of this receptor in the proper DC activation and in the regulation of SLAMF2 expression by cytoplasmic DNA, possibly, through IFN-β.

SLAMF2 engagement activates DCs

After characterizing the expression of SLAMF2, we investigated the function of SLAMF2 on DCs. To model the SLAMF2–SLAMF4 interaction, we used a mAb recognizing SLAMF2 (anti-human SLAMF2 Ab [αSF2]) and found that it activated IDCs in a dose-dependent manner (Supplemental Fig. 2A) as determined by the cell surface expression of the activation molecules CD80, CD83, and CD86 (Fig. 2A) and by the production of proinflammatory cytokines IL-6 and TNF-α (Fig. 2B). SLAMF2 engagement enhanced also the cytosolic DNA activation of DCs; however, this effect was not significant (Fig. 2A, 2B). Cell migration toward the CCR7 ligand MMP-β is an indicator of DC maturation. αSF2-treated IDCs migrated significantly better compared with control IgG-treated cells, whereas Ab treatment had an enhancing effect on DNA-DC migration (Fig. 2C). Increased migration correlated with significantly increased CCR7 and MMP-9 expression. In contrast, the MMP inhibitor TIMP-2 levels were downregulated upon αSF2 treatment (Fig. 2D). SLAMF2 engagement enhanced the capacity of...
DCs to stimulate T cells as aSF2-IDCs were able to significantly upregulate IL-2 production of cocultured T cells (Fig. 2E). To demonstrate that the DC-activating effect of aSF2 is specific, we used a recombinant SLAMF4 protein to treat IDCs. Significant DC activation was measured when this recombinant SLAMF4 protein was present in IDC cultures, indicating that SLAMF2 ligation has an activating effect on DCs (Supplemental Fig. 2B, 2C).

SLAMF2 engagement selectively promotes the survival of mature DCs

DCs are terminally differentiated cells unable to proliferate, and their destiny is to mature, activate T cells, and eventually die (39). Because the survival of DCs is connected to their activation status, in the next set of experiments the cell death of DCs was investigated. DCs were activated with cytosolic DNA or were left nonactivated and were treated with plate-bound aSF2 or nonspecific IgG. DC cell death was investigated by flow cytometry using 7AAD staining to exclude necrotic cells. The DNA-DC population showed increased cell death compared with IDCs (Fig. 3A). SLAMF2 engagement though significantly reduced the cell death of DNA-DCs at days 4 and 6 of culture (Fig. 3A). Therefore, SLAMF2 engagement rescues DNA-DCs from excessive cell death, resulting in the survival of the mature DC population.

FIGURE 1. SLAMF2 is upregulated on mature DCs. (A) mRNA expression (n = 10) and (B) cell surface expression (n = 5) of SLAMF2 on IDCs and on DNA-DCs activated by transfection of poly(dA:dT) for different time points. (C) Histograms show the distribution of SLAMF2 molecules on the surface of CD1a+ and CD1a– IDC and DNA-DC populations (n = 3, one representative experiment shown). (D) mRNA expression of SLAMF2 in IDCs and DNA-DCs treated with IFI16 or control siRNAs (n = 3). *p ≤ 0.05, ***p ≤ 0.005.

FIGURE 2. SLAMF2 engagement by specific Ab activates DCs. IDCs and DNA-DCs were treated with 5 μg/ml immobilized mouse control IgG or aSF2 for 24 h. (A) Expression of cell surface activation molecules CD80, CD83, and CD86. (B) Production of pro-inflammatory cytokines IL-6 and TNF-α. (C) MIP3-β–induced cell migration of DCs. (D) Gene expression of migration-related molecules CCR7, MMP-9, and TIMP-2. (E) IgG- or aSF2-pretreated IDCs and DNA-DCs were cocultured with total T cells for 24 h (IL-2) or for 72 h (IFN-γ), and the cytokine levels were measured from supernatants. n = 3, *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005.
SLAMF2 engagement protects mature DCs from cell death by inhibiting IFN-β production and caspase-4 expression

Next, we investigated the molecular events that result in increased survival of DNA-DC following engagement of SLAMF2. In our previous communication, we showed that DNA-DCs are able to produce large amounts of IFN-β compared with other mature DCs and IDCs (22). Because IFN-β is able to induce apoptosis of DCs (24, 29), we investigated whether IFN-β contributed to the death of DNA-stimulated DCs. Indeed, because of the presence of an Ab against the IFN receptor (IFNR) A and an Ab against IFN-β, the DNA-DC population became significantly more viable compared with those treated with control Ab (Fig. 3B). That the Abs used were functional was confirmed by demonstrating decreased mRNA expression (Supplemental Fig. 3A) and protein secretion (Supplemental Fig. 3B) of the IFN-β-inducible gene TRAIL.

Next, we tested whether SLAMF2 engagement is able to alter the production of IFN-β by DNA-DCs. Treatment of DNA-DCs with aSF2 (Fig. 3C) or SLAMF4 protein (Fig. 3D) significantly decreased IFN-β gene expression compared with controls. Treatment with aSF2 also significantly decreased IFN-β protein production (Fig. 3D) compared with the non-specific IgG-treated DNA-DCs. We measured the expression and production of TRAIL to track the effect of IFN-β inhibition and found significant downregulation of gene (Supplemental Fig. 3B) and protein levels (Supplemental Fig. 3C) of TRAIL following aSF2 treatment compared with the control IgG-treated group.

IFN-β induces mature DC apoptosis through caspase-11/caspase-3 activation (29). The human ortholog of caspase-11 is caspase-4, so in the next set of experiments we measured the expression of caspase-4 in DCs. DNA-DCs upregulated caspase-4 expression after 24–48 h of activation by cytosolic DNA compared with IDCs, and aSF2 (Fig. 4A) or SLAMF4 protein (Supplemental Fig. 3E) treatments were able to significantly inhibit the elevated expression of caspase-4. Similarly, using IFN-β blockade, the upregulation of caspase-4 was inhibited significantly (Fig. 4B).

Collectively, SLAMF2 engagement protects DCs from cell death by inhibiting the production of IFN-β and the subsequent expression of caspase-4.

SLAMF2 engagement protects mature DCs from cell death by promoting the expression and secretion of PI-9

Although we demonstrated that DNA-DCs escape the autocrine IFN-β–induced cell death by SLAMF2 engagement provided in vivo by SLAMF4+ effector/memory CD8+ T cells, it remains unknown how they escape the cytotoxicity by activated killer CD8+ T cells. Murine DCs produce SPI-6, which protects them against cytotoxicity by inhibiting granzyme B (18, 19). Accordingly, we measured the expression and secretion of the human ortholog of SPI-6, PI-9, by DCs. Indeed, IDCs and DNA-DCs treated with aSF2 (Fig. 4C) or with SLAMF4 protein (Supplemental Fig. 3F) displayed a rapid upregulation of PI-9 gene expression compared with controls. Similarly, protein secretion of PI-9 was significantly upregulated by aSF2 treatment compared with the IgG-treated controls (Fig. 4D). Based on these data, we conclude that DNA-activated DCs escape granzyme B–induced cell death by producing the inhibitor molecule PI-9.

**FIGURE 3.** SLAMF2 engagement rescues mature DCs from cell death by inhibiting the production of IFN-β and the expression of caspase-4. IDCs and DNA-DCs were treated with 5 μg/ml immobilized control IgG or aSF2 for the indicated time points. (A) Viable cell percentages of day 2, 4, and 6 DC cultures measured by flow cytometry using 7AAD staining to exclude necrotic cells (n = 5). (B) Viable cell percentages of day 2, 4, and 6 DC cultures treated with control Abs or with Ab mixture against IFN-β and IFNRA receptor (aIFNb, n = 3). (C) Relative IFN-β mRNA expression measured on the indicated time points of IgG- or aSF2-treated IDCs and DNA-DCs by quantitative PCR (n = 5). (D) Relative IFN-β mRNA expression measured on the indicated time points of control or SLAMF4 protein-treated IDCs and DNA-DCs by quantitative PCR (n = 3). (E) IFN-β production of the aSF2-treated DCs was measured by ELISA on the indicated time points from cell supernatants (n = 3). *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.005.
SLAMF4-bearing CD8+ T cells can provide a survival signal to DNA-activated DCs

Finally, we wished to determine the physiologic effect of SLAMF2 engagement on DCs by SLAMF4 expressed on T cells. To this end, we cocultured sorted blood-derived SLAMF4 naive or SLAMF4+ effector/memory CD8+ T cells with DNA-activated DCs, and the viability of DCs was detected 2 and 4 d later. Whereas SLAMF4 naive T cells had no effect on DC survival, we found that SLAMF4+ T cells were able to significantly prolong DC survival (Fig. 4C).

Collectively, these data support that DNA-DC/CD8+ T cell interaction though SLAMF4/SLAMF2 results in prolonged DC survival (Fig. 5).

Discussion

In this communication, we present evidence that SLAMF2 on human DCs serves not only as stimulatory molecule for immature DCs, but, more importantly, as a survival molecule protecting mature DCs from cell death during antiviral immune responses.

Virus invasion requires the rapid response of the immune system to inhibit the spreading of the infection. Cell death is an effective strategy to limit intracellular infections. The killing of infected cells by CD8+ T cells therefore is critical for immunity (19). DCs are the most potent APCs that stimulate both naive CD8+ T cells and memory CD8+ T cells to differentiate into CTLs (3, 11). By presenting the viral Ag to CTLs, DCs flag themselves as infected and serve as potential targets of cytotoxicity. Moreover, during the encounter with the pathogen, DCs become activated and produce large amounts of type I IFNs (predominantly IFN-β) to protect the neighboring cells from the infection, but meanwhile, they activate the IFN-β–induced apoptotic program. Thus, to fulfill their role as APCs, DCs need to develop effective protection against cell death.
DC maturation stimuli usually serve as survival signals. For example, CpG DNA upregulates the expression of the cellular inhibitor of apoptosis 1 and 2, whereas it downregulates the levels of active caspase-3 in a PI3K-dependent manner (41); HIV-infected DCs become resistant to NK-induced TRAIL-mediated apoptosis due to the upregulation of the cellular Flice-like inhibitory protein and cellular inhibitor of apoptosis 2 (42); LPS activates ERK, which regulates DC survival (43). Interestingly, we found elevated rates of cell death in DNA-DCs compared with IDCs, which was the consequence of the autocrine production of IFN-β. Mouse caspase-11, an ortholog of human caspase-4, is induced in hematopoietic cells by LPS and IFNs and activates caspase-1 and caspase-3 (44, 45). We found increased gene expression of caspase-4 in DNA-DCs compared with IDCs, explaining the excessive cell death of these mature DCs. Elevated caspase-4 expression was found to be inhibited by αSF-2 treatment and by blocking the IFN-β pathway, indicating that the autocrine production of IFN-β activates the pyroptotic pathway of cell death possibly through the AIM2 and/or IFI16 cytosolic DNA receptors (46). The effects of IFN-β on mature DC apoptosis could be controlled temporarily by SLAMF2 engagement during the intercourse of DCs and CD8+ T cells. To our knowledge, this is the first study to show the role of caspase-4 in IFN-β–induced cell death of human cells.

The virus-infected DCs need to fulfill two requirements to be eligible for SLAMF2-mediated survival, as follows: 1) they have to be matured enough to properly present Ag to T cells, and 2) they should be present at the right place to be able to make interactions with CD8+ CTLs and possibly NK cells that provide survival factors during contact with SLAMF2+ CD8+ cytotoxic T cells.

In the series of experiments presented above, to our knowledge, we show for the first time that SLAMF2 molecules serve as survival factors during contact with SLAMF4+ CD8+ cytotoxic T cells.

Using transfected dsDNA to mimic viral infections in human DCs (DNA-DCs), we previously observed massive amount of IFN-β production and effective CD8+ T cell activation by DNA-DCs (22). Simultaneously with the IFN-β production, DNA-DCs upregulate the expression of SLAMF2 molecules on the cell surface of effector/memory CD8+ T cells inhibits the IFN-β production and promotes the production of PI-9 by mature DCs, thus protecting DCs from either the IFN-β–induced apoptosis or granzyme B–induced cytotoxicity of T cells (4). Meanwhile, SLAMF2–SLAMF4 interaction can promote the cytotoxic effect of SLAMF4+ CD8+ T cells against infected cells.

In the experiment described above, to our knowledge, we show for the first time that SLAMF2 molecules serve as survival factors during contact with SLAMF4+ CD8+ cytotoxic T cells.

Using transfected dsDNA to mimic viral infections in human DCs (DNA-DCs), we previously observed massive amount of IFN-β production and effective CD8+ T cell activation by DNA-DCs (22). Simultaneously with the IFN-β production, DNA-DCs upregulate the expression of SLAMF2 molecules on the cell surface of effector/memory CD8+ T cells. This interaction results in rescuing DNA-DCs from excessive cell death through two distinct pathways: 1) through the inhibition of IFN-β production and IFN-β–induced apoptosis, and 2) by triggering the production of the granzyme B inhibitor PI-9.

SLAM family molecule interactions are difficult to explore because of the complex expression patterns of the members on different cell populations. Moreover, SLAMF2 expression is dynamically regulated; thus, time- and localization-dependent fine-tuning is crucial. The gene expression and protein levels of SLAMF2 seem to be regulated at different magnitudes, which could be due to the complex modulation of molecule that includes surface expression, internalization, and the production of a soluble form of SLAMF2. We showed higher SLAMF2 expression on dsDNA-activated DCs compared with IDCs. These data are in agreement with the original observation of elevated SLAMF2 expression on EBV-infected B cells (40) as well as with our previous work showing that DNA-activated DCs are very potent APCs (22). Moreover, expression differences of SLAMF2 CD1a+ and CD1a− DCs seem to follow the reported differences in their functional properties (38).

**Disclosures**

The authors have no financial conflicts of interest.

**References**


