



Published in final edited form as:

J Immunol. 2014 May 1; 192(9): 4436–4442. doi:10.4049/jimmunol.1302909.

Engagement of SLAMF2/CD48 prolongs the time frame of effective T cell activation by supporting mature dendritic cell survival[#]

Katalin Kis-Toth^{*} and George C. Tsokos

Division of Rheumatology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02115

Abstract

Signaling lymphocyte activation molecule family member 2 (SLAMF2/CD48) is a co-activator and adhesion molecule on cells with hematopoietic origin. It ligates mainly SLAMF4 on effector/memory CD8⁺ T cells and natural killer 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 DCs upregulate the expression of SLAMF2 molecules. Using anti-SLAMF2 antibody 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 antigen presenting cells to virus-specific effector T cells by prolonging the time frame of effective stimulation.

Keywords

dendritic cell; SLAMF2; CD48; SLAMF4; CD244; survival; cytotoxic T lymphocyte

Introduction

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 long-lasting protection against reinfection (2). DCs prime naïve CD8⁺ T cells to

[#]This work was supported by the NIH grant 2P01AI065687-06A1.

^{*}Correspondence: Katalin Kis-Toth, Harvard Medical School, Beth Israel Medical Center, 330 Brookline Avenue, CLS-928, Boston, MA 02115, Tel: 617 735 4176, Fax: 617 735 4170, kkistoth@bidmc.harvard.edu.

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 antigen 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 cytotoxic T cell (CTL) killing has also been described (2, 15–17), and granzyme B inhibitor serine protease inhibitor-6 (SPI-6) (human ortholog PI-9) has been shown to protect DCs against cytotoxicity (18, 19). The question though how DCs are able to present viral antigens 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 double-stranded DNA, 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, 26) (27, 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 antigen presentation to CTLs remains an unanswered question.

Signaling lymphocytic activation molecule family 2 (SLAMF2/CD48) is a glycosyl-phosphatidyl-inositol (GPI)-anchored protein found on the surface of several hematopoietic cells and serves as adhesion and co-stimulatory 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 bi-directional, most studies have focused on SLAMF2 as a ligand of SLAMF4 on natural killer (NK) cells and CD8⁺ effector/memory T cells reporting either activator or inhibitory effects (26, 32–35).

Here, we investigated the role of SLAMF2 in double-stranded DNA-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, 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 antigen presenting cell-dependent T cell activation during viral infection.

Materials and methods

Generation of monocyte-derived dendritic cells, cell culture and treatments

Human monocyte-derived DCs were generated from CD14⁺ blood monocytes isolated from peripheral blood mononuclear cells (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 Biotech, Auburn, CA). Purified CD14⁺ monocytes (95%) were plated at 2×10^6 cell/ml concentration and cultured in RPMI 1640 medium (Sigma, 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) given on days 0 and 2 and in a specific experiment on day 2 and 4 of extended culturing. Activation of IDCs were induced on day 5 by poly(dA:dT) (2.5 μ g/ml) complexed with LyoVec transfection reagent (Invivogen, San Diego, CA) for 24h or for the entire period of culture. For the anti-SLAMF2 treatment 5 μ g/ml BJ40 antibody (Biolegend) was coated or added soluble to IDCs or together with poly(dA:dT) on day 5 for the indicated time points. In some experiments 5 μ g/ml of recombinant human SLAMF4-Fc fusion protein or control protein (Sino Biological Inc. Beijing, China) was added to the cells for 24h.

In certain experiments IDCs and DNA-DCs were be treated with the IFN β blocking antibody cocktail anti-IFN β and anti-IFNRA (PBL Interferon Source, Piscataway, NJ) or purified mouse immunoglobulin G1 and rabbit IgG isotype control antibodies for the indicated time points. The antibodies 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 24h.

Flow cytometry

The identification of DC and T cell activation were 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) antibodies as compared to isotype-matched control antibodies (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 FACSCalibur flow cytometer (BD Biosciences Immunocytometry Systems, Franklin Lakes, NJ). Data analysis was performed using the FlowJo Flow Cytometry Analysis software (Ashland, OR).

Real-time quantitative reverse transcriptase-polymerase chain reaction (Q-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, MMP-9, MMP-12, 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 sec and 60 °C for 60 sec using Taqman assays (Applied Biosystems). All PCR reactions were run in triplicates with a control reaction containing no RT enzyme. The comparative Ct method was used to quantify transcripts relative to the endogenous control gene large ribosomal protein.

Enzyme-linked immunosorbent assay

IL-6, TNF- α , IFN- γ , IL-2 and TRAIL proteins were measured from cell supernatants using DuoSet human immunoassay kits (R&D Systems). IFN β protein was measured using the VeriKine Human IFN Beta ELISA Kit (PBL Interferon Source). PI-9 protein was measured using specific ELISA kit (Antibodies-online, Atlanta, GA). The optical density of the wells was determined using a microplate reader set at 450 nm.

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 μ m) were obtained from Sigma. MIP3- β chemokine (Peprotech) were diluted at 200 ng/ml in migration medium and added to the lower chambers in a final volume of 600 μ l. DCs were added to the upper chamber in a final volume of 250 μ l, and chemotaxis assays were conducted for 4h 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) by flow cytometry.

Transfection of small interfering RNA

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

Mixed leukocyte reaction

DCs were differentiated from monocytes as mentioned previously and labeled with carboxyfluorescein succinimidyl ester (CFSE). IDCs and DNA-activated DCs were co-cultured with blood-purified and sorted SLAMF4⁻ naïve or SLAMF4⁺ effector/memory CD8⁺ T cells in 1:4 ratio for 4 days 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 to the IDC or non-treated samples unless noted otherwise. For all experiments, the mean and the standard deviation (SD) are reported for at least n=3 independent experiments.

Results

SLAMF2 expression is upregulated by cytosolic DNA activation in dendritic cells

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 (0h time point in Fig. S1A) but it was rapidly downregulated following incubation with IL-4 and GM-CSF (monocyte-derived IDCs) (Fig. S1A). 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 pro-inflammatory-prone CD1a⁺ subpopulation of DCs compared to the rather inhibitory CD1a⁻ cells (Fig. 1D)(38). Since 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 (Fig. S1B). Previously we had identified IFI16 as a key intracellular DNA receptor responsible for the activation of DCs by cytosolic double-stranded DNA (22). Silencing of IFI16 in DNA-DCs (IDC: 79 \pm 13%, DNA-DC: 82 \pm 9% knockdown efficiency compared to control siRNA) inhibited the upregulation of SLAMF2 (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 immature dendritic cells

After characterizing the expression of SLAMF2 we investigated the function of SLAMF2 on DCs. To model the SLAMF2-SLAMF4 interaction we used a monoclonal antibody recognizing SLAMF2 (aSF2) and found that it activated IDCs in a dose-dependent manner (Fig. S2A) as determined by the cell surface expression of the activation molecules CD80, CD83 and CD86 (Fig. 2A), and by the production of pro-inflammatory 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 and B). Cell migration towards the CCR7 ligand MIP3 β is an indicator of DC maturation. aSF2-treated IDCs migrated significantly better compared to control IgG-treated cells, while antibody treatment had an enhancing effect on DNA-DC migration (Fig. 2C). Increased migration correlated with significantly increased CCR7 and matrix metalloproteinase (MMP)-9 expression. In contrast, the MMP inhibitor tissue inhibitor of MMP (TIMP)-2 levels were downregulated upon aSF2 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 co-cultured T cells (Fig. 2E). To demonstrate that the DC activating effect of the aSF2 antibody 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 (Fig. S2B and C).

SLAMF2 engagement selectively promotes the survival of mature dendritic cells

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 non-activated and were treated with plate-bound aSF2 or non-specific 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 to 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.

SLAMF2 engagement protects mature dendritic cells from cell death by inhibiting IFN β production and caspase-4 expression

Next, we investigated the molecular events which 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 to 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, the presence of an antibody against the IFN receptor (IFNR) A and an antibody against IFN β the DNA-DC population became significantly more viable compared to those treated with control antibody (Fig. 3B). That the antibodies used were functional was confirmed by demonstrating decreased mRNA expression (Suppl. Fig. 3A) and protein secretion (Suppl. Fig. 3B) of the IFN β -inducible gene TNF-related apoptosis-inducing ligand (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 to controls. Treatment with aSF2 antibody also significantly decreased IFN β protein production (Fig. 3D) compared to 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 (Suppl. Fig. 3B) and protein levels (Suppl. Fig. 3C) of TRAIL following aSF2 treatment compared to 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 24–48h of activation by cytosolic DNA compared to IDCs, and aSF2 (Fig. 4A) or SLAMF4 protein (Fig. S3E) 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 dendritic cells from cell death by promoting the expression and secretion of protease inhibitor-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 serine protease inhibitor-6 (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, protease inhibitor-9 (PI-9), by DCs. Indeed, IDCs and DNA-DCs treated with aSF2 antibody (Fig. 4C) or with SLAMF4 protein (Fig. S3F) displayed a rapid upregulation of PI-9 gene expression compared to controls. Similarly, protein secretion of PI-9 was significantly upregulated by aSF2 treatment compared to 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.

SLAMF4-bearing CD8⁺ T cells can provide a survival signal to DNA-activated dendritic cells

Finally, we wished to determine the physiologic effect of SLAMF2 engagement on DCs by SLAMF4 expressed on T cells. To this end we co-cultured sorted blood-derived SLAMF4⁻ naïve or SLAMF4⁺ effector/memory CD8⁺ T cells with DNA-activated DCs and the viability of DCs were detected 2 and 4 days later. While SLAMF4⁻ naïve 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 through SLAMF4/SLAMF2 results in prolonged DC survival.

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 anti-viral 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 antigen presenting cells that stimulate both naïve CD8⁺ T cells and memory CD8⁺ T cells to differentiate into CTLs (3, 11). By presenting the viral antigen 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 antigen presenting cells, DCs need to develop effective protection against cell death. In the series of experiments presented above we show for the first time that SLAMF2 molecules serve as survival factors during contact with SLAMF4⁺ CD8⁺ cytotoxic T cells.

Using transfected double-stranded DNA 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 to interact with the SLAMF4 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: (a) through the inhibition of IFN β production and IFN β -induced apoptosis, and (b) 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 which include

surface expression, internalization and the production of a soluble form of SLAMF2. We showed higher SLAMF2 expression on double-stranded DNA-activated DCs compared to 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 antigen presenting cells (22). Moreover, expression differences of SLAMF2 CD1a⁺ and CD1a⁻ DCs seem to follow the reported differences in their functional properties (38).

DC maturation stimuli usually serve as survival signals. For example, CpG DNA upregulates the expression of the cellular inhibitor of apoptosis (c-IAP) 1 and 2, while 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 c-IAP2 (42); LPS activates ERK, which regulates DC survival (43). Interestingly, we found elevated rates of cell death in DNA-DCs compared to 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 to IDCs, explaining the excessive cell death of these mature DCs. Elevated caspase-4 expression was found to be inhibited by aSF2 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: (a) they have to be matured enough to professionally present antigen to T cells and (b) they should be present at the right place to be able to make interactions with CD8⁺ CTLs and possibly NK cells which provide SLAMF4 to engage SLAMF2. In this setting our model which depicts SLAMF2 as a survival factor (Figure 5) can explain how effector/memory CD8⁺ SALMF4⁺ T cells are able to activate and rescue the antigen-carrying DCs from killing by the same CTLs that they induce. This in turn will prolong the time frame of effective stimulation of the expanding population of antigen-specific CD8⁺ T cells (2, 15–17).

The IFN β -induced cell death and caspase-11 involvement as well as the role of SPI-6 in DCs have been investigated almost exclusively in mouse cells and cell lines. Our work expands these investigations to human DCs and more importantly, it identifies the involved molecular events.

Given the critical role in antigen presentation and T cell activation, DCs have attracted major attention for the improvement of vaccine efficacy, including the use of adjuvants to optimally activate DCs and consequently boost immunity. We hope that with the data presented in this communication new strategies can be developed to maximize the antigen presenting and T cell activating capacity of DCs, thus to enhance the effectiveness of DC-based vaccine therapies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

IDC	immature dendritic cell
MDC	mature dendritic cell
SLAM	Signaling Lymphocyte Activation Molecule
aSF2	anti-human SLAMF2 antibody

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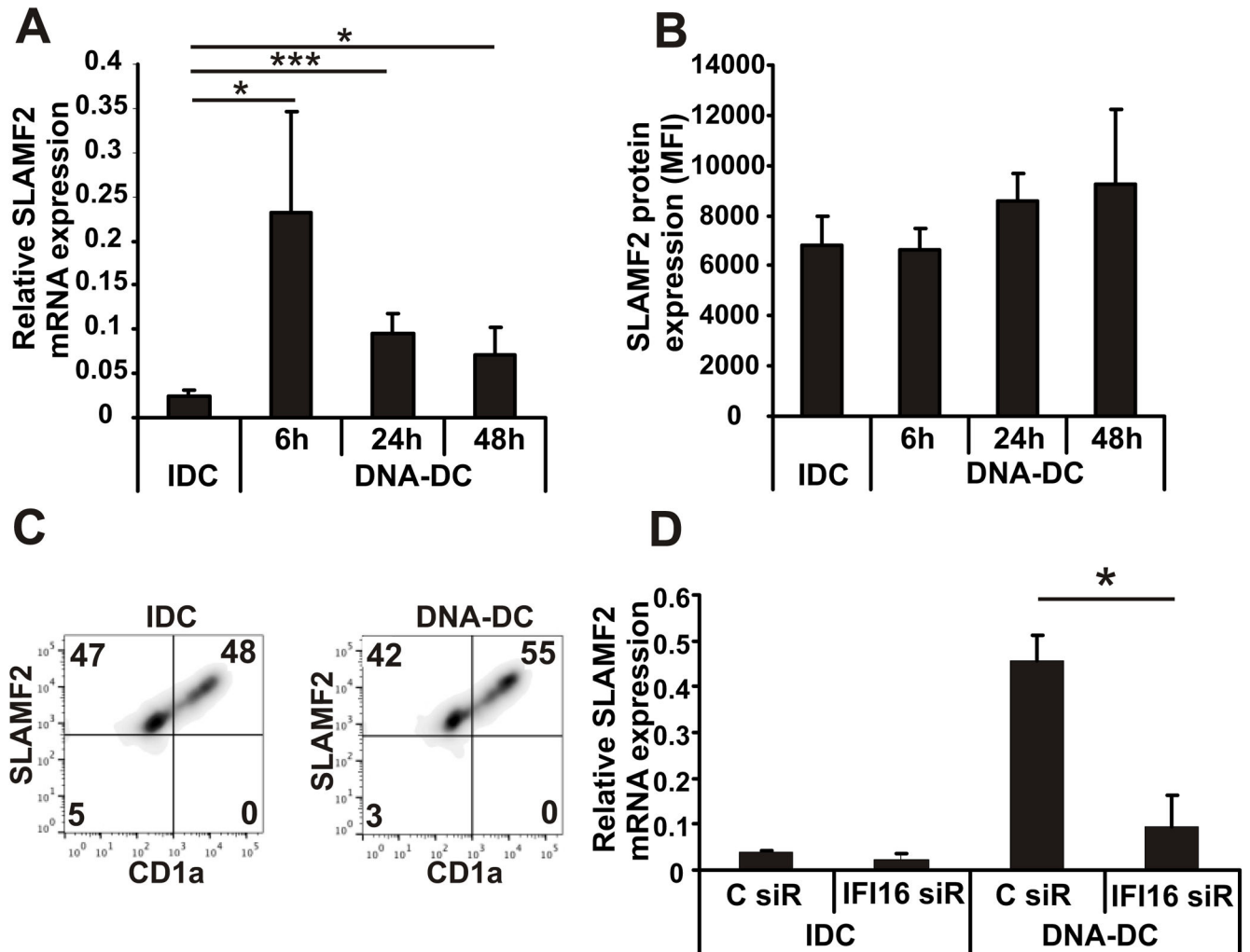


Figure 1. SLAMF2 is upregulated on mature dendritic cells

(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

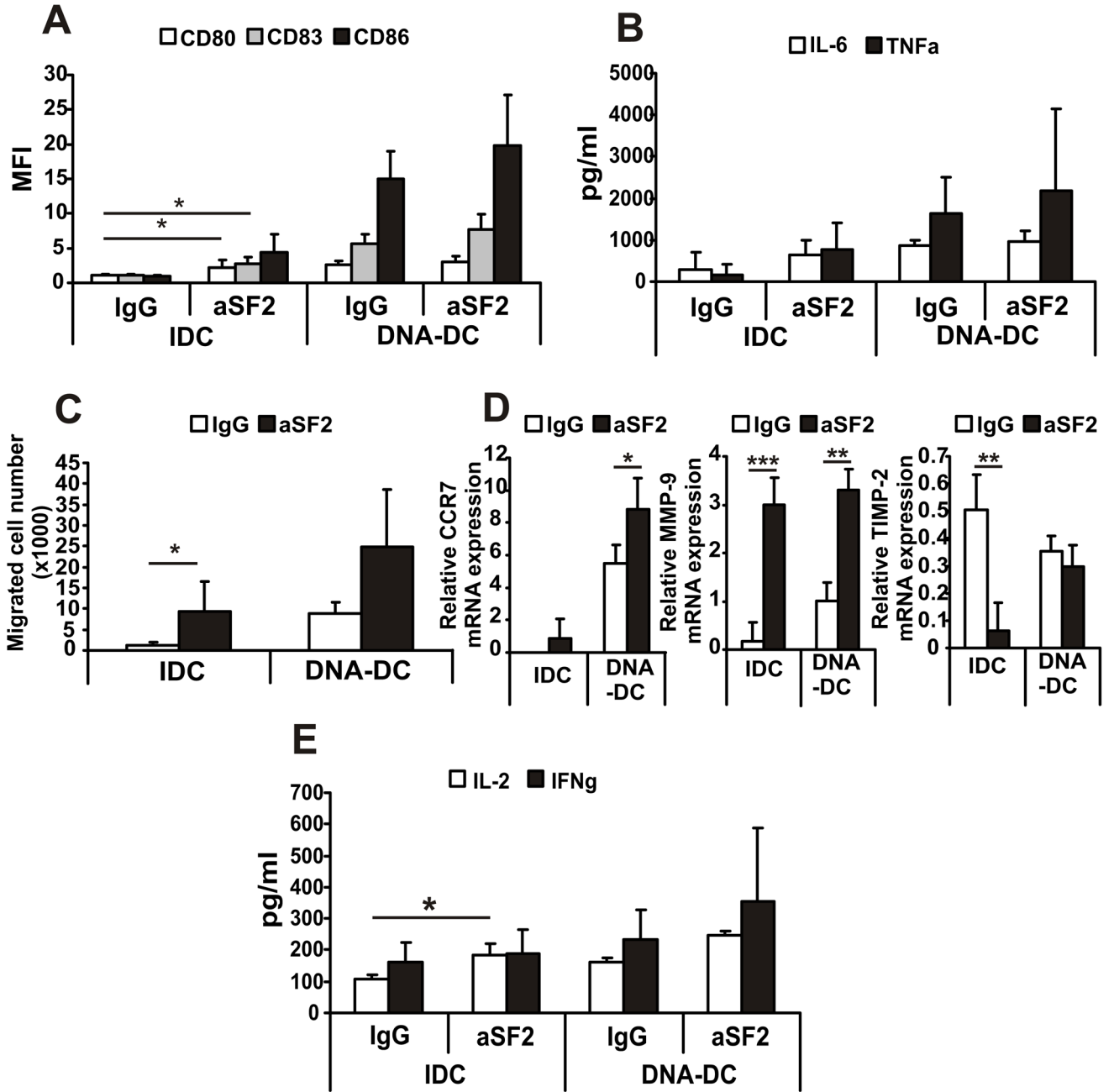


Figure 2. SLAMF2 engagement by specific antibody activates dendritic cells
 IDCs and DNA-DCs were treated with 5 μg/ml of immobilized mouse control IgG or anti-SLAMF2 antibody (aSF2) for 24h. (A) Expression of cell surface activation molecules CD80, CD83 and CD86. (B) Production of pro-inflammatory cytokines IL-6 and TNFα. (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-pre-treated IDCs and DNA-DCs were co-cultured with total T cells for 24h (IL-2) or for 72h (IFNγ) and the cytokine levels were measured from supernatants. n=3, * p 0.05; ** p 0.01; *** p 0.005

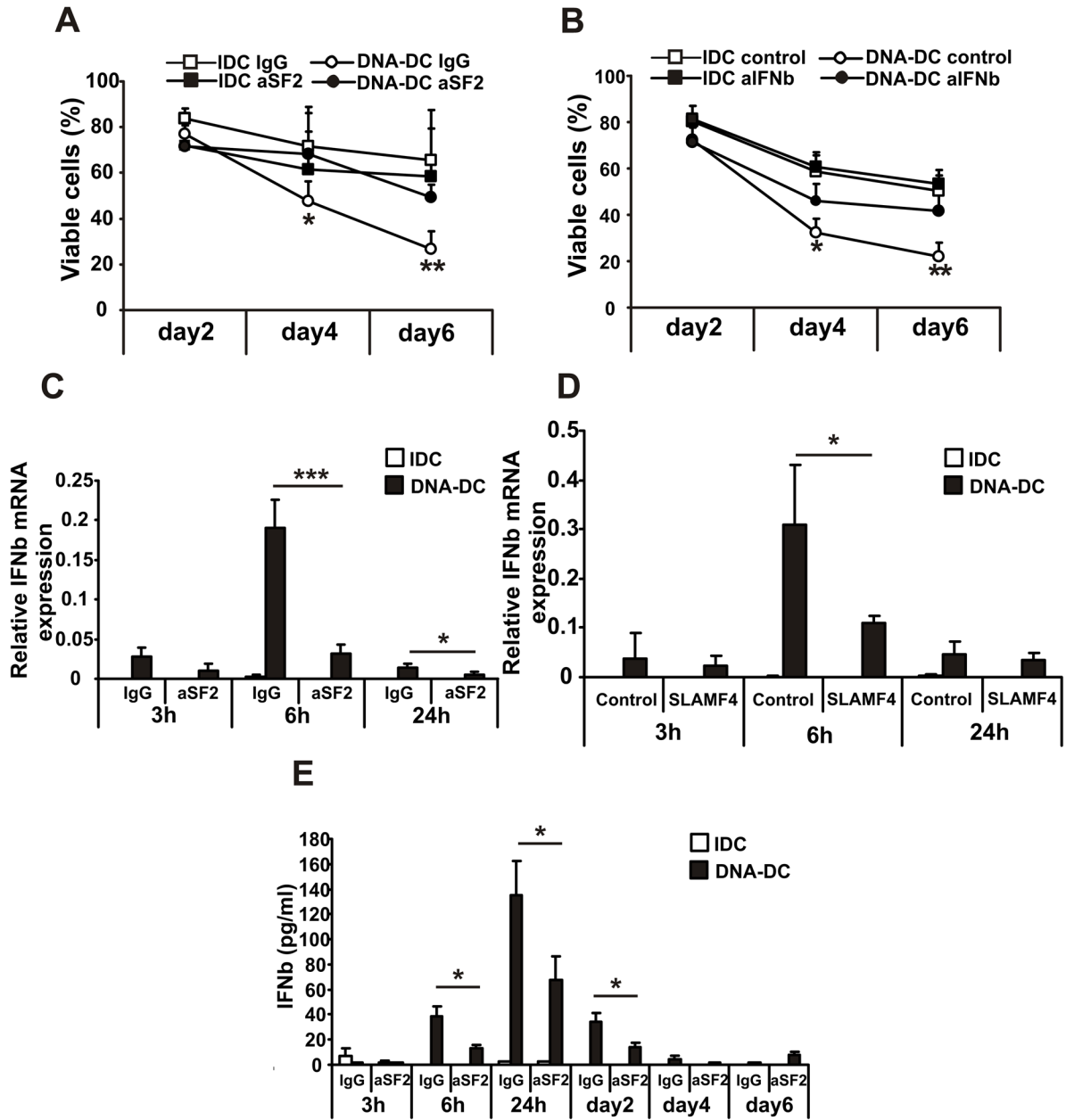


Figure 3. SLAMF2 engagement rescues mature dendritic cells from cell death by inhibiting the production of IFN β and the expression of caspase-4

IDCs and DNA-DCs were treated with 5 μ g/ml of immobilized control IgG or aSF2 antibodies for the indicated time points. (A) Viable cell percentages of day2, day4 and day6 DC cultures measured by flow cytometry using 7AAD staining to exclude necrotic cells (n=5). (B) Viable cell percentages of day2, day4 and day6 DC cultures treated with control antibodies or with antibody cocktail against IFN β and IFNRA receptor (aIFN β , n=3). (C) Relative IFN β mRNA expression measured on the indicated time points of IgG or aSF2-treated IDCs and DNA-DCs by Q-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 Q-PCR (n=3). (E) IFN β production of the aSF2-treated DCs were measured by ELISA on the indicated time points from cell supernatants (n=3). * p 0.05; ** p 0.01; *** p 0.005

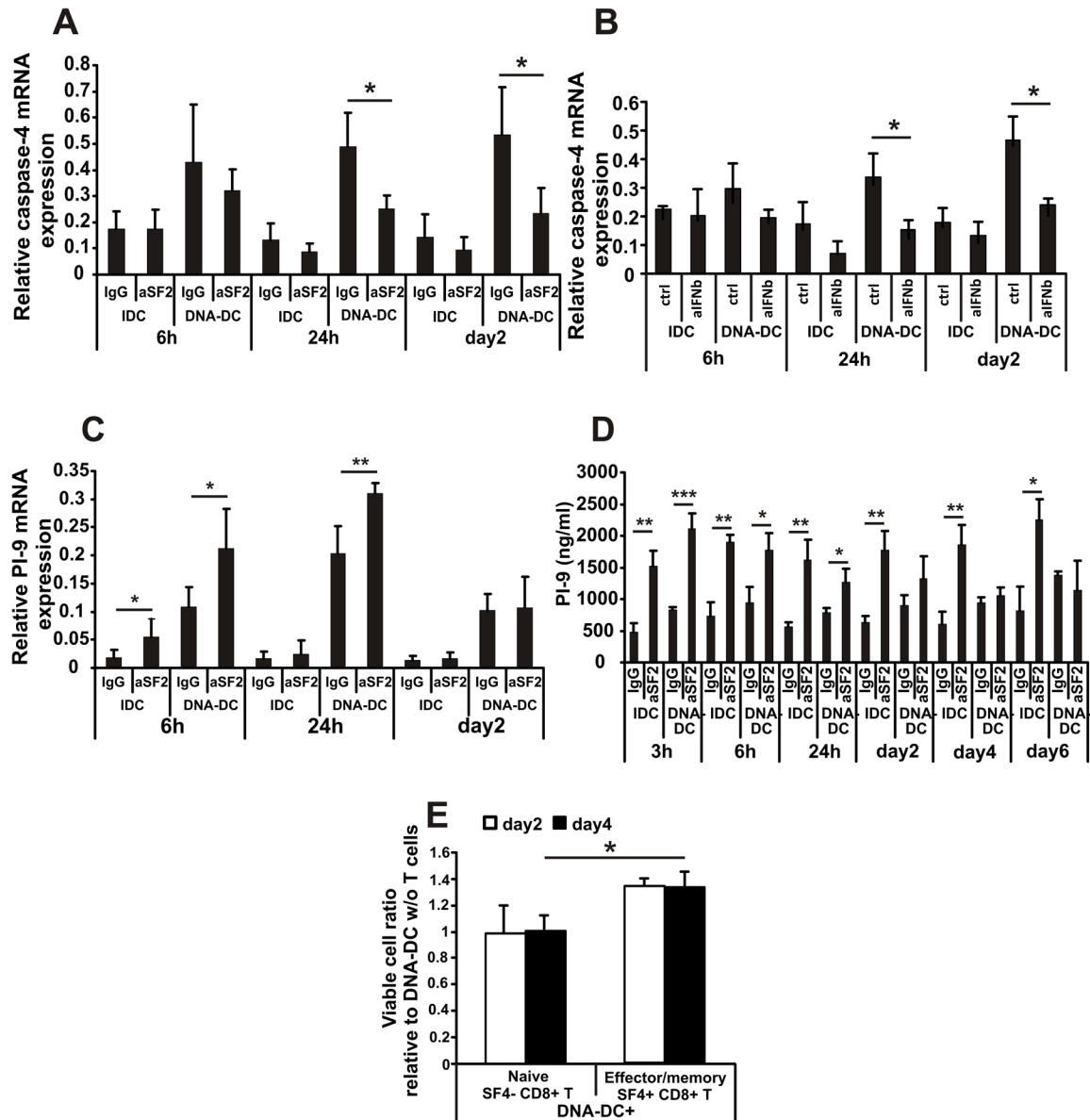


Figure 4. SLAMF2 engagement promotes the expression and secretion of protease inhibitor PI-9 of mature dendritic cells; SLAMF4⁺ CD8⁺ T cells promote DNA-DC survival

(A) Relative caspase-4 mRNA expression of IDCs and DNA-DCs treated with IgG or aSF2 antibody for the indicated time points measured by Q-PCR (n=3). (B) Relative caspase-4 mRNA expression of IDCs and DNA-DCs treated with control antibodies or with antibody cocktail against IFN β and IFN α receptor for the indicated time points (n=3). (C) Relative PI-9 mRNA expression of IDCs and DNA-DCs treated with IgG or aSF2 antibody for the indicated time points (n=3). (D) Secreted PI-9 protein measured by ELISA (n=3). (E) CFSE-labeled DNA-DCs were co-cultured with sorted SF4⁻ CD8⁺ naïve T cells or SF4⁺ CD8⁺ effector/memory T cells (1:4 DC:T cell ratio) for 2 and 4 days and the viability of DCs were measured by flow cytometry. The viable cell ratio relative to the DNA-DC viability without any T cell co-culture is plotted (n=3). * p 0.05; ** p 0.01; *** p 0.005

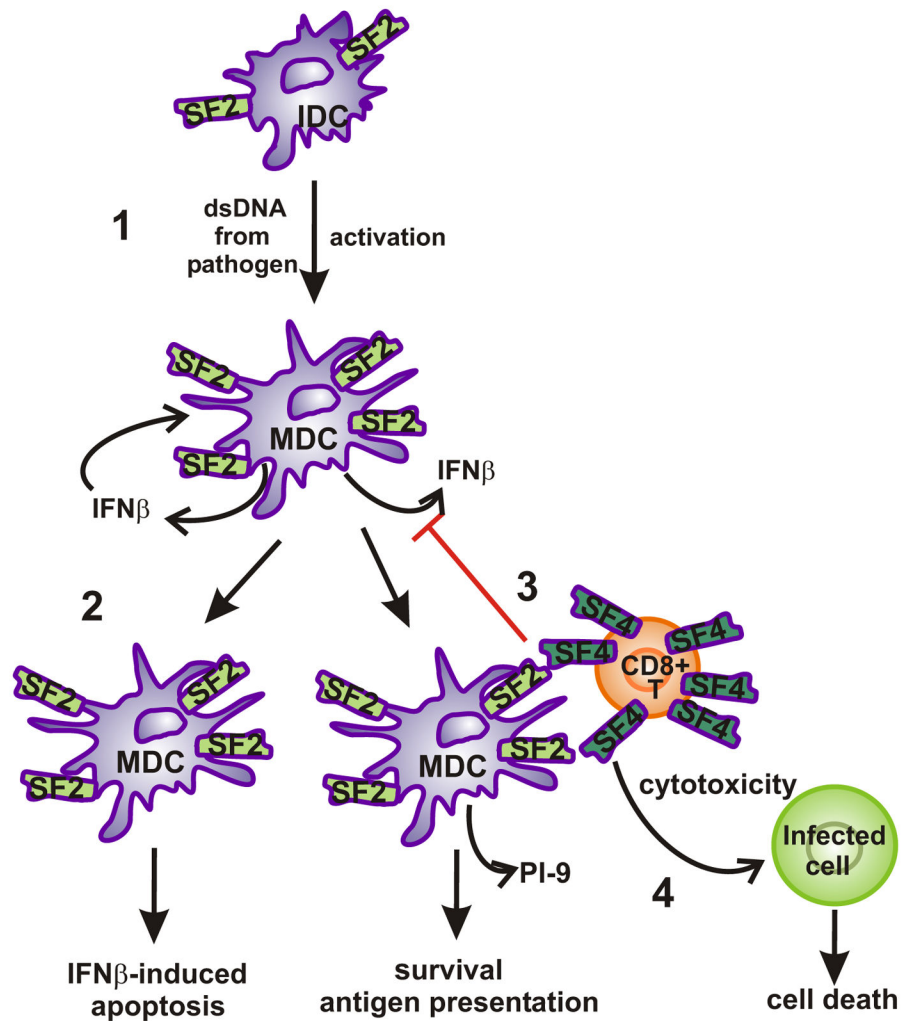


Figure 5. Hypothesis of SLAMF2-SLAMF4 interaction on dendritic cells

(1) Upon DNA activation from pathogen or other sources (e.g. self-DNA from dead cells) immature DCs become activated, produce IFN β and upregulate the expression of SLAMF2 molecules on the cell surface. (2) Without SLAMF2 engagement by SLAMF4, mature dendritic cells die by IFN β -induced apoptosis. (3) SLAMF2 engagement by SLAMF4 on the surface of effector/memory CD8⁺ T cells inhibits the IFN β production and promotes the production of protein inhibitor-9 by mature DCs, thus protects DCs from either the IFN β -induced apoptosis and from 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.