

Signaling Lymphocytic Activation Molecule Is Expressed on Mature CD83⁺ Dendritic Cells and Is Up-Regulated by IL-1 β ¹

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Signaling lymphocyte activation molecule (SLAM), a 70-kDa costimulatory molecule that mediates CD28-independent proliferation of T cells and IFN- γ production, has been identified on human T cells, immature thymocytes, and a subset of B cells. We have found that SLAM is expressed on mature but not immature dendritic cells (DC). However, the SLAM-associated protein, is missing in DC. SLAM surface expression is strongly up-regulated by IL-1 β . Addition of IL-1 β to the DC maturation mixture also increases the stimulatory properties of DC. These findings provide a new marker for DC maturation and help to explain two areas of DC biology. First, SLAM is a receptor for the measles virus, previously shown to infect DC. Second, SLAM could possibly contribute to the enhanced immunostimulatory functions of DC that are observed following the addition of IL-1. *The Journal of Immunology*, 2001, 167: 1989–1995.

Dendritic cells (DC)³ are the most potent APCs presently known and are widely distributed throughout the non-lymphoid and lymphoid tissues (1, 2). Importantly, DC are capable of inducing T cell-mediated immune responses by priming in vivo helper and killer T cell subsets and are therefore considered to be “nature’s adjuvant” (3).

The cardinal properties of the myeloid lineage-derived DC include the following functional and phenotypical characteristics: 1) the ability to take up, process, and present Ags; 2) the ability to migrate and at the same time mature; and 3) the ability to interact with, stimulate, and direct T lymphocyte responses (4, 5). Functional, mature DC are derived in vivo or in vitro from circulating precursor cells after a period of maturation. During this period, manifold activities on the level of gene regulation take place within the precursor cells, which ultimately result in the generation of mature DC. In addition to their functional qualities, DC are also characterized by the expression of a specific array of marker molecules. These include the accessory/costimulatory gene products CD40, CD80, CD86 as well as MHC class I and II molecules and CD83 (1, 6, 7).

To become potent T cell stimulators, DC have to mature. Immature DC, which reside in the peripheral tissue, capture and process Ags and subsequently migrate to secondary lymphoid organs, whereas mature DC present processed peptides very efficiently to rare Ag-specific T cells (8). During this maturation, a variety of different DC-specific gene products are up-regulated. This includes

adhesion and costimulatory molecules, MHC class I and class II molecules, and CD83.

Apart from the interaction between the TCR and MHC-peptide complex, also costimulatory signals are crucial to potentially stimulate T cells. These include molecules expressed on the cell surface of T cells such as CD28 and ICOS, as well as the recently identified novel signaling lymphocytic activation molecule (SLAM), and their corresponding receptors on APCs (9). SLAM, which has also been designated CDw150 or IPO-3 (10, 11), belongs to the CD2 subfamily of the Ig supergene family (12). The potential biological importance of SLAM is highlighted by the finding that mutations or deletions of a SLAM-associated protein, SAP (also called SH2D1A or DSHP), causes severe illness, the X-linked lymphoproliferative disease (13–15). Furthermore, SLAM has very recently been identified as a cellular receptor for the measles virus (16).

CD45RO⁺ T cells, immature thymocytes, and subsets of B cells express SLAM (12) and its expression is rapidly up-regulated after B and T cell stimulation (17). The engagement of SLAM can trigger T cell proliferation, can trigger production of IFN- γ (18–21), and can augment DC-dependent spread of HIV (22). Different isoforms of SLAM, including a soluble, secreted, and a cytoplasmic form, have been identified (12, 18). Furthermore, SLAM has been shown to be its own ligand (12, 17). The soluble isoform of SLAM can be detected in serum and synovial fluid and induces proliferation and Ig synthesis in B cells (12, 17).

However, SLAM has not been studied on the critical DC system of APCs, where it would act as the required ligand for SLAM signaling in B and T cells. In this study, we report that SLAM is expressed upon DC maturation, although in the absence of SAP. We present evidence that SLAM can possibly contribute to the capacity of DC to stimulate lymphocytes dependent on their maturation stage and characterized the induction of SLAM expression which might help to further understand the interaction between DC and T cell.

Materials and Methods

Cell culture medium

RPMI 1640 (BioWhittaker, Walkersville, MD), supplemented with glutamine (300 μ g/ml; BioWhittaker), penicillin/streptomycin (20 μ g/ml), 10 mM HEPES (pH 7.5; Sigma, St. Louis, MO), and 1% of heat-inactivated

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³ Abbreviations used in this paper: DC, dendritic cell; SLAM, signaling lymphocytic activation molecule; SAP, SLAM-associated protein; IL-1RA, IL-1R antagonist.

(56°C for 30 min) human plasma from a single AB donor, obtained from the Department of Transfusion Medicine (Erlangen, Germany) was used as standard medium.

Generation of DC

PBMC (5×10^7) isolated from buffy coats were sedimented in Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) and seeded onto an IgG-coated ($10 \mu\text{g/ml}$; Sigma) 100-mm culture dish and incubated at 37°C in 5% CO_2 . After 1 h, the nonadherent cell fraction was recovered, and the adherent cells were further incubated for 7 h in medium containing 1% human plasma. Then, a second nonadherent cell fraction was removed. The first step of DC differentiation (i.e., conversion of adherent monocytes to immature DC) was induced by adding the cytokines GM-CSF (800 U/ml; Novartis Research Institute, Vienna, Austria) and IL-4 (1000 U/ml; Genzyme, Cambridge, MA) to the medium containing 1% human plasma. Cytokines were added again on day 3 in 5 ml fresh medium (containing 4000 U GM-CSF and 5000 U IL-4) per dish. On day 5, all nonadherent cells were collected, counted, and transferred into new culture dishes at a density of $0.3\text{--}0.5 \times 10^5$ cells/ml. For the second step of DC differentiation (i.e., maturation of immature DC into fully mature and stable DC), the medium containing 1% human plasma, GM-CSF (400 U/ml), IL-4 (500 U/ml), TNF- α (25 ng/ml; Boehringer Ingelheim, Vienna, Austria), and PGE_2 (1 ng/ml; Cayman Chemicals, Ann Arbor, MI) was supplemented either with or without 1 ng/ml IL-1 β (Sigma). For certain stimulation studies, agonistic anti-CD40 mAbs (Cymbus Biotechnology, Chandlers Ford, U.K.) and anti-SLAM mAbs A12 (DNAX, Palo Alto, CA) (11) were added simultaneously to the maturation mixture composed of TNF- α and PGE_2 with or without IL-1 β (1 ng/ml).

In some experiments, DC were cultured either in the presence of an IL-1 β -neutralizing Ab (catalog no. 0796; Immunotech, Marseilles, France) or in the presence of the human IL-1 receptor antagonist (IL-1RA; R&D Systems, Abingdon, U.K.) to investigate the influence of IL-1 β on the SLAM expression. The neutralizing Ab or the IL-1RA was added 15 min before the maturation mixture was added to the cell cultures. Cells were then cultured as described above.

FACS analyses

Phenotypic analyses of cells (1×10^5) were performed by flow cytometry using saturating concentrations of the following mAbs: anti-SLAM (DNAX), CD83 (Immunotech), CD54 (Dianova, Hamburg, Germany), and CD14 and CD80 (BD Pharmingen, Hamburg, Germany). The isotype controls IgG1a, IgG2a, and IgG2b were obtained from BD Biosciences (Mountain View, CA) and were run in parallel. Ten thousand cells were analyzed on a FACScan (BD Biosciences). Nonviable cells were gated out on the basis of their light scatter properties.

RNA isolation and RT-PCR

Cultured cells were harvested and washed in PBS at different time points of DC generation. RNA of each sample was prepared using the acidic phenol extraction method. Samples were stored at -80°C until used. Reverse transcription was performed using the Superscript system (Life Technologies, Eggenstein, Germany) according to manufacturer's instructions. Two to 4 ng of the resulting cDNA were used as template and amplified with *Taq* polymerase (PerkinElmer, Langen, Germany). A RT-PCR for β -actin and a negative control with no cDNA template were run in parallel with each experiment. To detect isoforms of SLAM and SAP, 40 cycles were performed (initial denaturation at 94°C for 2 min, 30 s at 94°C , 30 s at 55°C , and 1 min at 72°C). The following primers were used: for membrane-bound SLAM: sense, 5'-ATC ACT GGA GAA CAG TGT-3' and antisense, 5'-CCC AGC ATA CAC TGC CC-3'; for soluble SLAM: sense, 5'-ATC ACT GGA GAA CAG TGT-3' and antisense, 5'-TTC GTT TTA CCT GAG GGG TCT G-3'; for SAP: sense, 5'-GCC TGG CTC CAG TAG CAG CGG CAT CTC CC-3' and antisense, 5'-ATG TAG AAA AGT CCA TTT CAG CTT TGA C-3'; for the CD3- ξ chain: sense, 5'-GAA GAT CTA GAG TGA AGT TCA GCA GGA G-3' and antisense, 5'-GCT CAT GAT TAG CGA GGG GGC AGG GCC-3'; and for β -actin: sense, 5'-GAA CTT TGG GGG ATG CTC GC-3' and antisense, 5'-CGG GAA ATC GTG CGT GAC AT-3'. PCR products were electrophoresed using a 1% agarose gel and visualized with ethidium bromide.

Allogeneic MLR

CD4^+ T cells (2×10^5 /well) were negatively isolated using a CD4 T cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany) from buffy coats and were stimulated with mature allogeneic DC, which were either matured in the presence or absence of IL-1 β . T cells and DC were cocultured for 4 days in 200 μl RPMI 1640 supplemented with 5% human serum

from a single AB donor in 96-well cell culture dishes. Then, cells were pulsed with [^3H]thymidine (1 $\mu\text{Ci}/\text{well}$; Amersham, Braunschweig, Germany) for 8–16 h. The culture supernatants were harvested onto glass fiber filters using an IH-110 harvester (Inotech, Dottikon, Switzerland) and filters were counted in a 1450 microplate counter (PerkinElmer Wallac, Gaithersburg, MD).

Allogeneic cord-blood MLR

Cord-blood PBMC, obtained from human cord blood of healthy donors after informed consent was given, were isolated by centrifugation over a density gradient (Lymphoprep; Nycomed Pharma, Oslo, Norway). On day 6, DC were matured with the maturation mixture in the presence or absence of IL-1 β . Cells which did not receive the maturation stimulus remained immature and were used as controls. On day 7, mature and respectively immature DC were harvested, washed twice, and added in graded doses to 2×10^5 allogeneic T cells/well in 96-well flat-bottom plates and cocultured for 4–5 days in RPMI 1640 supplemented with gentamicin, glutamine, and 5% heat-inactivated human pool serum. The proliferation was determined by addition of [^3H]thymidine (4 $\mu\text{Ci}/\text{ml}$ final concentration) for the last 12–16 h of culture.

Induction of peptide-specific CTL

Purified CD8^+ T cells were obtained by magnetic cell sorting using CD8 microbeads according to the manufacturer's instructions (Miltenyi Biotec). Autologous DC from HLA-A2.1 $^+$ healthy donors were either pulsed with HLA-A2.1-restricted influenza matrix peptide (GILGVVFTL, purchased from Clinalfa, L  ufelfingen, Switzerland; 10 $\mu\text{g}/\text{ml}$ for 4 h at 37°C at 1×10^6 DC/ml) or left untreated. These cells were then incubated with T cells at a DC:T cell ratio of 1:10 for 7 days without the addition of any cytokines. IFN- γ -producing effector T cells were then quantified by ELISPOT analyses. Briefly: 5×10^4 /well CD8^+ T cells were added in triplicates to nitrocellulose-bottom 96-well plates (MAHA S4510) precoated with the primary anti-IFN- γ mAb (1-D1K; Mabtech, Stockholm, Sweden) in 50 μl ELISPOT medium (RPMI 1640 containing 5% heat-inactivated human serum)/well. After addition of influenza matrix peptide (10 $\mu\text{g}/\text{ml}$) and incubation for 20 h, wells were washed six times, incubated with the biotinylated IFN- γ -specific mAb (7-B6-1; Mabtech) for 2 h, washed, and stained with a Vectastain Elite kit (Vector Laboratories, Burlingame, CA). Spots were evaluated and counted using a special computer-assisted video imaging analysis system (Carl Zeiss Vision, Eching, Germany).

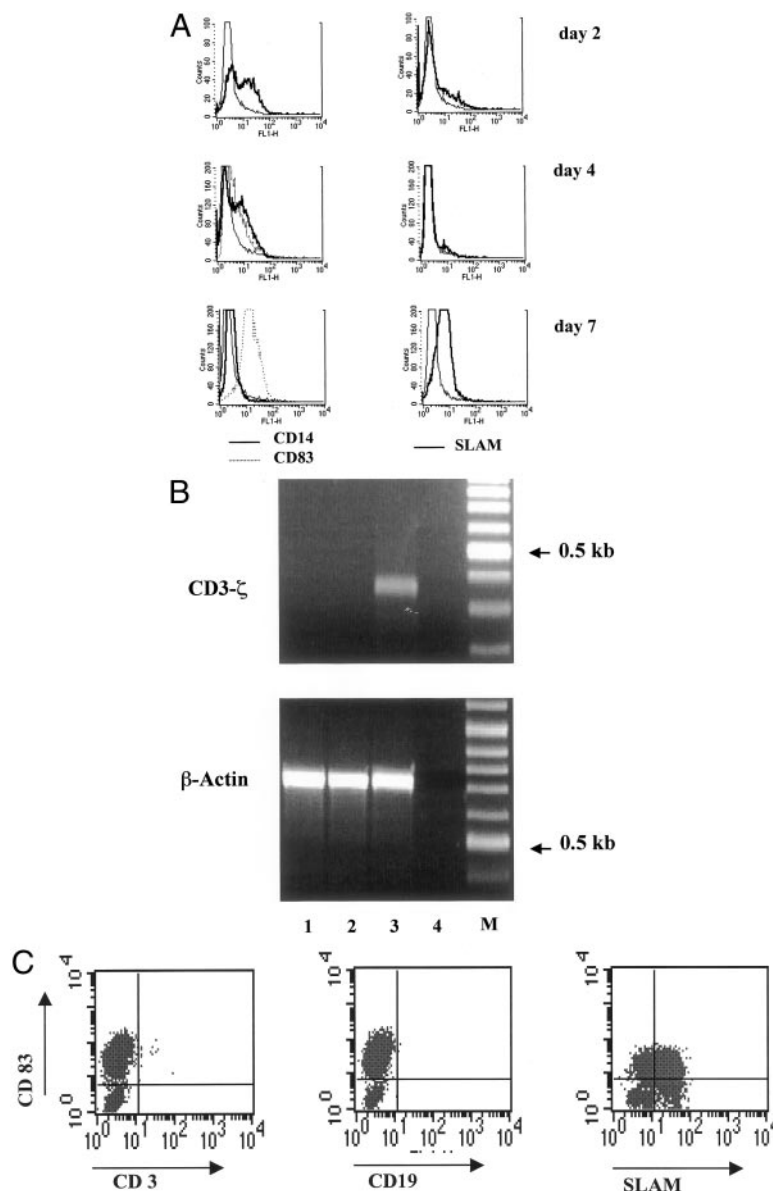
Results

Mature DC express SLAM on their cell surface

To investigate SLAM expression on the cell surface during DC maturation, we generated DC in vitro following an improved two-step protocol (23). Briefly, the DC generation includes a first step, whereby Ig-adherent monocytes are converted into immature DC under the aegis of GM-CSF and IL-4 over 4 days, followed by a final maturation step induced by a mixture composed of TNF- α and PGE_2 with or without IL-1 β . The generated DC showed all the typical phenotypical and functional properties including stellate morphology, nonadherence to plastic, up-regulation of MHC I and II and costimulatory molecules (24), CD83 (25), and intracellular p55 (26), and a very high capacity to stimulate allogeneic CD4^+ and CD8^+ T cell responses as described elsewhere (23, 27).

To follow SLAM expression during DC maturation, FACS analyses were performed on days 2, 4, and 7 (Fig. 1A). DC analyzed on days 2 and 4 represent DC precursors, whereas day 7 analyses represent finally differentiated mature DC. Cells were stained with mAbs specific for SLAM, CD83, a well-characterized marker for mature DC, and CD14, the marker for monocytes and macrophages. The FACS data clearly demonstrated that SLAM was only found on mature DC and was up-regulated in parallel with the well-characterized DC marker CD83. In contrast, DC precursors that were analyzed on days 2 and 4 expressed neither SLAM nor CD83 (see Fig. 1A). To exclude the possibility that contaminating T cells were responsible for the SLAM expression, RT-PCR analyses were performed. The absence of T cells in these DC cultures was confirmed using CD3- ξ chain-specific primers (Fig. 1B). In addition DC cultures were analyzed by two-color FACS using

FIGURE 1. A, SLAM is expressed on mature DC. *Left panel*, Expression of the surface molecules CD14 (bold solid line) and CD83 (dotted line). *Right panel*, Expression of SLAM (bold solid line). DC were analyzed on days 2, 4, and 7. Ig controls were run in parallel (thin solid lines). SLAM expression was only detectable on mature DC (i.e., day 7). During the DC generation, the DC precursors lost the CD14 molecule on their cell surface. After the final DC maturation, induced with a specific mixture composed of $\text{TNF-}\alpha$ and PGE_2 , CD83, the typical marker for mature DC, was strongly up-regulated. B, PCR analyses of immature DC (lane 1), mature DC (lane 2), and T cells (lane 3) using a CD3- ζ chain-specific primer pair; β -actin has been used as a standard control. Lane 4, Negative control; M, DNA marker. C, Two-color FACS analyses clearly show that the DC preparations are not contaminated with T cells (CD3) or B cells (CD19). SLAM is expressed by CD83 $^+$ DC.



CD3, CD19, CD83, as well as SLAM-specific Abs (Fig. 1C). These data clearly show that the DC-specific SLAM expression is not due to a T or B cell contamination. CD83-positive DC do express SLAM. Taken together, these data demonstrate for the first time that SLAM is expressed on the cell surface of mature DC but not of immature DC.

Detection of SLAM mRNA isoforms in mature DC

To identify which SLAM isoforms are expressed in DC, total cellular RNA from immature and mature DC extracts was analyzed by RT-PCR. This highly sensitive RNA assay revealed that both membrane-bound-SLAM and soluble SLAM were expressed in mature DC (Fig. 2, lane 2), but not in immature DC (Fig. 2, lane 1). These results confirmed the FACS data. Moreover, the message for the SAP which is associated with SLAM in T cells (15) could only be detected in T cells (Fig. 2, lane 4) but not in immature or mature DC (Fig. 2, lanes 1–3). In addition to the 48-h time point (see Fig. 2, lane 2), the SAP expression was also analyzed after 6, 12, and 24 h. Also, at these earlier time points SAP expression could not be detected. In addition, stimulation of DC precursors on day 4 with anti-CD40 mAbs did not alter the expression pattern of SLAM isoforms or SAP in these mature DC (Fig. 2, lane 3).

IL-1 β enhanced SLAM expression on mature DC

Final DC maturation was induced by a cytokine mixture composed of GM-CSF, IL-4, $\text{TNF-}\alpha$, and PGE_2 . In contrast to the observation by Jonuleit et al. (23), in our cell culture system one proinflammatory cytokine, $\text{TNF-}\alpha$ or IL-1 β , was sufficient to generate phenotypically mature DC. In fact, DC matured in the presence of $\text{TNF-}\alpha$ only, without the addition of IL-1 β , expressed high levels of CD83. In addition, these mature DC were also SLAM positive.

Further analyses demonstrated that the addition of IL-1 β to the standard cytokine mixture composed of GM-CSF, IL-4, $\text{TNF-}\alpha$, and PGE_2 significantly enhanced the expression of SLAM (Fig. 3). At an IL-1 β concentration of 1 ng/ml, the SLAM expression was ~50% enhanced. Decreasing IL-1 β concentrations resulted in a lower SLAM expression, whereas higher concentrations had no additional effects (data not shown). In contrast, the expression levels of CD83 and CD25 were only slightly affected by the addition of IL-1 β (Fig. 3).

To investigate the IL-1 β effects in more detail, increasing concentrations of an IL-1 β -neutralizing Ab or the recombinant human IL-1RA were added to the cell cultures shortly before the induction of the final DC maturation. As shown in Fig. 4,

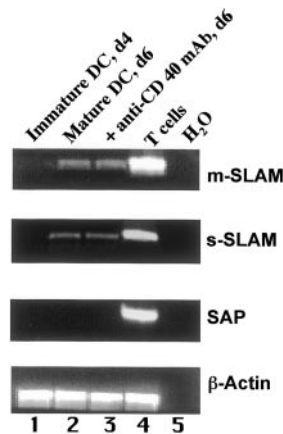


FIGURE 2. Detection of SLAM- and SAP- specific mRNAs by RT-PCR. Total RNA was isolated from DC extract on days 4 and 6 and transcribed into cDNA. Specific transcripts were amplified with primers specific for membrane-bound SLAM (m-SLAM), soluble SLAM (s-SLAM), SAP. β -Actin was used as control. Lane 1, Immature DC isolated on day 4; lane 2, mature DC isolated on day 6; lane 3, mature DC that were stimulated on day 4 with anti-CD40 mAbs (100 ng/ml) in addition to the standard mixture (TNF- α and PGE₂) and isolated on day 6; lane 4, T cells stimulated for 24 h with Con A (10 μ g/ml) and irradiated feeder cells; lane 5, negative control.

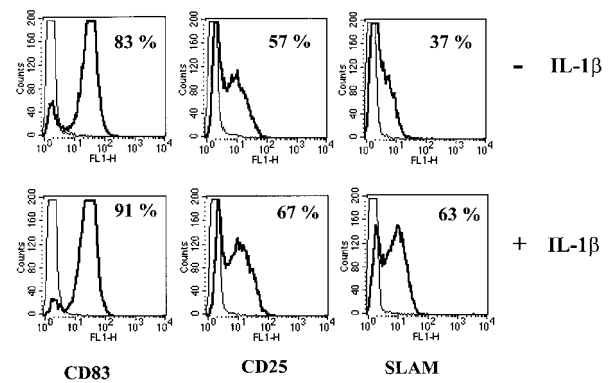


FIGURE 3. IL-1 β significantly enhanced SLAM expression on mature DC. DC precursors were cultured as described in *Materials and Methods*. The final step of DC maturation was initiated on day 4 with the standard mixture (TNF- α and PGE₂) or in addition to IL-1 β (1 ng/ml). The expression of CD83, CD25, and SLAM was analyzed by FACS. The addition of IL-1 β to the standard mixture strongly increased the SLAM expression.

administration of IL-1RA results in a clear reduction of SLAM surface expression. Similar results were obtained with IL-1-neutralizing Abs (data not shown). These data clearly demonstrate that IL-1 plays a major role in the induction of SLAM surface expression on maturing DC.

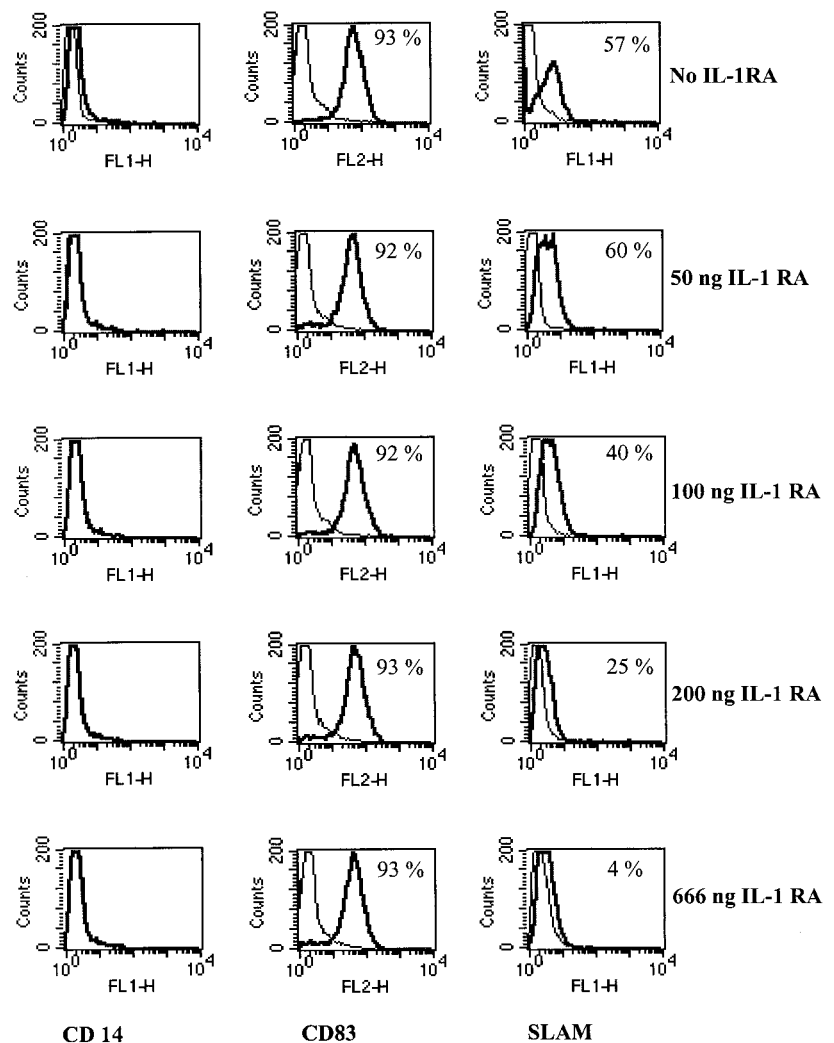


FIGURE 4. Inhibition of IL-1 β leads to a reduced expression of SLAM on mature DC. Recombinant human IL-1RA was added 15 min before the addition of the maturation mixture (TNF- α , IL-1 β , and PGE₂). The expression of CD14, CD83, and SLAM was analyzed by FACS. The addition of IL-1RA strongly inhibited the cell surface expression of SLAM.

ICAM-1 is up-regulated by anti-CD40 mAbs in concert with anti-SLAM mAbs

The functional consequences of SLAM engagement were analyzed by studying the effects of SLAM on the cell surface phenotype as well as the T cell stimulatory properties of DC on T cells. Agonistic anti-SLAM mAbs, or anti-CD40 mAbs, or the combination of both were added concurrently to the maturation mixture during the final DC maturation. Stimulation with anti-SLAM mAbs or anti-CD40 mAbs alone did not increase the basic level expression of the adhesion molecule ICAM-1 (CD54) and was comparable to the mock and IgG control (15% of cells were positive). Interestingly, the expression of ICAM-1 was clearly increased by the co-stimulation via SLAM and CD40 (30% of cells were positive). In contrast, the CD80 expression was only enhanced by anti-CD40 (from 14 to 24%) when compared with mock-treated cells. The combination of both anti-SLAM and anti-CD40 mAbs had no additional effect. Finally, the CD83 expression was not affected by CD40 and/or SLAM engagement. Furthermore, microscopic inspections revealed that in the presence of anti-SLAM and anti-CD40 mAbs DC gained the capacity to form large DC clusters (data not shown). DC that were only incubated with anti-CD40 mAbs also formed cell aggregates but these aggregates were considerably smaller than those generated in the presence of anti-CD40 and anti-SLAM mAbs. Anti-SLAM or mock treatment did not lead to the formation of cell clusters. These data suggest that

the up-regulation of the ICAM-1 expression may increase the capacity of DC to form membrane-membrane interactions.

DC matured in the presence of IL-1 β show an increased stimulation of allogeneic T cell and peptide-specific CTL

The most distinctive functional characteristic of DC is their potent capacity to stimulate T cells. Therefore, we studied whether or not IL-1 β affects the ability of DC to induce CD4⁺ T cell proliferation in a primary allogeneic MLR. As depicted in Fig. 5A, addition of IL-1 β to the maturation mixture increased the stimulatory capacity of these DC. Interestingly, this increase could be inhibited by the administration of IL-1RA or an inhibitory anti-IL-1 Ab. No difference was observed when CD8⁺ T cells were analyzed (data not shown).

To further investigate the effect of IL-1 β , primary allogeneic cord-blood MLR analyses were performed. As shown in Fig. 5B, addition of IL-1 β strongly increased the capacity of these DC to stimulate naive T cells. In addition, when these T cells were counted after the cocultivation with differentially matured DC, it became clear that IL-1-matured DC increased the T cell number far better than cells that were matured without IL-1 (Fig. 5C). Finally, the effect on influenza matrix peptide M1-specific CTL was analyzed using the ELISPOT assay. As demonstrated in Fig. 5D, in the presence of IL-1 the DC showed an increased capacity to stimulate influenza matrix peptide-specific memory cells. In

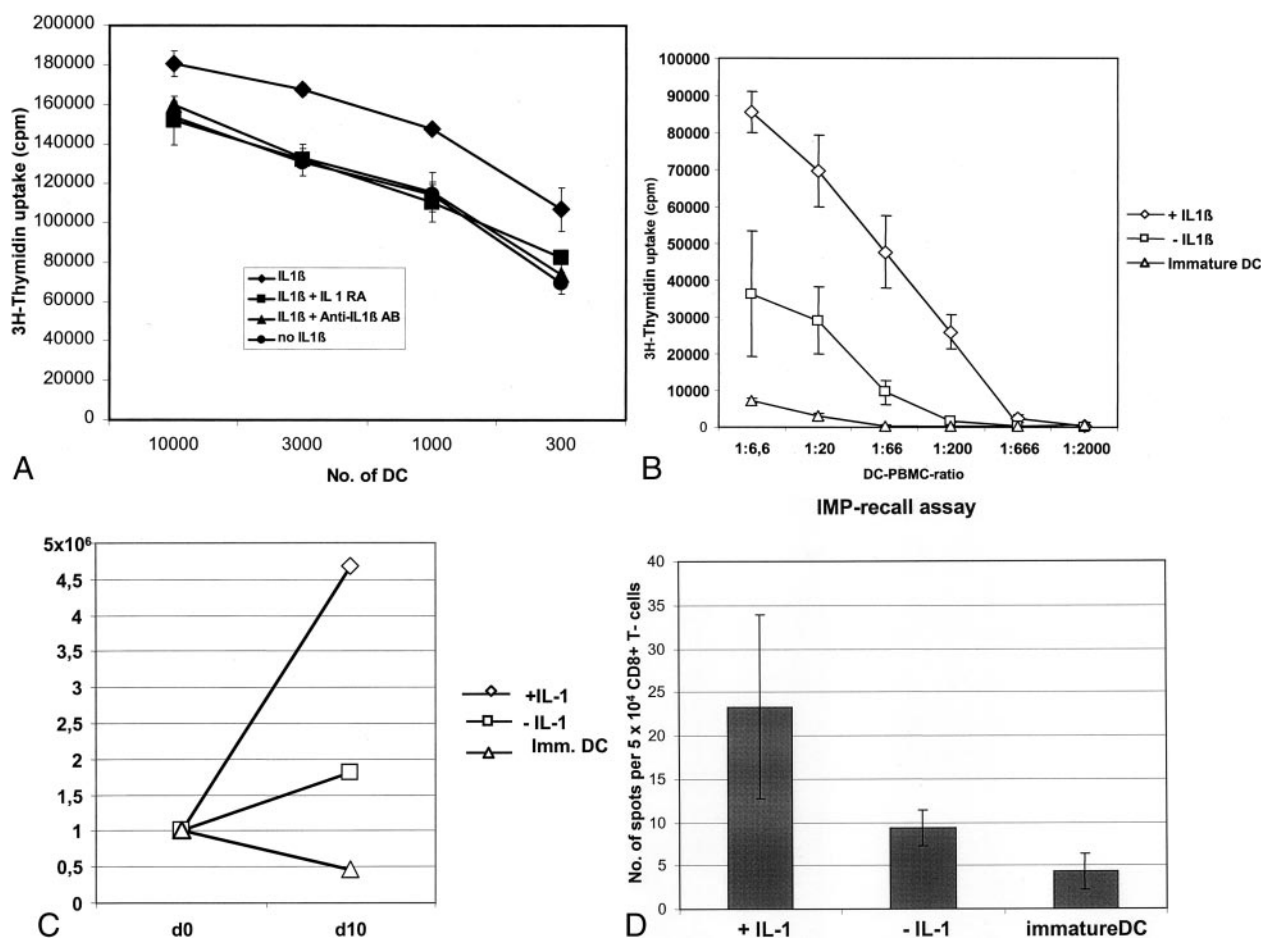


FIGURE 5. A, The presence of IL-1 β in the DC maturation mixture enhances the allostimulatory capacity of DC. Mature DC were incubated with CD4⁺ T cells and cultured in the absence or presence of IL-1 β , and IL-1 enhances the stimulatory capacity of DC. IL-1RA as well as inhibitory anti-IL-1 Abs inhibit this enhancement. B, Primary cord-blood MLR. The presence of IL-1 in the DC maturation mixture strongly enhances the proliferation of naive T cells. Immature DC were used as controls. C, IL-1-treated DC induce a stronger expansion of naive T cells when compared with untreated or immature DC. D, IL-1-treated DC are better stimulators of influenza matrix peptide (M1)-specific memory CTL. All tests were performed at least in triplicates.

conclusion, it could be speculated that the up-regulation of SLAM by IL-1 β leads to an increased DC-induced T cell response.

Discussion

In this study, we show for the first time that SLAM expression is induced during DC maturation. The expression of CD83, a cell surface molecule which represents one of the best known markers for mature DC (7, 25, 28), is also up-regulated during DC maturation and its expression is closely mirrored by the SLAM expression. Although immature DC do not express SLAM, its expression is up-regulated in mature DC, representing a new phenotypical marker for mature DC. Noteworthy, the SLAM expression could also be confirmed on mature cord blood-derived DC as well as on mature isolated CD11c-positive blood DC (data not shown). Noteworthy, mature DC and CD45RO⁺ T cells expressed SLAM at comparable levels, 64 and 72%, respectively. On the other hand, SLAM was less abundant on B cells (48%).

Qualitative RT-PCR analyses confirmed these data and showed that only mature DC express the membrane and the soluble isoform of SLAM. Both of these isoforms have been shown to be agonistic (12). Previous studies have demonstrated that CD83-specific mRNA was already present in immature DC on day 5 (29, 30) as opposed to the SLAM-specific mRNA which was not present in those DC precursors (Fig. 2).

Interestingly, SLAM expression was clearly increased upon addition of IL-1 β . The proinflammatory cytokine IL-1 β is, like TNF- α , an important cytokine for the final maturation of DC. The present study shows that the maturation effect of IL-1 β on DC is reflected by the enhancement of SLAM expression and that the SLAM expression is specifically regulated in DC.

It is interesting to note that it has been known for some time, from studies using DC isolated from murine tissue, that IL-1 has a distinct effect on maturing DC. Koide et al. (31) reported that IL-1 enhances the clustering and T cell stimulatory capacity of spleen DC. Heufler et al. (32) found that IL-1 enhances the stimulatory capacity of murine Langerhans cells matured in the presence of GM-CSF. Later, Romani et al. (27) reported that IL-1 also enhanced the stimulatory capacity of DC generated from adherent PBMC using GM-CSF and IL-4. Our finding that IL-1 β induces SLAM on maturing DC appears to provide the first mechanistic explanation of these earlier findings.

The RT-PCR analyses also revealed that SAP, which binds in T cells to the intracellular domain of SLAM and regulates the recruitment of the phosphatases Src homology 2 domain-containing tyrosine phosphatase in T cells (15), is missing in DC. This suggests that in DC the SLAM-dependent signaling pathways differs from those in T cells. Nevertheless, the precise SLAM-dependent signaling events in DC still need to be elaborated. Interestingly, also B cells do not express SAP (Ref. 33, and data not shown), indicating that APCs may have a different SLAM-dependent signaling cascade.

We found that some biological effects of SLAM ligation occur in concert with CD40 stimulation. Under these experimental conditions, SLAM enhanced the expression of ICAM-1 and concurrently the adhesion properties of DC.

This study outlines some potential key functions for SLAM on DC. First, SLAM is expressed on mature DC representing a new marker for mature DC and, second, SLAM may be responsible for the enhanced T cell stimulation. Since SLAM is expressed on both DC and T cells and has biological effects on both cell types, it could be envisaged that SLAM not only plays an essential role during the maturation of DC but additionally also in the T cell activation during the DC-T cell interaction in vivo.

Interestingly, SLAM has recently been identified as a measles virus receptor (16). It has previously been shown that measles virus can infect DC and thereby heavily interfere with the biology of DC. This includes the suppression of cell-mediated immunity by interfering with survival and function of the infected DC, leading to immunosuppression (34–36). Also, the reduced capacity to produce crucial cytokines such as IL-12 has been reported (36). Measles virus is still a major killer of children and the severe immunosuppression induced by this virus increases the risk of secondary infections in these patients and consequently leading to the high mortality. The Edmonston strain of measles virus, and the derived vaccine strains from it, use CD46 as cellular receptor (37). This molecule is expressed on all nucleated cells; nevertheless, most clinical isolates cannot use this receptor (38). SLAM on the other hand can be used as a receptor by measles viruses, including the Edmonston strain (16). The selective expression of SLAM on T and B cells and as we show now in this paper also on DC is consistent with their susceptibility to clinical isolates of measles virus. Therefore, DC probably represent a reservoir for measles virus infection and serve as a vector to transport the virus to the lymph nodes where it encounters and infects lymphoid cells. Since measles virus can infect DC and subsequently cause immunosuppression, the inhibition of the interaction between SLAM on DC and the virus might be an interesting way to protect the infected individuals.

In summary, our findings that SLAM is expressed on mature DC may not only help to further understand the cross-talk between DC and T cells but also to gain further insights into the interaction of DC and measles virus.

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