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The Novel Cyclophilin-Binding Drug Sanglifehrin A Specifically Affects Antigen Uptake Receptor Expression and Endocytic Capacity of Human Dendritic Cells

Andrea M. Woltman, Nicole Schlagwein, Sandra W. van der Kooij, and Cees van Kooten

Sanglifehrin A (SFA) is a recently developed immunosuppressant that belongs to the family of immunophilin-binding ligands. SFA is a cyclophilin A-binding immunosuppressive drug with a novel, but unidentified, mechanism of action. Several reports exist about the effect of SFA on T cells, but its effect on the initiators of the immune response, i.e., dendritic cells (DCs), is relatively unknown. Therefore, we examined the effect of SFA on the differentiation and function of human monocyte-derived DCs. Unlike the well-known cyclophilin A-binding immunosuppressant cyclosporin A, which did not affect DC phenotype, differentiation of DCs in the presence of SFA resulted in CD14-CD1a DCs with normal DC morphology, viability, and a proper capacity to activate allogeneic T cells. However, DCs generated in the presence of SFA demonstrated reduced macropinocytosis and lectin-mediated endocytosis, which was in line with a decreased expression of C-type lectins, including mannose receptor, C1qRP, DC-ASGPR, and especially, DC-SIGN. In contrast, FcαRI (CD89) and FcγRII (CD32) were increased by SFA. The explicit effect of SFA on the expression of Ag uptake receptors and Ag capture by DCs makes SFA unique among immunophilin-binding immunosuppressive drugs. The Journal of Immunology, 2004, 172: 6482–6489.

Dendritic cells (DCs) are the most potent APCs. They are highly motile cells that operate at the interface of innate and acquired immunity with a key role in the initiation and direction of immune responses (1, 2). DCs constitutively patrol through the blood, peripheral tissues, lymph, and secondary lymphoid organs. In peripheral tissues, DCs act as sentinels against foreign Ags, but they can also take up tissue-derived self Ags, which plays an important role in tolerance induction and maintenance to self Ags (3).

Efficient Ag internalization, either through receptor-mediated endocytosis or fluid-phase endocytosis (i.e., macropinocytosis) is a specific attribute of immature DCs. Macropinocytosis represents a critical Ag uptake pathway allowing DCs to rapidly and nonspecifically sample large amounts of surrounding fluid (4). Receptor-mediated endocytosis, in contrast, is initiated by the engagement of specific receptors, triggering a cascade of signal transduction that is required for actin polymerization and effective engulfment. Receptors for Ag capture, such as C-type lectins and Fc receptors, vary in their ligand specificity and mode of delivery to Ag-processing compartments. In addition, depending on the route of internalization and the source of Ag, the internalization process can lead to DC maturation and subsequent T cell activation (5).

Because Ag uptake and presentation together with the regulation of costimulation are essential for the induction of both tolerogenic and immunogenic responses, both processes provide a target for the action of immunosuppressive drugs. Immunophilin-binding immunosuppressive drugs, including cyclosporin A (CsA), FK506, and rapamycin (RAPA), initially selected for their effects on T cells, also have profound immunomodulatory effects on DC function. RAPA induces strong apoptosis in DCs (6–8) and impairs Ag uptake via both receptor-mediated endocytosis and macropinocytosis, whereas the calcineurin-inhibitors CsA and FK506 demonstrated some effects on DC maturation without affecting their survival or Ag uptake capacity (8–11).

Sanglifehrin A (SFA) is a recently developed immunosuppressive macrocyclic compound produced by the actinomycetes strain Streptomyces sp. A92-308110 (12, 13). Like CsA, SFA binds to the immunophilin cyclophilin A but with an affinity that is 20-fold higher than that of CsA (12). Like other immunophilin-binding drugs, the immunosuppressive activities of SFA and its mechanism of action have been mostly studied on T cells. The cyclophilin-SFA complex does not interact with calcineurin and does not affect calcium-dependent IL-2 production (14). Rather, it has been demonstrated to inhibit IL-2-dependent T cell proliferation, which resembles the action of RAPA (15). Furthermore, SFA shows some inhibitory activities on IgG production by B cells, on TNF-α production by monocytes (14), and on IL-12 production by DCs (16).

In the present study the effect of SFA on human DC differentiation and function was investigated in detail. SFA influenced the differentiation of human monocyte-derived DCs resulting in cells that normally lost their expression of CD14 and lacked the expression of CD1a. Importantly, DCs generated in the presence of SFA demonstrated reduced macropinocytosis and lectin-mediated endocytosis, which was in line with a decreased expression of C-type lectins, such as mannose receptor (MR) and DC-specific ICAM-3-grabbing nonintegrin (SIGN). In contrast, FcαRI (CD89) and FcγRII (CD32) were increased by SFA. Its explicit effect on the expression of Ag uptake receptors and Ag capture makes SFA unique among immunophilin-binding immunosuppressive drugs.
Materials and Methods
Reagents
SFA was provided by Novartis Pharmaceuticals (Basel, Switzerland). A stock solution (10⁻³ M) of SFA was made in methanol, which was further diluted in medium directly before use at the concentrations indicated. CsA (Neoral, Novartis Pharmaceuticals) and RAPA (Calbiochem, Cambridge, MA) were used at 10⁻⁶ M final concentration. Propidium iodide was purchased from Molecular Probes (Leiden, The Netherlands) and used at 10 μg/ml final concentration.

Generation monocyte-derived DCs
DCs were generated as previously described (9). In brief, human monocytes were isolated from buffy coats obtained from healthy donors using ficoll-hypaque (Sigma-Aldrich, St. Louis, MO) and Percoll (Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation, followed by plastic adherence (2 h). DCs were generated in 6-well culture plates (Costar, Cambridge, MA) in RPMI 1640 containing 10% heat-inactivated FCS and penicillin/streptomycin supplemented with 5 ng/ml GM-CSF (Leucomax, Novartis Pharmaceuticals) and 10 ng/ml IL-4 (PeproTech, Rocky Hill, NJ) for at least 6 days. SFA-DCs were generated by addition of SFA from day 0 (monocyte stage) onward, unless otherwise indicated.

Analysis of cell surface molecules by FACS
Cells were harvested and washed in buffer containing 1% BSA, 1% heat-inactivated normal human serum, and 0.02% NaN₃. FACS analysis was performed using mAbs against CD1a (Leu-6) and CD14 (Leu-M3)(both BD Biosciences, San Jose, CA). HLA-DR (clone B8.11.2), CD86 (IT2.2; BD PharMingen, San Diego, CA), FcγRII/CD16 (3G8), FcγRI/CD32 (IV.3 and AT10), and FcγRI/CD64 (nr.22) (all provided by J. G. J. van de Winkel, Universitair Medisch Centrum, Utrecht, The Netherlands), MR/CD206 (D547.3; kindly provided by F. Koning, Leiden University Medical Center, Leiden, The Netherlands), FcγRI/CD89 (D21; see Ref. 17), DC-SIGN/CD209 (AZN-D1, a gift of Y. van Kooyk, VU Medisch Centrum, Amsterdam, The Netherlands), DC-asialoglycoprotein receptor (ASGPR) (AG-5; a gift of S. Sacland, Schering-Plough, Dardilly, France), C1qRP/CD93 (R139; see Ref. 18 and kindly provided by A. J. Tenner, University of California, Irvine, CA). Staining was visualized by PE-conjugated goat anti-mouse Ig (Dako, Glostrup, Denmark) and assessed for fluorescence intensity by flow cytometry (FACSCalibur, CellQuest software; BD Biosciences). Dead cells, characterized by propidium iodide uptake, were excluded from analysis.

Allogeneic MLR
Responder T cells used for allogeneic MLR assays were isolated by sheep erythrocyte rosetting of mononuclear cells obtained from healthy donors. Stimulator cells were irradiated (50 Gy) and added in graded doses to 1.5 × 10⁵ allogeneic T cells in 96-well U-bottom tissue culture plates in RPMI 1640 containing 10% heat-inactivated FCS (0.2 ml/well). Cell proliferation was quantified by incubating the cells during the last 8 h of the 5-day cultures with 1 μCi (37 kBq) of [methyl-3H]thymidine (NEN, Boston, MA). Results are presented as the mean cpm ± SD obtained from triplicate cultures, or as mean cpm ± SEM from five independent experiments.

Ag uptake assays
Fluid phase endocytosis (macropinocytosis) was measured as the cellular uptake of 100 μg/ml Lucifer yellow (LY) dipotassium salt (Molecular Probes) or 0.2 μg/ml BSA-FITC (BSA-FITC, 1:12 molar ratio; Sigma-Aldrich), whereas lectin-mediated endocytosis was measured as the cellular uptake of 0.2 μg/ml α-D-mannosylated-BSA-FITC (manBSA-FITC, 1:2.5 molar ratio; Sigma-Aldrich) or 100 μg/ml dextran-FITC (dextran-FITC, m.w. 40,000; Molecular Probes). Approximately 5 × 10⁵ DCs were incubated at 37°C in the presence of culture medium or dextran-FITC. Lectin-mediated uptake was blocked by 15 min preincubation with 50 mM D-mannose (Sigma-Aldrich). Negative controls were incubated with the respective Ag at 4°C. Ag uptake was stopped by extensive cold washing in PBS-1% and FCS-0.02% Na₂CO₃, and cell surface fluorescence was quenched with trypan blue (Sigma-Aldrich). Ag uptake was evaluated by flow cytometry.

Statistical analysis
Phenotypic analyses of DCs cultured either with or without SFA and presented as histograms are representative for at least five independent experiments with different donors. Statistical differences in endocytic capacity of DCs cultured either in the presence or absence of SFA (10⁻⁶ M) were analyzed after 30 min of incubation and are representative for at least six independent experiments. Mean fluorescence intensities were analyzed with Student’s t test for paired samples. Results were considered significant if p < 0.05.

Results
Effects of the cyclophilin-binding compounds CsA and SFA on DC morphology and phenotype
Monocytes were cultured in the presence of IL-4 and GM-CSF. After 6 days of culture, a nonadherent cell population with protruding veils typical for DCs was obtained. When similar cultures were performed in the presence of the cyclophilin-binding drugs CsA (10⁻⁶ M) or SFA (10⁻⁶ M), or the solvent methanol, cells showed a comparable DC morphology (Fig. 1A). Addition of SFA or methanol did not affect cell yields, whereas CsA slightly decreased cell survival as determined by trypan blue exclusion (Fig. 1B).

DCs, either cultured under normal conditions or with CsA, demonstrated the typical DC phenotype: both CD1a and DC-SIGN were clearly induced, CD14 was completely down-regulated, and DCs demonstrated a relative low expression of CD86 and HLA-DR, which is characteristic for immature DCs (Fig. 2). In contrast, SFA inhibited the induction of CD1a (p = 0.005), reduced the induction of DC-SIGN (p = 0.025), but did allow the down-regulation of CD14. Although SFA seemed to increase the expression of CD86 and HLA-DR, a significant difference compared with control cultures could not be observed (p > 0.05). Thus, although both CsA and SFA are known to exert their immunomodulatory effect via binding to cyclophilin A, only SFA does significantly change the DC phenotype.

SFA does not affect T cell stimulatory capacity of DCs in allogeneic MLR
Next, the effect of SFA on the T cell stimulatory capacity of DCs was investigated in allogeneic MLRs. Addition of SFA to control conditions did not affect cell yields. Monocytes were cultured for 6 days with IL-4 and GM-CSF under control conditions (+), or in the presence of CsA (10⁻⁶ M), SFA (10⁻⁶ M), or methanol (1/10000 dilution). A. The morphology of the cells (original magnification ×200) and the relative cell yields obtained by trypan blue exclusion are shown. B. The mean ± SEM cell recovery compared with control cultures of three independent experiments with different donors is shown.
MLRs showed a dose-dependent inhibition of T cell proliferation (IC_{50}, 10^{-8} – 10^{-7} M) (Fig. 3A). Addition of 10^{-6} M SFA to day 6 immature DCs did not decrease their T cell stimulatory capacity (data not shown), which was in line with the lack of significant changes in the expression of costimulatory molecules or with LPS-induced or CD40 ligand-induced up-regulation of these molecules (data not shown).

DCs generated in the presence of 10^{-6} M SFA from day 0 onward demonstrated a slightly decreased T cell stimulatory capacity (Fig. 3B). Cocultures of SFA-DCs with allogeneic T cells in a 1:10 ratio resulted in a relative T cell proliferation of 93% (range from 76% to 112%, p > 0.05) as compared with coculture with control DCs. Only cocultures of SFA-DCs and T cells in a 1:300 ratio resulted in a significantly decreased T cell proliferation (reduction: 54 ± 11%, p = 0.0065).

**SFA decreases both lectin-mediated and fluid phase endocytosis**

We next analyzed whether Ag uptake was influenced by SFA. Lectin-mediated endocytosis and fluid phase endocytosis were investigated by analyzing the uptake of dextran_{PITC} or manBSA_{PITC} and LY or BSA FITC, respectively. Ligand specificity was confirmed by blocking studies with α-mannose, which demonstrated a strong inhibition in the uptake of dextran_{PITC} without changing the uptake via fluid phase endocytosis (Fig. 4A).

DCs cultured in the presence of SFA showed significantly decreased lectin-mediated endocytosis (reduction: mean 47%; range from 25% to 70%, p = 0.0045) of dextran_{PITC} (Fig. 4B) and manBSA_{PITC} (data not shown), which was already detected after 5 min of incubation and became more clear upon longer incubation time (Fig. 4C). Interestingly, also the fluid phase endocytic capacity was significantly decreased by SFA (reduction: mean 46%, range 30% to 62%, p < 0.0001) (Fig. 4, D and E). Dose-response analysis revealed that SFA inhibited the fluid phase and lectin-mediated endocytosis to the same extent (IC_{50}, 10^{-8} – 10^{-7} M) (Fig. 4F). The inhibitory effects on Ag uptake were not due to a generic toxic effect because the incidence of cell death on day 6 of culture was not significantly affected by SFA, as determined by trypan blue staining and propidium iodide uptake (Fig. 1B and data not shown).

**SFA interferes with the induction of C-type lectins during DC development**

Surface bound C-type lectins are responsible for the uptake of glycosylated Ags. DC-SIGN is one of a growing number of C-type lectins that can be found on the surface of DCs. The decreased ability of SFA-DCs to take up glycoproteins like dextran_{PITC} and manBSA_{PITC} may be explained by the down-regulation of C-type lectins, as observed for the decreased expression of DC-SIGN (Fig. 2). Therefore, the effect of SFA on the expression of the MR/CD206, which belongs to the type I family of C-type lectins, and of the type II lectins DC-ASGPR, DC-SIGN/CD209, and C1qRP/CD93 was investigated in more detail. Control DC cultures demonstrated a high expression of MR and DC-SIGN and a moderate expression of DC-ASGPR and C1qRP, as shown previously by others (4, 19–21). As observed for the expression of DC-SIGN, DCs generated in the presence of SFA showed a decreased expression of all lectins tested (Fig. 5A). A detailed dose-response analysis revealed that significant inhibitory effects on the expression of MR and DC-SIGN could be observed from 10^{-8} M SFA (Fig. 3B), SFA down-regulated the expression of DC-SIGN ~100-fold more efficiently than the expression of MR.

Less pronounced effects were observed when SFA was added later during the differentiation period. Although SFA decreased the expression of lectins, of lectin-mediated uptake (Fig. 5C) and of fluid phase endocytosis (data not shown) when added to already differentiated DCs, this reduction was less pronounced compared with cells exposed to SFA from day 2 onward and especially from day 0 onward.

RAPA is another immunophilin-binding immunosuppressive drug known to inhibit the endocytic capacity of human DCs (8). The presence of RAPA during DC development resulted in cells with a relatively low expression of MR and a reduced endocytic capacity (Fig. 6A). In contrast to SFA, RAPA did not decrease CD1a or DC-SIGN expression, but rather increased the expression of the latter molecules. Moreover, RAPA strongly induced cell death (50% reduced cell yield compared with control), which is also clear from forward/side scatter plots (Fig. 6B) and propidium iodide staining (data not shown) (6). The very low cell viability of cells cultured in the presence of RAPA may contribute to the differences in function and phenotype.

**SFA specifically increases the expression of FcγRII (CD32) and FcαRI (CD89)**

Next to C-type lectins, Fc receptors also play an important role in Ag capture. The expression of the FcαR (FcαRI/CD89) and the FcγR (FcγRII/CD16, FcγRII/CD32, FcγRI/CD64) were analyzed upon DC generation either in the presence or absence of SFA.
starting at a concentration of 10^{-8} M. As immature cells, scattered throughout the body, DCs have the capacity to recognize and take up a wide range of self and non-self Ags. DCs use different mechanisms, including fluid phase and receptor-mediated endocytosis, for the uptake of Ags, such as C-type lectin and Fc receptor-mediated uptake. DCs discriminate self from non-self using conserved pattern-recognition receptors, which recognize molecular patterns at the cell surface of all microorganisms. Receptors of this type include Toll-like receptors (22, 23) and C-type lectins (24). Some of the C-type lectins are shared with other cell types, such as the MR, whereas other receptors are more DC restricted, e.g., DEC205/CD205, which has very low expression on human blood DCs; Langerhans cells and in vitro-cultured DCs; Langerin/CD207, which is exclusively expressed on Langerhans cells; DC-SIGN/CD209 expressed on interstitial DCs; and a small subset of blood DCs, including an ASGPR expressed on interstitial DCs and BDCA-2 on plasmacytoid DCs (19, 25).

SFA is the first immunosuppressive drug that shows inhibitory effects on the expression of DC-SIGN. DC-SIGN is an adhesion receptor important for several DC functions (26). DC-SIGN can interact with the carbohydrate-bearing self-glycoproteins ICAM-2 and ICAM–3 to mediate chemokine-induced transmigration of DCs across endothelium (27) and transient adhesion between DCs and T cells enabling complete TCR engagement (21), respectively. In addition, C-type lectins like DC-SIGN recognize pathogens through their carbohydrate profiles and internalize the pathogen for Ag processing and presentation (25, 28, 29). Detailed knowledge about pathogenic targets as well as cellular ligands, including the identity of the carbohydrate structure they recognize, is lacking for most of these receptors. Concerning pathogen-recognition, DC-SIGN is involved in binding of HIV-1, CMV, and Ebola virus resulting in enhanced cis and trans infection (21, 30, 31). Recently, it was found that DC-SIGN also recognizes nonviral pathogens, including Mycobacterium tuberculosis, Helicobacter pylori, Leishmania mexicana, and Schistosoma mansoni (32, 33). It appears that viral pathogens target DC-SIGN for transmission, whereas nonviral pathogens modulate DC-induced immune activation (34). Next to DC-SIGN, the MR has been shown to recognize mycobacteria and fungi/protozoa (35), and probably also other C-type lectin-pathogen interactions will be discovered in the future. This means that an altered expression of C-type lectins such as DC-SIGN and MR may have profound effects on the infection efficiency and subsequent immune response to these types of pathogens.

Next to C-type lectins, DCs use Fc receptors to efficiently internalize Ag. Fc receptors evolved to bind the Fc fragment of Igs for uptake of Ags opsonized with specific Abs. IgG and IgA, which bind to FcγR and FcαR, respectively, are the most abundant Ig isotypes present in human blood. Two types of FcγRs exist: activating and inhibitory FcγR. Activating FcγRs include FcγRI, FcγRIIb, and FcγRIII (36). FcγRIIa, which does not exist in mice but does exist in human, inhibits cell activation through immunoreceptor tyrosine-based inhibitory motifs. Human immature monocyte- and CD34-derived DCs only express FcγRII/CD32 (9, 37, 38)(Fig. 6), but the relative expression of activating and inhibitory isoforms of CD32 is not known. Ligation of FcγR is known to

Discussion

Modulation of T lymphocyte function by SFA is well documented. The present study demonstrates that SFA also interferes with DC function. SFA strongly decreases the efficacy of Ag uptake by DCs through at least two means: by fluid phase endocytosis (or macropinocytosis), as shown by LY uptake, as well as by lectin-mediated endocytosis, as shown by dextran^{FITC} uptake. The decreased uptake of dextran^{FITC} can be explained by the inhibitory effect of SFA on the expression of C-type lectins, such as MR and DC-SIGN. Interestingly, SFA increased the expression of the Fc receptors CD89 and CD32. Both the inhibitory as well as the stimulatory effects of SFA were dose-dependent and already observed with 10^{-8} M. Control DCs showed expression of CD32 and CD89 surface molecules, but did not express CD16 and CD64 (Fig. 7A), as previously demonstrated (9). DCs generated in the presence of SFA were also negative for CD16 and CD64, but demonstrated an increased expression of CD32 and CD89. CD32 and CD89 were up-regulated to the same extend and in a dose-dependent manner, starting at a concentration of 10^{-8} M SFA (Fig. 7).
induce the maturation of mouse DCs, whereas targeting FcγRs on human DCs only partially triggers DC maturation (38, 39). IgG-induced semimature human DCs do not produce IL-12, but demonstrated an increased production of IL-10 in a CD32-dependent
manner (38), making these DCs rather tolerogenic than immunogenic (40, 41).

The best characterized receptor for IgA in humans is FcoRI/CD89. Both in vivo and in vitro, it has been shown that CD89 is
expressed on interstitial DCs, but not Langerhans cells (9, 42, 43). As was found for CD32 cross-linking on DCs, CD89 triggering with IgA immune complexes resulted in rapid internalization and subsequent semimaturation of DCs, characterized by a partially increased CD86 expression and high IL-10 production (42).

The molecular mechanism by which SFA exerts its immunomodulatory effects remains to be elucidated. With regard to the effects of SFA on DC function, it seems that SFA is more closely related to RAPA than to CsA, as found for its effect on T cells (14, 15). Calcineurin-inhibition with either FK506 or the calcineurin-A

FIGURE 5. The effect of SFA on the expression of C-type lectins. A, DCs were generated under control conditions or in the presence of SFA (10⁻⁶ M) or methanol. Cell surface expression of the indicated C-type lectins was analyzed by flow cytometry as described in Materials and Methods. Data shown are representative of five independent experiments. B, DCs were generated in the presence of different concentrations of SFA. On day 6, cells were analyzed for their expression of MR and DC-SIGN by flow cytometry. Data shown are the mean (± SEM) of relative expressions of MR or DC-SIGN compared with the expression on control DC of three independent experiments with different donors. C, Monocytes were cultured for 7 days with IL-4 and GM-CSF. SFA (10⁻⁶ M) was added to the cultures on day 0, day 2, or day 6. Cell surface expression of the indicated proteins and uptake of dextranFITC was analyzed by flow cytometry as described in Materials and Methods. Experiment was performed in duplicate and is representative of three independent experiments.
binding drug CsA affected neither Ag uptake receptor expression nor the endocytic activity of DCs (8–10) (data not shown). In contrast, we observed that RAPA, in accordance with previous reports (8), decreased the expression of MR and also reduced fluid phase (data not shown) and lectin-mediated endocytosis as was found for SFA. However, RAPA also dramatically induced cell death (6), which may account for the decreased functional capacity of the cells. SFA increased the expression of CD32, an effect that has not been observed with RAPA (8). Delayed exposure of developing DCs to SFA showed less pronounced effects on DC function and phenotype. This observation may explain the apparent discrepancy between the present data and the recent study of Steinschulte et al. (16) in which it was demonstrated that CD1a expression was not significantly affected when SFA was added on day 2 of DC differentiation. The latter study did not comment on Ag receptor expression or endocytosis. Furthermore, both RAPA (our unpublished observation and Ref. 8) and SFA are very active when present during DC development, but when added to already differentiated immature DCs the effects on phenotype and endocytosis are less and sometimes even absent. These data suggest that SFA influences the development of the endocytic machinery by targeting a signaling molecule already present in monocytes. In view of the effect on endocytosis and the decreased ability to produce IL-12 (16) it is most likely that this target remains present in differentiated DCs.

Controversial data exist about the effect of CsA on the T cell stimulatory capacity of DCs. It seems that both CsA (11) and SFA, if so, only moderately decrease their ability to stimulate T cells. It has been suggested that RAPA partially reduces the T cell stimulatory capacity of human DCs in allogeneic MLRs (8), but the observed reduction in T cell proliferation may be explained by a decreased DC viability due to RAPA-induced apoptosis (6, 8). The tendency of a decreased potency of SFA-DCs to activate T cells can be the consequence of a decreased expression of DC-SIGN, as adhesion molecules are important in DC-T cell interactions (21), and/or the consequence of a decreased production of growth factors such as observed for IL-12 (16). However, also inevitable carry over effects of the drug cannot be excluded. The role of CD1

![FIGURE 6. RAPA affects cell phenotype, function, and viability. DCs were generated under control conditions or in the presence of either SFA or RAPA (10⁻⁶ M). Cell surface expression of the indicated proteins and uptake of dextranFITC (A) were analyzed by flow cytometry as described in Materials and Methods. Cells were gated on forward/side scatter (B) and propidium iodide-positive cells were excluded from analysis. Experiment was performed in duplicate and is representative of two independent experiments.]

![FIGURE 7. SFA dose dependently increases the expression of FcγRII and FcεRI. A, DCs were generated under control conditions or in the presence of SFA (10⁻⁷ M). Cell surface expression of the indicated Fc receptors was analyzed by flow cytometry as described in Materials and Methods. Data shown are representative for one of five independent experiments. B, DCs were generated under control conditions or in the presence of SFA (10⁻⁷–10⁻³ M). On day 6, cells were analyzed for their expression of CD32 (FcγRII) and CD89 (FcoR) by flow cytometry. Shown are the relative expressions of CD32 and CD89 compared with the expression on control DC of two independent experiments with different donors.]
molecules on DCs remains largely unknown. Nevertheless, it is not expected that CD1a plays a major role in the stimulation of allo-
geneic T cells. However, the lack of CD1a on SFA-DCs may have an
effect on Ag presentation as they present lipid Ags to T cells (44), unlike the evolutionary related MHC class I and II molecules, which display peptide Ags.

In conclusion, its explicit effect on the expression of Ag uptake receptors and Ag capture without affecting DC viability makes SFA unique among immunophilin-binding immunosuppressive

drugs. Until now, nothing is known about the in vivo effects of

SFA on Ag uptake and presentation. Blocking DC endocytic activity by reducing the expression of C-type lectins would reduce the likelihood of T cell priming through indirect presentation and

may be a relevant strategy to induce immunosuppression. In ad-
dition, down-regulation of C-type lectins, especially DC-SIGN

may be useful in viral therapies.

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