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CD85j (Leukocyte Ig-Like Receptor-1/Ig-Like Transcript 2) Inhibits Human Osteoclast-Associated Receptor-Mediated Activation of Human Dendritic Cells[1]

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Immature dendritic cells (DCs) derived from freshly isolated human monocytes were used to evaluate the effect of the inhibiting receptor CD85j (leukocyte Ig-like receptor-1/ILT2) on activation induced by cross-linking of the human osteoclast-associated receptor (hOSCAR). CD85j and hOSCAR were expressed consistently at the same density on monocytes and on monocyte-derived DCs (both immature and mature). Cross-linking of hOSCAR, which activates via the FcR-associated γ-chain, induced Ca^{2+} flux in DCs. Concomitant cross-linking of anti-CD85j mAb abolished this early activation event. Likewise, CD85j stimulation strongly reduced IL-8 and IL-12 production by hOSCAR-activated DCs. Inhibition of DCs via CD85j also impaired their ability to enhance Ag-specific T cell proliferation induced by hOSCAR. Finally, because hOSCAR prevents apoptosis of DCs in the absence of growth/survival factors, CD85j cross-linking was able to counteract completely this antiapoptotic effect and to reduce Bcl-2 expression enhanced by hOSCAR stimulation. Thus, CD85j is an inhibiting receptor that is functional in human DCs. ***The Journal of Immunology,*** **2005, 174: 6757–6763.**

Dendritic cells (DCs)[6] are APCs that play a central role in the initiation of primary immune responses (1, 2). Upon stimulation, DCs secrete cytokines that are crucial for the regulation of innate resistance and adaptive immunity and undergo a process of maturation characterized by remarkable morphological and functional changes. The maturational state affects the ability of DCs to take up, process, and present Ags. Immature DCs located in peripheral tissues act as immune sentinels and are able to capture and process Ags due to their high endocytic activity (3, 4). Following activation and maturation induced by proinflammatory signals, DCs migrate to the T cell areas of secondary lymphoid organs, where they are able to present Ags to naive T cells. The outcome of the immune response (immune priming or tolerance) depends on the maturational status of DCs (5), which is generally believed to be regulated by endogenous factors such as proinflammatory cytokines (TNF and IL-1) (3, 6–8) or by exogenous factors such as pathogen-associated molecular patterns (e.g., LPS, dsRNA, and Cpg-DNA) (9, 10) recognized by specific receptors on the DC surface, such as the family of TLRs.

DC activity also can be regulated by both activating and inhibiting receptors that transduce signals via ITAM (11) and ITIM (12, 13), respectively. ITIM-bearing receptors negatively regulate cell functions when coligated with stimulating receptors that signal through ITAM. This growing family of receptors was described first in lymphocytes (14–16). However, in recent years, an increasing number of receptors that are members of the lectin family and of the Ig superfamily and are linked to ITAM/ITIM signaling have been described on myeloid cells and DCs, such as FcγR, C-type lectin receptors (DC immunoactivating receptor), Ig-like transcript (ILT)3, ILT4, and FDF03 (17–22).

Among ITIM-bearing inhibiting receptors, the leukocyte Ig-like receptor (LIR)/ILT molecular family (Ref. 23 and reviewed in Ref. 24) is encoded by at least 10 genes, and these proteins belong to the Ig superfamily. The products of some of these genes, such as LIR-1/ILT2 (CD85j), are surface membrane inhibiting receptors. CD85j/LIR-1/ILT2 is detected on the surface of a proportion of NK cells (23–77%) and of T lymphocytes (20%), on all B lymphocytes, monocytes, and myeloid DCs (14, 25–27). CD85j consists of four C2 Ig domains and exhibits a molecular mass of 110 kDa under both reducing and nonreducing conditions, suggesting that it is a noncovalently linked monomer (25, 26). Its inhibiting function is mediated by tyrosine phosphorylation of four ITIM-like sequences in its cytoplasmic tail. Tyrosine phosphorylation by $p56^{lck}$ in T cells leads to recruitment of Src homology protein (SHP)-1, SHP-2, and Src homology 2 domain-containing inositol phosphatases (22). Recruitment of these phosphatases down-regulates the signaling mediated by activating receptors (23, 27, 28).

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[6] Abbreviations used in this paper: DC, dendritic cell; ILT, Ig-like transcript; SHP, Src homology protein; OSCAR, osteoclast-associated receptor; LIR, leukocyte Ig-like receptor; h, human; mono-DC, monocyte-derived DC; m, mouse; GAM, goat anti-mouse; $DiOC_6$, 3,3-dihexyloxacarbocyanine iodide; SIRP, signal regulatory protein.

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We have described previously that CD85j is present and functional in the cytoplasm of all T cells, independently of its surface expression (14, 29). Ligands for CD85j include the nonclassical class I HLA-G protein (30), some alleles of *HLA-A* and *-B* loci, and the human CMV *UL18* gene product, a viral homolog of HLA class I (26). CD85j shows a differential affinity for these ligands, e.g., 1000 times higher for UL18 than for HLA-A2 and 4 times higher for HLA-G than for classical HLA molecules (31, 32). Although it is unknown whether the different affinity could play a role in CD85j function, the interaction of CD85j with UL18 leads to activation of T lymphocytes, resulting in the lysis of human (h)CMV-infected cells, despite the defined inhibiting functions of CD85j (33). Likewise, UL18-expressing transfectants are lysed by human NK clones (34).

Several groups have reported the presence of the LIR/ILT family of receptors on APCs. It has been shown that these receptors function as negative regulators of monocyte and DC activation, presumably through recruitment of SHP-1 (20, 27, 35*)*.

We recently described the human osteoclast-associated receptor (hOSCAR), a novel immune receptor associated with the FcRγ chain (FcRγ) and involved in endocytosis and Ag presentation through the MHC class II pathway in monocyte-derived DCs (mono-DC) (36). Its association with the ITAM-bearing FcRγ confers to hOSCAR the capacity to activate myeloid cells as shown by its ability to trigger calcium flux and cytokine release (36). We also showed that hOSCAR ligation induced phenotypic and functional maturation of DCs, elicited cytokine and chemokine secretion, and synergistically amplified the TLR-induced ability of DCs to prime naive T cell proliferation (37). Unlike hOSCAR, mouse (m)OSCAR is expressed only on osteoclasts (38), which, like certain DC subsets, derive from the myeloid lineage. Data from different groups strongly suggest that in vivo ligation of mOSCAR on osteoclasts is essential for the differentiation of these cells. The existence of an endogenous ligand for mOSCAR on osteoblasts has been inferred from this work (38).

In this study, mono-DCs have been activated by cross-linking of several surface molecules (CD1a, HLA-DR, and hOSCAR) with mAb, and the inhibiting ability of CD85j has been tested subsequently. hOSCAR (36, 37) provided the strongest activating signal for DCs and was therefore chosen for all of the experiments. We show that CD85j inhibits intracellular calcium mobilization and cytokine secretion triggered by ligation of hOSCAR. Moreover, CD85j prevents the rescue from apoptosis and the cooperation between DCs and Ag-specific T cells that is mediated by hOSCAR.

Materials and Methods

Cell cultures

Human peripheral blood samples were obtained from healthy donors according to institutional guidelines. PBMCs were purified by Ficoll-Hypaque density gradient centrifugation. Monocytes and $CD4^+$ T lymphocytes were isolated by positive selection with magnetic beads coated with mAb to CD14 and CD4 (MACS; Miltenyi Biotec). To induce DC differentiation, purified monocytes were cultured for 5 days in the presence of 200 ng/ml recombinant human GM-CSF and 10 ng/ml recombinant human IL-4 (Schering-Plough Research Institute). These cells are termed immature DCs. Maturation was achieved by culturing immature DCs for an additional 24 h in the presence of 10 ng/ml LPS (Sigma-Aldrich). Cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS, 5 mM L-glutamine, and 50 IU/ml penicillin-streptomycin (from here on referred to as complete medium).

Antibodies

mAb used for immunophenotypic and functional analyses were as follows: anti-CD85j purified Ab (clone GHI/75; BD Pharmingen) or ascites (clone HP-F1, provided by Dr. M. Lopez-Botet, Molecular Immunopathology Unit, Universitat Pompeu Fabra, Barcelona, Spain); anti-OSCAR mAb 11.1CN5; anti-CTLA-4 (CH7.3-624 ascites, provided by Dr. A. Lanzavecchia, Institute for Research in Biomedicine, Bellinzona, Switzerland); anti-CD31 (clone Moon-1, provided by Dr. F. Malavasi, Laboratory of Immunogenetics, University of Torino, Italy); anti-HLA-DR (D1.12, a gift from Dr. C. Gelin, Institut National de la Santé et de la Recherche Médicale, Unité 396, Institut d'Hématologie, Hôpital Saint-Louis, Paris, France); and anti-Bcl-2 (DakoCytomation). Other mAb used for surface phenotype analysis were PE-conjugated anti-CD14, anti-CD1a, and anti-HLA-DR (BD Pharmingen).

Flow cytometry

The surface phenotype of monocytes and mono-DCs was assessed by flow cytometric analysis (FACSCalibur; BD Biosciences). The secondary reagent was PE-labeled goat anti-mouse (GAM) antiserum (Southern Biotechnology Associates).

Measurement of intracellular Ca^{2+} flux

Intracellular Ca^{2+} mobilization was determined as described previously (19), with modifications. Briefly, mono-DCs were loaded with 10 μM Fluo-3AM and 5 μM Pluronic F-27 (Molecular Probes) for 30 min at 37°C. mAb (10 μg/ml) was added to human serum-saturated cells and then cross-linked with 20 μg/ml GAM antiserum (Jackson ImmunoResearch). For inhibition experiments where anti-hOSCAR was used in combination with HP-F1, anti-CTLA-4, or anti-CD31, the latter mAb were added 30 s before anti-hOSCAR. Cells were analyzed subsequently by flow cytometry (FACSCalibur) to detect Ca^{2+} fluxes. Only viable cells (based on forward scatter criteria) and Fluo-3AM-loaded cells (based on 405- vs 530-nm emission spectra) were analyzed. A positive control was obtained by addition of ionomycin.

Cell activation and cytokine assays

Anti-hOSCAR, purified anti-CD85j, and anti-CD31 (as isotype- matched control Ab) were coated overnight at 4°C on flat-bottom plates at a final concentration of 20 μg/ml in PBS. The ascites HP-F1 and anti-CTLA-4 (as ascites control) were used at 1/100 dilution. In inhibition experiments, HP-F1 and purified anti-CD85j, or anti-CD31 and anti-CTLA-4, were coated in the same well together with anti-hOSCAR at the concentrations indicated above. Immature DCs were plated at 1×10^6 cells/ml. *Escherichia coli* LPS was used at the final concentration of 10 ng/ml. Supernatants were collected after 24 h and tested for the presence of IL-8 and IL-12 p40 by ELISA (Diaclone Research).

Proliferation assays

DCs and $CD4^+$ T lymphocytes were obtained as described above. Immature DCs were prelabeled with 10 μg/ml mAb and cross-linked with GAM antiserum (Jackson ImmunoResearch) for 30 min in the cold, washed, and pulsed with recall Ags (*Candida albicans* bodies, 3×10^5/ml, or purified protein derivative, 10 μg/ml). Proliferative responses were measured by culturing 3×10^4 pretreated immature DCs in the presence of 10^5 $CD4^+$ autologous T lymphocytes in 0.2 ml of complete medium, in 96-well flat-bottom microtiter plates. Cultures were pulsed with 0.5 μCi of [^{3}H]thymidine (Amersham Biosciences) on day 4 and harvested 18 h later. Dry filters with scintillation fluid were counted in a gamma counter.

Detection of apoptosis

After DC differentiation, cells were collected and washed four times with PBS to remove GM-CSF and IL-4. Immature DCs were stimulated for 3 days with coated mAb in complete medium, as described above. Cultures were harvested, and the apoptotic cells were detected using the Annexin V^{FITC} kit (BD Pharmingen). To measure mitochondrial membrane potential, another indicator of apoptosis, cells were incubated in complete medium containing 25 nM 3,3-dihexylaxocarbocyanine iodide ($DiOC_6$) (Molecular Probes) for 30 min at 37°C in the dark, followed by flow cytometric analysis. Intracellular labeling with FITC-conjugated mAb to Bcl-2 (DakoCytomation) was performed as described elsewhere (39).

Statistical analyses

Differences in cell proliferation and cytokine production between hOSCAR-activated DCs and DCs treated concomitantly with anti-CD85j and anti-hOSCAR mAb were observed. To assess their statistical significance, Student's t test was used with a level of $p < 0.05$.

Results

CD85j is expressed on the surface of freshly isolated monocytes and of immature and mature DCs

Fresh monocytes, isolated by positive selection using magnetic beads coated with anti-CD14 mAb, were cultured for 5 days with GM-CSF and IL-4 (immature DCs), and further stimulated for 1 day with LPS (mature DCs). Cells were tested for the expression of cell surface markers. Freshly isolated monocytes and immature and mature DCs from 15 donors consistently expressed the inhibiting receptor CD85j and the activating receptor hOSCAR, at similar levels (Fig. 1). CD14, an LPS receptor, was down-regulated upon differentiation toward DCs and remained low in both immature and mature DCs, whereas CD1a and HLA-DR were up-regulated sharply in comparison with fresh monocytes. All cell types also expressed CD31 at about the same level (Fig. 1).

In the experiments described below, immature DCs have been used because they yield the best response to both activating and inhibiting stimuli.

CD85j inhibits Ca^{2+} flux induced by the activating receptor hOSCAR

Ligation of hOSCAR was a better activating stimulus than CD1a and HLA-DR (data not shown). As shown in Fig. 2, ligation of hOSCAR triggered a rapid Ca^{2+} flux in immature DCs (*a*). hOSCAR-induced Ca^{2+} flux was prevented completely in the presence of anti-CD85j mAb (HP-F1 or GHI/75) (Fig. 2*b*). In contrast, Ca^{2+} flux induced by cross-linking of hOSCAR was not affected by ligation of the inhibiting receptor CTLA-4 and of CD31 (Fig. 2, *c* and *d*). mAb to CTLA-4 that is not expressed on the DC surface (data not shown) and to CD31 were used in the form of ascites and of purified Abs, respectively, and thus provided isotype-matched controls for the inhibition experiments using HP-F1 (ascites) and GHI/75 (purified Abs). HP-F1 alone did not cause Ca^{2+} mobilization (Fig. 2*e*), whereas ligation of activating molecules, such as CD1a or HLA-DR (22, 27), yielded a low level of Ca^{2+} flux (Fig. 2*f*) that was inhibited by anti-CD85j mAb (data not shown). Thus, the strong signal produced by hOSCAR was used as a model of activation in all of the experiments.

CD85j down-regulates cytokine production induced by hOSCAR

Because stimulation via hOSCAR triggered the production of high amounts of IL-8 and IL-12 p40 by immature DCs (36, 37), we investigated the ability of CD85j cross-linking to inhibit the release of these cytokines. Cell stimulation was obtained by mAb coated onto culture plates to avoid the use of GAM antiserum that could interfere with cytokine detection in the supernatants. Concomitant coating with anti-CD85j, used both as purified mAb and as ascites, yielded a significant decrease of IL-8 and IL-12 p40 secretion (Fig. 3), when compared with stimulation with anti-hOSCAR alone. Cross-linking of CTLA-4 and of CD31 did not affect cytokine secretion mediated by hOSCAR (Fig. 3). In addition, as a positive control for immature DC functions, stimulation via LPS was also performed (Fig. 3). LPS, as shown previously (36), yielded the highest level of cytokine production that was not inhibited by coligation of CD85j (data not shown).

CD85j inhibits the ability of DCs to stimulate Ag-specific T cells

Because cross-linking of CD85j with other receptors decreases cytokine secretion by DCs, we investigated whether inhibition via this receptor affected the cooperation of DCs with T cells.

Because CD85j also inhibits T cell function (14), to prevent a direct effect of HP-F1 mAb on T lymphocytes, immature DCs were pulsed with HP-F1 mAb and GAM antiserum, washed to remove unbound Abs, and then added to purified autologous $CD4^+$ T cells stimulated with recall Ags. In the presence of recall Ags, anti-hOSCAR mAb enhanced $CD4^+$ cell proliferation that was abrogated by the concomitant cross-linking of CD85j on DCs (Fig. 4). Similarly to cytokine secretion, T cell proliferation was not affected by cross-linking of CTLA-4 and CD31. DCs not stimulated by Ag, and DCs or $CD4^+$ T cells alone did not proliferate (Fig. 4).

CD85j prevents the rescue from apoptosis mediated by hOSCAR

In the absence of survival factors such as GM-CSF and IL-4, immature DCs underwent apoptosis from which they were rescued by

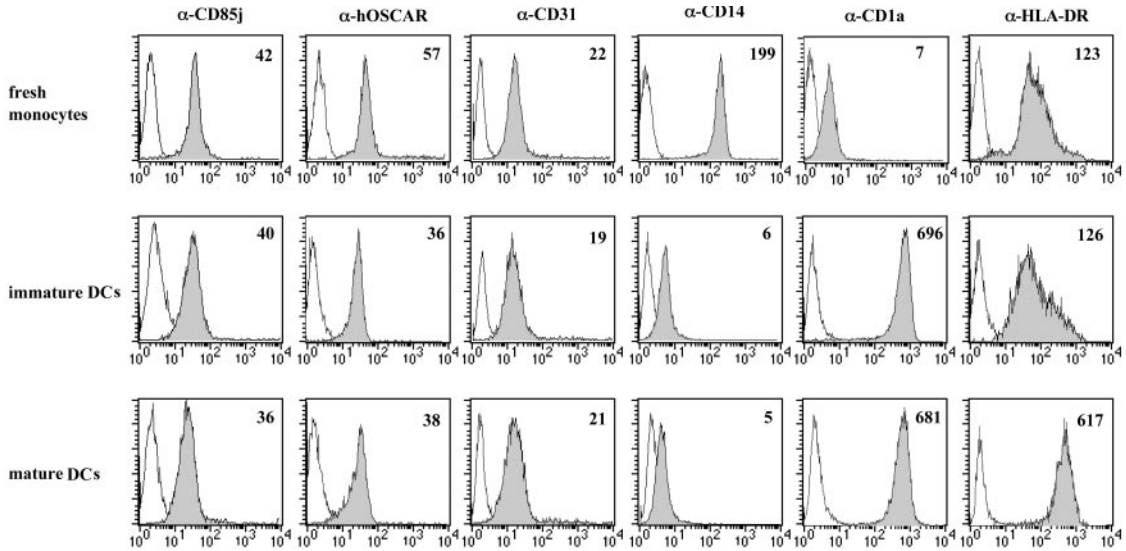

FIGURE 1. Surface phenotype of freshly isolated monocytes and immature and mature DCs. Flow cytometry analyses show that CD85j, hOSCAR, and CD31 are expressed at similar density on freshly isolated monocytes, on cells cultured for 5 days with GM-CSF and IL-4 (immature DCs), and following 24-h stimulation with LPS (mature DCs). In contrast, CD14 is sharply down-regulated on DCs in comparison with monocytes, whereas CD1a and HLA-DR are up-regulated. Numbers indicate mean fluorescence intensity (shaded histograms) with an identical setting of the instrument for all cells analyzed. Empty histograms refer to the binding of an isotype-matched control mAb.

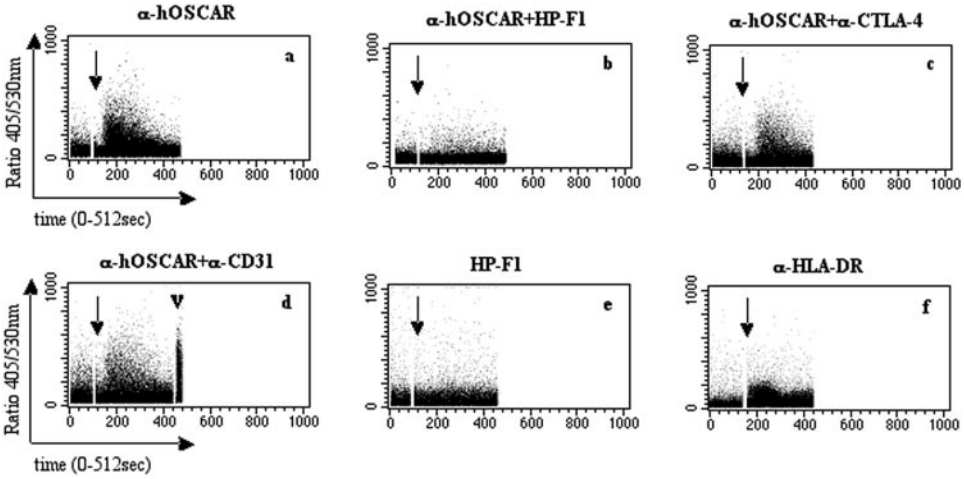

FIGURE 2. Ligation of CD85j in immature DCs inhibits hOSCAR-mediated intracellular Ca^{2+} mobilization. Intracellular Ca^{2+} flux, as determined by the 405/530-nm ratio in Fluo-3AM-loaded cells, is measured from the onset of the experiment. Primary Ab binding and the addition of GAM antiserum is used to allow receptor cross-linking (arrows). In these conditions, cross-linked anti-hOSCAR leads to intracellular Ca^{2+} mobilization (*a*) that is abrogated by concomitant addition of HP-F1 (*b*). Cross-linking of CTLA-4 (*c*), as ascites control, and of CD31 (*d*), as isotype-matched control Ab, does not affect Ca^{2+} influx induced by anti-hOSCAR. Ligation of HP-F1 alone has no effect on Ca^{2+} mobilization (*e*). Cross-linking of HLA-DR yields a moderate Ca^{2+} flux (*f*). Arrowhead in *d* corresponds to the positive control obtained after ionomycin addition. Data are from one representative experiment of five performed.

cross-linking of hOSCAR (37). The ability of CD85j to prevent the rescue of DC apoptosis mediated by hOSCAR was therefore investigated.

Immature DCs were cultured with medium alone and in the presence of plastic-coated mAb. In these conditions or in the presence of irrelevant mAb, immature DCs underwent spontaneous apoptosis after 3 days of culture. DC stimulation via hOSCAR significantly reduced the number of apoptotic cells, detected by Annexin V^{FITC} staining or $DiOC_6$ uptake (see Fig. 5 and Ref. 37). The concomitant cross-linking of CD85j and hOSCAR reconstituted DC apoptosis to levels similar to those of cells cultured in the absence of survival factors (Fig. 5). Likewise, CD85j cross-linking decreased the expression of Bcl-2, an antiapoptotic molecule, the expression of which is enhanced by hOSCAR stimulation (Fig. 5).

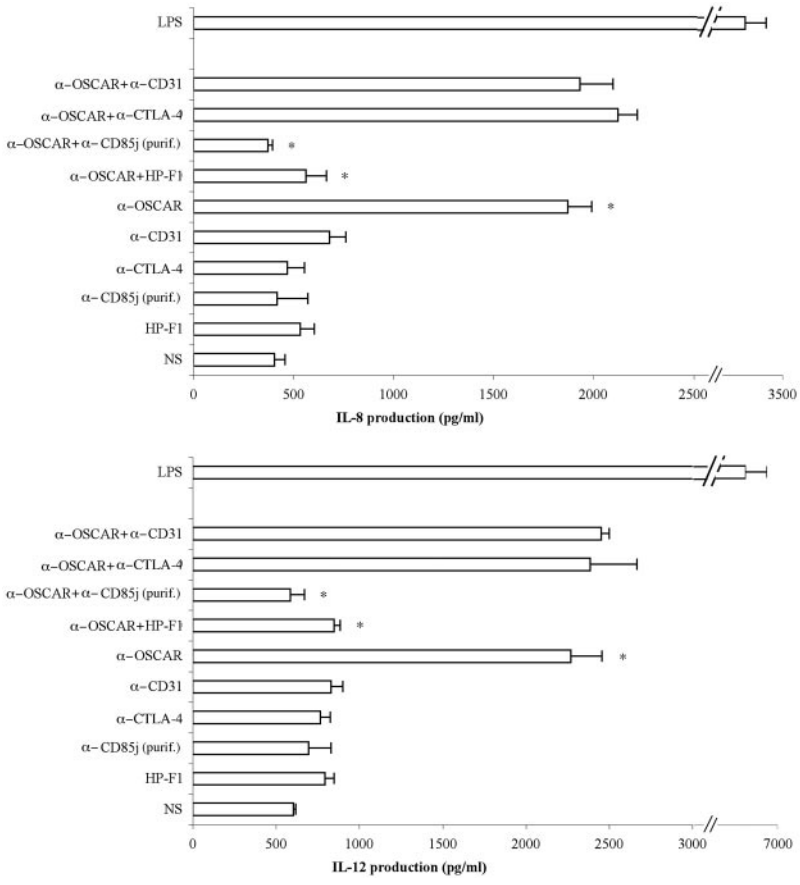

FIGURE 3. Cross-linking of CD85j in immature DCs down-regulates the secretion of IL-8 and IL-12 p40 induced by hOSCAR. Immature DCs are stimulated with coated mAb or LPS, as described in *Materials and Methods*. After 24 h, supernatants are tested by ELISA for the presence of IL-8 and IL-12 p40. Data are means ± SD of triplicate samples from one representative experiment of three performed with similar results. NS, Nonstimulated. ∗, Statistically significant differences ($p < 0.05$) between hOSCAR-stimulated DCs in the absence or presence of anti-CD85j mAb.

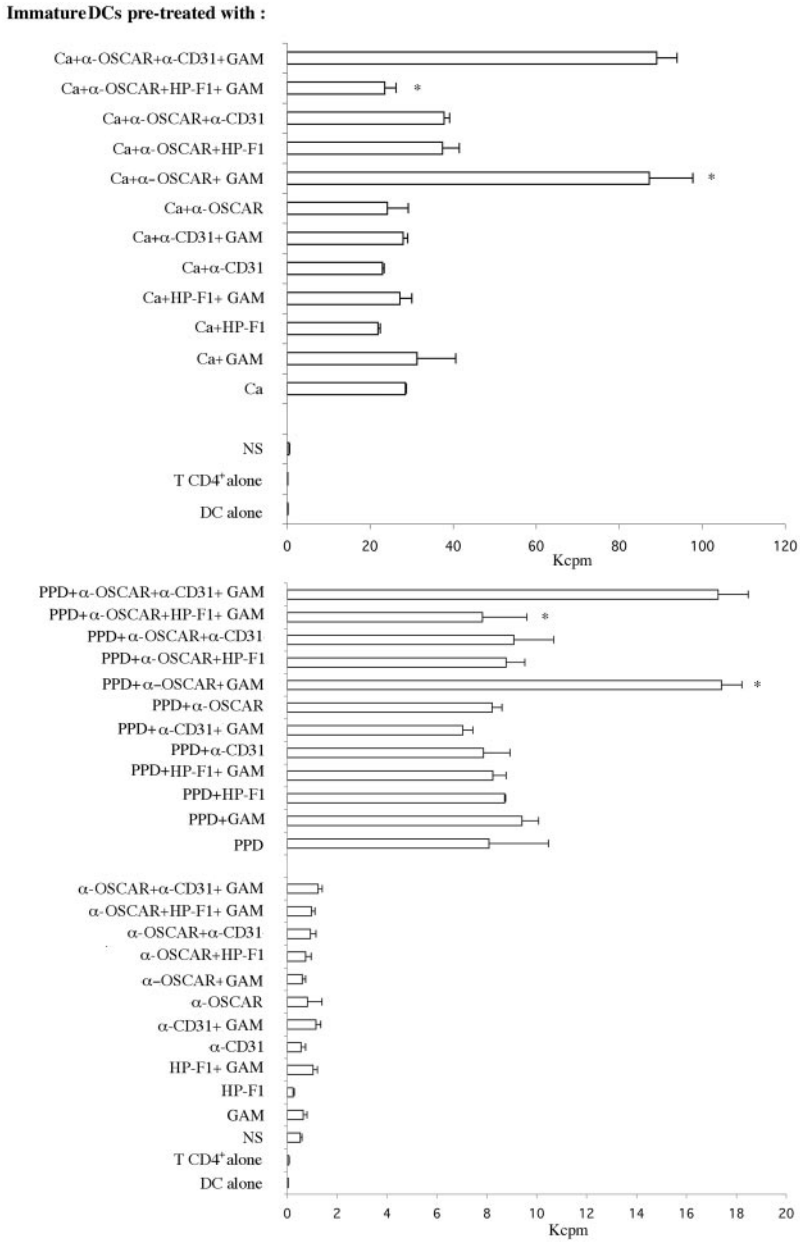

FIGURE 4. CD85j ligation on immature DCs inhibits hOSCAR-mediated proliferation of autologous Ag-specific $CD4^+$ T lymphocytes. Immature DCs are pretreated with the indicated mAb, washed, and pulsed using the recall Ags *C. albicans* (Ca) and purified protein derivative (PPD). Subsequently, autologous $CD4^+$ T lymphocytes are included in the assay. After 5-day culture, lymphocyte proliferation is measured by [^{3}H]thymidine uptake. hOSCAR-induced proliferation of $CD4^+$ cells, in the presence of recall Ags, is inhibited by the concomitant addition of HP-F1 mAb. $CD4^+$ T cells do not proliferate in the absence of Ag. Both stimulating factors, hOSCAR and Ag, are necessary on DCs for CD85j to inhibit T cell proliferation. Data are the means ± SD of triplicate samples from one representative experiment of three performed with similar results. *, Statistically significant difference ($p < 0.05$) of stimulation in the absence or presence of anti-CD85j mAb.

Discussion

In this study, the level of CD85j expression on DCs in the course of their differentiation from monocytes has been evaluated. CD85j was expressed in cells from all donors at similar levels and at all differentiation stages. These findings raised the issue of whether or not CD85j is functional in DCs. To evaluate the inhibitory function mediated by CD85j, hOSCAR was chosen among other activating molecules, such as CD1a and HLA-DR, because it provided the strongest activating signal for DCs. It is known that hOSCAR transduces an activating signal by interacting with an ITAM adapter, namely the FcR-associated γ-chain, providing a target for phosphatases recruited by CD85j. In immature DCs, the simultaneous cross-linking of CD85j, but not of CD31, significantly reduced hOSCAR-induced activation measured by Ca^{2+} flux or cytokine production. The only report on a regulation exerted by inhibiting receptors on cytokine production by monocytes and DCs deals with signal regulatory protein (SIRP)-α/CD47 ligation leading to inhibition of IL-12 and TNF-α, but not of IL-8 production induced by bacterially derived products such as *Staphylococcus aureus* Cowan I or LPS (40, 41). In this study, we show that activated DCs produce lower amounts of IL-8 and IL-12 when CD85j is cross-linked. Thus, a role for CD85j in rendering DCs tolerogenic is conceivable.

The down-regulation of cytokine production by hOSCAR-stimulated DCs suggested that the cooperation with $CD4^+$ T lymphocytes might also be affected when CD85j is engaged. To gather information on this issue, immature DCs were pulsed with various mAb plus or minus GAM antiserum and washed to remove unbound Abs, to rule out a direct effect of the anti-CD85j mAb on T cells. Subsequently, Ab-primed DCs were added to purified autologous $CD4^+$ T cells in the presence of recall Ags. In these experimental conditions, CD85j cross-linking on DCs strongly reduced the proliferation of Ag-specific T cells. CD85j engagement did not down-regulate the expression of Ag-presenting molecules such as HLA-DR on DCs (data not shown). This suggests that CD85j did not impair the ability of DCs to process or present Ags but, rather, that the reduction of cytokine production affected T cell proliferation. In support of this contention, it should also be noted that,

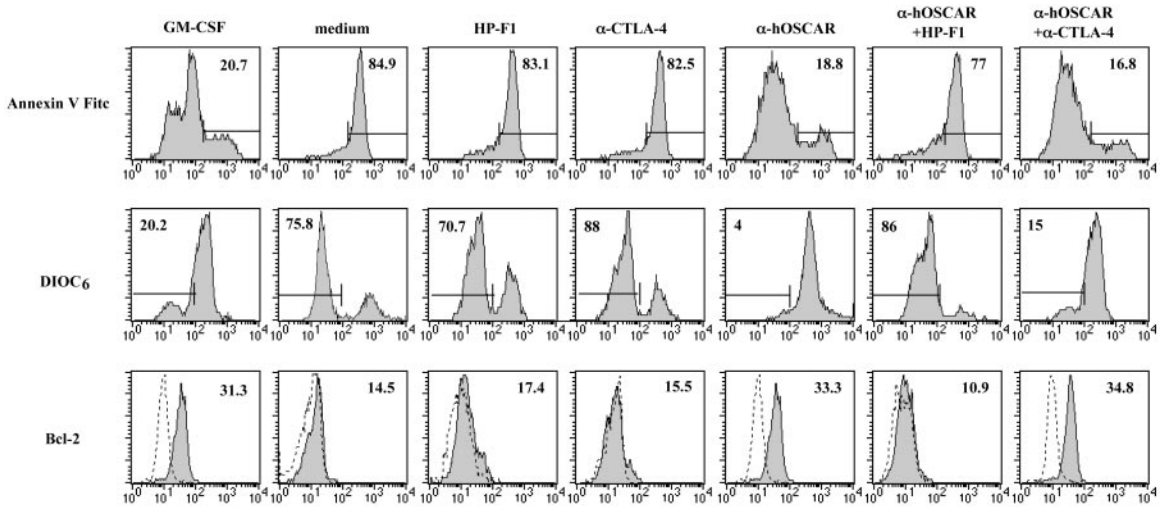

FIGURE 5. Cross-linking of CD85j abrogates hOSCAR-induced cell survival of immature DCs. Immature DCs are washed extensively to remove GM-CSF and IL-4, before stimulation with plastic-coated mAb, as described in *Materials and Methods*. After 3-day stimulation, cells are analyzed for annexin V binding, $DiOC_6$ uptake, and intracellular staining for Bcl-2. Numbers in the corner indicate the percentage of cells positive for annexin V and negative for $DiOC_6$ that correspond to early and late events of apoptosis, respectively. As for Bcl-2 staining, numbers indicate the specific mean fluorescence intensity of the different samples (shaded histogram), and the dotted lines show the binding of an isotype-matched control mAb. Data are representative of one of three independent experiments.

although T cells did not proliferate in the absence of recall Ags, the inhibiting effect of CD85j occurred when DCs were stimulated by both Ag and hOSCAR, and not when cells were stimulated by Ag alone. This suggests that hOSCAR sustains T cell proliferation indirectly by cytokine production that is down-regulated when CD85j is engaged. We also explored whether or not the loss of $CD4^+$ cell proliferation is due to the presence of Treg cells. However, no differences in the percentage of $CD4^+CD25^{high}$ cells has been observed (data not shown).

In the absence of exogenous factors such as GM-CSF, DCs rapidly undergo apoptosis. Stimulation via hOSCAR yielded an antiapoptotic effect, as suggested by the up-regulated Bcl-2 expression in hOSCAR-stimulated DCs, and provided a survival signal to these cells. Engagement of CD85j prevented the rescue from apoptosis exerted by hOSCAR. Thus, CD85j could play a role in the termination of the immune response, because this is no longer sustained by Ag presentation conducted by DCs that undergo cell death following engagement of the receptor.

In conclusion, the inhibition mediated by CD85j was significant when activation was provided by hOSCAR. Therefore, it appears that CD85j interacts closely with hOSCAR on the cell surface. Phosphatases recruited by CD85j may exert an immediate and direct effect on kinases recruited by the ITAMs of FcRγ. Although CD31 (PECAM-1) is an adhesion molecule with ITIMs (42), CD31 was unable to block hOSCAR-FcRγ activation. This is probably due to the properties of the anti-CD31 mAb used. The Moon-1 mAb used in this study is specific for the second Ig domain of the CD31 molecule (43). This domain is not involved in Ca^{2+} mobilization that, instead, is triggered by fifth and sixth Ig domains (44).

Unlike the stimulation provided by hOSCAR, CD85j failed to inhibit the activation induced by LPS (data not shown). This is probably due to the maximum effect yielded by LPS, which recognizes at least two receptors on DCs, namely TLR4 (45) and CR3 (46), and provides activation using several distinct pathways (47). This could be relevant in vivo, during the acute phase of infection sustained by LPS-producing bacteria. In this phase, LPS activates DCs that sustain the immune response at the highest level. In contrast, when a successful immune response leads to bacterial clearance and to lack of LPS, CD85j inhibits activating stimuli and therefore may contribute to the termination of the immune response. However, studies on SIRP-α inhibition of LPS stimulation suggest that PI3K activation via SIRP-α is essential to block cytokine production via TLR4 (41). Therefore, CD85j should not use this molecular pathway.

Although the ligand of hOSCAR is presently unknown, there is strong evidence from the data in mice that it is an endogenous molecule (38). Thus, it is conceivable that hOSCAR acts to induce DC maturation in the presence of its ligand. hOSCAR-activated DCs improve their survival and up-regulate the production of immune modulators, such as chemokines and cytokines (37). CD85j abrogated these responses and, consequently, could be a component of a regulatory loop that is active in a physiologic context. In addition, CD85j presumably inhibits other activating pathways and thus could play a more general role. CD85j broadly recognizes several HLA class I alleles and loci, including nonclassical class I molecules such as HLA-G (30–32). Interestingly, HLA-G is expressed strongly by fetal tissues and plays an essential role in maintaining the fetal graft during pregnancy by blocking locally the maternal immune response (48, 49). CD85j might therefore be involved in the abrogation of DC activation in the course of maternal tolerance.

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Disclosures

The authors have no financial conflict of interest.

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