Fc receptor γ-chain activation via hOSCAR induces survival and maturation of dendritic cells and modulates Toll-like receptor responses

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ABSTRACT

We previously reported the characterization of human OSCAR (hOSCAR), a novel Fc receptor γ-chain (FcRγ)-associated receptor expressed by myeloid cells. Here we show that ligation of hOSCAR by specific antibodies promotes dendritic cell (DC) survival by an ERK- and PI3K-dependent pathway, linked to expression of the Bcl-2 and Bcl-xL anti-apoptotic molecules. Crosslinking of hOSCAR leads to maturation of DC, as demonstrated by up-regulation of maturation markers, decrease in dextran uptake capacity, and secretion of immune effectors such as IL-8/CXCL8, IL-12 p40, MCP-1/CCL2 and MDC/CCL22. Stimulation of hOSCAR acts in conjunction with the Toll-like receptor (TLR) ligands, LPS, R-848 and poly(I:C), to increase the expression of maturation markers, and to modulate cytokine release. A PI3K-dependant up-regulation of IL-10 release is observed with all the TLR ligands used, whereas regulation of IL-12 production is variable depending on the TLR stimulated. hOSCAR engagement on DC did not significantly increase the proliferation of naive T cells, however when co-incubated with TLR ligands an enhanced proliferation was observed. The percentage of IFN-γ-producing T cells is decreased when hOSCAR engagement is combined with LPS stimulation. Altogether these data suggest that hOSCAR may modulate the responses of both innate resistance and adaptive immunity.
INTRODUCTION

Dendritic cells (DC) are potent antigen presenting cells which have an unique ability to prime naive T cells \(^1\). Additionally, they secrete immune-modulating factors that play a key role in the initiation and regulation of both innate resistance and adaptive immunity. DC undergo striking changes in function and morphology depending on their maturation state and localization \(^1\). Immature DC, located in peripheral tissues, are considered as immune sentinels and are able to capture and process antigen \(^2\). Upon activation and maturation induced by pro-inflammatory signals, such as pathogen-associated molecular patterns (PAMP) \(^3\), they migrate to the T cell areas of secondary lymphoid organs, where they are able to present antigen to naive T cells \(^4,5\). The outcome of the immune response (either immune priming or tolerance) is directly linked to the maturation status of DC \(^6\), which is generally believed to be induced by endogenous factors such as pro-inflammatory cytokines (TNF or IL-1\(\beta\)) \(^7,8\) or by exogenous products such as PAMPs (e.g. LPS, LTA or viral-RNA mimics e.g. poly(I:C)) \(^9\).

DC activity can be regulated by both activating and inhibiting immune receptors that transduce signals through immunoreceptor tyrosine-based activating motifs (ITAM, consensus: D/Ex\(_7\)D/Ex\(_2\)Yx\(_2\)L/Ix\(_7\)Yx\(_2\)L/I) \(^10,11\) and through immunoreceptor tyrosine-based inhibitory motifs (ITIM, consensus: I/V/L/SxYx\(_2\)L/V) \(^12,13\), respectively. This growing family of receptors was first described in lymphocytes \(^14-16\), but in recent years an increasing number of receptors of the lectin and the Immunoglobulin Superfamilies (IgSF) linked to ITAM/ITIM signalling expressed on myeloid cells and DC has been described \(^17,18\). These data indicate that cells of the myeloid lineage have a large number and variety of receptors regulating their activity. These receptors signal through activating or inhibiting pathways that regulate the amplitude and the duration of the immune response triggered by pathogenic stimuli \(^19-21\).
We recently described hOSCAR, a novel immune receptor associated with the Fc receptor γ-chain (FcRγ) and involved in endocytosis and antigen presentation through the MHC class II pathway in monocyte-derived dendritic cells (mono-DC). Its association with an ITAM-bearing chain confers to hOSCAR the capacity to activate myeloid cells as shown by its ability to trigger calcium flux and cytokine release. As hOSCAR is expressed by both immature and LPS-matured mono-DC, we proposed that hOSCAR may have a biological role on human DC probably different to the previously described activating receptors such as the Fc receptors (FcR) and TREM-2, the expression of which is down-regulated after activation of DC. In contrast to the human receptor, mouse OSCAR (mOSCAR) is only expressed on osteoclasts, which are derived, like certain DC subsets, from the myeloid lineage. Data from different groups strongly suggest that in vivo ligation of mOSCAR on osteoclasts is essential for differentiation of these cells. An endogenous ligand for OSCAR on osteoblasts has been inferred from this work. Of interest, TREM-2, an activating receptor that uses the ITAM-bearing adapter DAP12, was first described in DC and shown to promote cell survival and a partial maturation phenotype. TREM-2 has also been shown to be involved in osteoclast differentiation and function. These data underline the relevance of receptors associated with either FcRγ or DAP12 in the biology of cells of the myeloid lineage, including osteoclasts and DC.

In this study we show that hOSCAR ligation induces phenotypical and functional maturation of DC. Cytokine and chemokine secretion was induced in DC by hOSCAR ligation, although no significant effect was noted on the ability of the treated DC to prime naive T cell proliferation. However, when hOSCAR ligation was associated with treatment by certain Toll-like receptor (TLR) ligands, the ability of DC to support naive T cell proliferation was synergistically amplified. Finally, the inflammatory effects in DC of the TLR4-ligand LPS, but not those of the TLR7/8-ligand R-848 and of the TLR3-ligand poly(I:C), were attenuated.
by hOSCAR ligation which induced the production of anti-inflammatory cytokines such as IL-10 and lead to a lower level of Th1 polarization of naive T cells.
MATERIALS AND METHODS

Cell culture
Mono-DC were produced by culturing purified blood monocytes for 5 days in the presence of 200 ng/ml rhGM-CSF (Schering-Plough Research Institute, Kenilworth, NJ) and 10 ng/ml rhIL-4 (Schering-Plough Research Institute), as previously described. Cells were cultured in RPMI 1640 (GIBCO BRL, Gaithersburg, MD) supplemented with 10% (vol/vol) heat-inactivated FBS (Eurobio, Les Ulis, France), 2 mM L-glutamine, 100 µg/ml gentamicin (Schering-Plough, Levallois-Perret, France) (hereafter referred to as complete medium).

Peripheral blood CD11c⁺ myeloid DC were purified by negative depletion of PBMC with anti-CD3 (OKT3), -CD8 (OKT8), -CD14 (MOP9.25), and anti-CD19 (4G7) mAbs (all ascites from our laboratory), purified anti-CD56 (NKH-1, Beckman Coulter, Miami, FL), anti-CD16, -glycophorin A mAbs (both from Immunotech, Marseille, France), and magnetic beads (Dynabeads M450, Dynal, Oslo, Norway). Enriched cells were further FACS-sorted as Lineage⁻ CD4⁺ (Beckman Coulter, Marseille, France) and CD11c⁺ (Becton Dickinson, San Jose, CA 95131 USA) (>98% purity). The lineage negative antibody cocktail is composed of FITC-conjugated anti-CD3, -CD15 (Dako, Glostrup, Denmark), -CD14, -CD16, -CD19, -CD56 (Becton Dickinson), and -CD35 (BD Pharmingen, San Diego, CA).

Blood naive CD4⁺ CD45RA⁺ T cells were prepared from PBMC by two round of negative selection using magnetic beads (Dynal). The depletion was performed with anti-CD45RO (UCHL1), -CD8, -14, -CD19, -CD40 (89), -HLA-DR (L243), (all ascites from our laboratory), purified anti-CD56, -CD16, -CD35, -glycophorin A (all from Immunotech). Purity of the preparation was controlled by flow cytometer analysis of double labeling with anti-CD3-FITC (Dako) and anti-CD45RA-PE (Beckman Coulter) and was ≥96%.
In vitro stimulation of mono-DC

Anti-hOSCAR mAb and F(ab’)2 fragments mAb were produced by as previously described. Anti-hOSCAR mAb and F(ab’)2 (clone 11.1CN5), and irrelevant mAb MOPC21 (Sigma) or anti-CD13 (Immunotech) as isotype controls were coated for 4 h at 37°C on flat-bottom plates with a final concentration of 20 µg/ml in PBS. Immature DC were plated at a concentration of 1 x 10^6 cells/ml. The following activating factors were used at final concentrations of 20 ng/ml rhTNF (Genzyme, Boston, MA), 10 ng/ml *E. coli* lipopolysaccharide (LPS) (Sigma), 25 µg/ml polyninosinic-polycitidylic acid, (poly(I:C), InvivoGen, San Diego, CA), 10 µM R-848 (imidazoquinoline resiquimod synthesized in our laboratory Schering-Plough). Supernatants and cells were collected after 24 h and tested by ELISA and flow cytometry, respectively. All mAb used for tissue culture were shown to be endotoxin free, as determined by Limulus-Amebocyte Assay (BioWhittaker, Walkersville, MD). To block the effect of LPS, polymixin B (Sigma) was added to the culture at 10 µg/ml.

Flow cytometry

Cell staining was performed using PE-conjugated mouse mAb anti-CD54 (Bioscience), anti-CD40, -CD83, -DC-LAMP (Immunotech), anti-CD25, -CD80, -CD86, -HLA-DR (BD PharMingen) and FITC-conjugated mouse mAb anti-CCR7 (BD PharMingen). The staining with anti-DC-LAMP was performed using the cytofix/cytoperm kit (BD PharMingen).

Incorporation of FITC-dextran

Mono-DC were harvested after 48-h of stimulation, as described above, resuspended in complete medium supplemented with 25 mM Hepes (Life Technologies, Paisley Park, UK) and incubated at 4 or 37°C. FITC-dextran (M_r = 40,000 kDa ; Molecular Probes Eugene, OR) was added at a final concentration of 100 µg/ml for 30 min. The cells were washed four times...
with cold PBS, 5% SVF, 0.01% NaN₃, and were analysed immediately by flow cytometer. The maturation of mono-DC was assessed by staining with PE-conjugated mouse mAb anti-CD86 (BD Pharmingen).

**Detection of apoptosis**

After mono-DC differentiation, the cells were harvested and washed four times in PBS to remove GM-CSF and IL-4. Mono-DC stimulated with coated mAb for 3 days were harvested and the apoptotic cells were detected using the FITC-annexin V kit (BD Pharmingen).

To measurement mitochondrial membrane potential, cells were incubated in complete medium containing 25 nM 3,3-Dihexyloxocarbo-cyanine iodide (DiOC₆(3) (Molecular Probes, Eugene, OR) for 30 min at 37°C in the dark followed by flow cytometer analysis.

Intracellular labelling with FITC-conjugated mouse mAb anti-Bcl-2 (Dako) or with rabbit polyclonal Ab anti-Bcl-x (BD Biosciences), followed by PE-conjugated goat anti-rabbit (Sigma) were performed as described elsewhere 30. In blocking experiments, inhibitors (10 µM LY294002, 10 µM wortmannin, 20 µM PD98059, all from Calbiochem) were added 60 min before stimulation.

For biochemical analysis, mono-DC were stimulated as described above. After 3 days, cells were harvested and lysed in reducing sample buffer, and cell lysates were analysed by SDS-Page and reducing Western blot, as previously described 22. The Bcl-x isoforms were detected using polyclonal Ab anti-Bcl-x and donkey anti-rabbit conjugate (Vector, Burlingame, CA).

**Measurement of mono-DC secreted cytokines and chemokines**

Supernatants of cells stimulated for 24 h were collected and tested by ELISA for production of IL-1β, IL-6, IL-8/CXCL8, IL-10, IL-12 p40, IL-12p70, TNF, MCP-1/CCL2,
IP10/CXCL10, GM-CSF (OptEIA kits, BD Pharmingen) and MDC, M-CSF (Duoset kit, R&D Systems, Minneapolis, MN).

**Semi-quantitative RT-PCR**

Cells were lysed and total RNA was extracted using the RNeasy kit (Qiagen, Maryland). Genomic DNA contamination was tested by amplification (quantitative PCR, Light Cycler,) of the non-transcribed part of the human CD4 promoter (forward primer: 5’-TTCCACACTGGGCCACCTAT, reverse primer: 5’-TTGTGGGCTTACCACCTGCTG, probe: CACTGGACACAATTGCCCTCAGG). Single-stranded cDNA was synthesized using a mix of random hexamer primers (Invitrogen, San Diego, CA) and oligo (dT)15 (Promega, Madison, WI) and the Superscript II RNase-H reverse transcriptase (Invitrogen). Real-time quantitative PCR was performed with a dsDNA-binding dye, SYBR® green I, in an Icycler IQ (Bio-Rad, Hercules, CA), in 50 µl reactions (25 µl QuantiTect SYBR® Green PCR kit 2x (Qiagen), 0.4 µM each primer plus cDNA). The following primers were used: human MIG/CXCL9 5’-GAGATCCCACCACCGTCTTATC (forward) and 5’-CCTGTGAGATGAAAGGTAAGTGGGT (reverse), human IP10/CXCL10 ABI PRISM primer pairs (Applied Biosystems, Foster City, CA), human RANTES/CCL5 5’-TCCCGAACCCATTTCTTCTCT (forward) and 5’-CCCAGCAGTCGTCTTTGTCA (reverse), human TARC/CCL17 5’-CCCTTAGAAAGCTGAAGACGTG (forward) and 5’-TTGGGGGTCCGAACAGATG (reverse), human MDC/CCL22 5’-TTGGGCTGACCGCTTCAAG (forward) and 5’-AGACGCTGTCTTCCATGTTGG (reverse). Experiments were performed in triplicate. Real-time data were acquired and analyzed using Icycler IQ Optical System software (Bio-Rad) with automatic adjustment of the baseline and threshold parameters. Gene expression levels were determined using cycle threshold values (Ct), normalized by the average expression of the housekeeping gene.
GAPDH (forward primer: 5’-TGCCACCACCAACTGCTTAG, reverse primer: 5’-GGATGCAGGGATAGTGTC) and the results are expressed as gene relative expression, by applying the formula 1.8\((C_{T \text{ GAPDH}} - C_{T \text{ gene of interest}}) \times 10000\).

**DC-T cell culture, mixed leukocyte reaction (MLR) and T cell orientation**

In MLR, 5.10⁴ allogenic CD45RA⁺ naive T cells were cultured with serial dilutions of mono-DC, irradiated by 3,000 rad, and pre-incubated overnight with coated mAb and/or activators as described above. The cells were cultured in 96-half-area-well plates (Corning, Acton, MA), in quadruplicates. The highest ratio is 2T:1DC, and the number of mono-DC was decreased by 2/3 third for each dilution. After 5 days, the cultures were pulsed with [³H]-thymidine (1 µCi/well; Amersham, Buckingham, UK) for 16 h.

To study the polarization of naïve T lymphocytes, 1.10⁶/ml CD45RA⁺ T cells were cultured with 1.10⁵/mL mono-DC stimulated as described above. At 5 days, human rIL-2 (a generous gift from Dr. C. Caux-Menetrier) was added at 10 U/ml, and the cells were expanded for 9 days. The quiescent T cells were then stimulated with 50 ng/ml PMA and 2 µg/mL ionomicin (Sigma) for 6 h, with the additional presence of GolgiPlug (Pharmingen) for the last 4-h of culture, before staining with with FITC-anti-IFN-γ (Pharmingen) and PE-anti-IL-4 (Becton Dickinson) mAb.

For the detection of secreted cytokines, quiescent T cells were restimulated with coated anti-CD3 (UCHT1, BD Pharmingen), and anti-CD28 (Sanquin, Amsterdam, Netherland). Supernatants were tested by ELISA for production of IFN-γ, IL-4 and IL-5 (OptEIA kits, BD Pharmingen).

**Statistical analyses**
Statistical analyses were performed in Microsoft Excel 5.0 (Microsoft Corporation, Redmond, WA) using two-tailed student's $t$ tests. A $p$ value $< 0.05$ was considered statistically significant. For the T cell orientation assay, statistical analyses were performed using two-tailed student's $t$ tests with pair-wise comparison, to compare the percentage of IFN-γ-positive cells after stimulation with TLR ligands alone or in combination with hOSCAR ligation independently on donor variability.
RESULTS

**hOSCAR cross-linking promotes DC survival**

Some activating signals, such as LPS\(^{31}\) or anti-TREM-2 stimulation\(^{26}\), have been shown to trigger prolonged survival of mono-DC. We showed that hOSCAR cross-linking induces \([\text{Ca}^{++}]_i\) flux and cytokine release in monocytes and mono-DC\(^{22}\). We therefore investigated whether ligation of hOSCAR increased the survival of mono-DC cultured in the absence of GM-CSF. Mono-DC stimulation by plastic-coated anti-hOSCAR mAb that induced receptor aggregation and triggering of the ITAM-signalling pathway\(^{32}\) resulted, as observed at 72 h, in morphological changes, adherence, and conservation of cellular integrity (Fig. 1A). A high percentage of anti-hOSCAR treated mono-DC survived in the absence of GM-CSF for more than 10 days (Fig. 1B).

After 3 days without exogenous survival factors, a high proportion of apoptotic mono-DC (annexin V-positive cells) could be observed after culture in the presence of plastic-coated MOPC21 isotype control mAb or anti-CD13 (Fig. 1C). In these culture conditions, mono-DC were also unable to maintain their mitochondrial membrane potential, as shown by the low level of DiOC\(_6\)(3) incorporation. Only a small proportion of mono-DC cultured for three days in the presence of plastic-coated anti-hOSCAR underwent apoptosis and the majority of these cells incorporated DiOC\(_6\)(3), indicating that their mitochondrial membrane potential was maintained (Fig. 1C). A comparable level of survival was also observed in mono-DC cultured in the presence of TNF or GM-CSF. To exclude that contaminating LPS was responsible for the rescue from apoptosis, mono-DC were cultured in the presence of polymixin B, and no inhibition of survival induced by anti-hOSCAR was observed (data not shown).

Proteins from the Bcl-2 family, particularly Bcl-2 and the Bcl-x\(_L\) isoform, are known cell death antagonists\(^{33,34}\), whereas the shorter Bcl-x\(_S\) isoform has an opposite role. The survival
effect of hOSCAR, as well as that of the positive controls TNF and GM-CSF, was characterised at day 3 by concomitant expression in mono-DC of Bcl-2 and Bcl-x, as shown by intracellular staining (Fig. 1C). As shown by Western blot, the staining for Bcl-x was mainly due to the presence of the anti-apoptotic long isoform Bcl-xL of 28kDa (Fig. 1D).

Treatment of mono-DC with the ERK inhibitor PD98059 and the two phosphoinositide-3-OH-kinase (PI3K) inhibitors, LY294002 (Fig. 1E) or wortmannin (data not shown), inhibited survival (as assessed by annexin V binding) and Bcl-2 expression induced by hOSCAR ligation. In a similar fashion, Bcl-x expression was also maintained by a PI3K-, ERK-dependent pathway (data not shown). These observations indicate that hOSCAR induces survival of mono-DC, at least in part by maintaining expression of the anti-apoptotic molecules of the Bcl-2 family through activation of the PI3K and ERK signalling pathways.

**hOSCAR ligation up-regulates the maturation marker expression on DC**

Immature mono-DC were stimulated by LPS or by mAb coated onto culture plates, an efficient way of cross-linking the appropriate receptors. After 24-h stimulation, phenotypical analysis of these cells was performed by flow cytometry (Fig. 2).

Both coated F(ab')2 and whole mAb anti-hOSCAR were able to trigger the activation of mono-DC as shown by up-regulation of the co-stimulatory molecule CD86 (Fig. 2A). The up-regulation of CD86 expression induced by F(ab')2 and whole anti-hOSCAR was consistently observed but of lower intensity compared to that observed in mono-DC activated by LPS. Unlike anti-hOSCAR, the two isotype-matched controls, MOPC21 (irrelevant mAb) or anti-CD13 (a known DC surface receptor), had no significant effect on the activation state of mono-DC. Cross-linking of hOSCAR was required for the triggering of activation, since no effect was observed with soluble mAb. All mAb used in this study were negative for endotoxin contamination as measured by the Limulus assay. The activating effect of
anti-hOSCAR was maintained in the presence of polymixin B and absent when soluble mAb was used, excluding any role of undetectable levels of endotoxins (Fig. 2A).

Upon hOSCAR ligation on mono-DC, we also observed up-regulation of CD40, CD80 and HLA-DR, and slightly increased expression of CD54 (Fig. 2B). In addition, a subpopulation of mono-DC stimulated by anti-hOSCAR up-regulated CD83 and the intracellular maturation marker DC-LAMP. With some donors (2 out of 5), a slight up-regulation of CCR7 was also observed (data not shown).

The activating ability of hOSCAR was then studied on freshly isolated blood CD11c+ DC (Fig. 2C). hOSCAR ligation triggered slight up-regulation of CD80, and an increase of CD86 and CD83 expression. The increase in CCR7 expression, upon hOSCAR crosslinking, was higher and more reproducibly observed on CD11c+ DC than on mono-DC.

hOSCAR stimulation enhanced the ability of the TLR ligands LPS, R-848, and poly(I:C) to induce DC maturation and up-regulation of co-stimulatory marker expression (CD25, CD80, CD83, CD86) (Fig. 3), but no alteration in the levels of HLA-DR, CD40, and CD54 (data not shown) were seen. This effect was more pronounced when sub-optimal doses of TLR ligands were used and the increase of expression was particularly high with poly(I:C), a less potent mono-DC stimulator, than with LPS and R-848. Nevertheless the up-regulation of expression is moderate, and may be due to an additive effect between ITAM and TLR signalling.

**Maturation of mono-DC by anti-hOSCAR decreases their ability to uptake FITC-dextran**

The ability to internalise and process antigen is a constitutive property of immature DC that has been shown to decrease upon cellular maturation 7. In our experiments the majority of mono-DC, cultured alone or in the presence of the isotype controls MOPC21 and anti-CD13, were positive for FITC-dextran uptake (80.5% ± 6.7, 74.2% ± 10.7, and 84.6 ± 6.1,
respectively) (Fig. 4B), and negative for CD86 expression (Fig. 4A). The percentage of FITC-dextran positive cells decreased upon DC maturation (Fig. 4A). Almost all the LPS-matured mono-DC strongly expressed CD86 and were unable to uptake FITC-dextran. After treatment with anti-hOSCAR or TNF, a 39.9% ± 8.3 and a 59.3% ± 5.3 reduction, respectively, in dextran-positive mono-DC was observed (p<0.001). After stimulation of mono-DC by anti-hOSCAR and TNF two populations were identifiable: CD86-negative cells that were still able to incorporate FITC-dextran and fully matured CD86-positive mono-DC that had no macropinocytic/endocytic activity.

**hOSCAR activation induces the secretion of cytokines and chemokines**

We investigated the ability of hOSCAR ligation on mono-DC to trigger cytokine and chemokine secretion (Table 1). Stimulation of this receptor triggered production of high amounts of IL-8/CXCL8 (Fig. 5), which were not produced in response to isotype controls (MOPC21 anti-CD13) or soluble anti-hOSCAR. Coated F(ab')2 and whole anti-hOSCAR mAb in the presence of polymixin B were able to trigger the activation of DC as potently as the whole mAb, demonstrating that the activation observed was not mediated by FcR engagement or by contaminating endotoxin (Fig. 5A). hOSCAR ligation also induced secretion of M-CSF and small amounts of IL-12 p40 (Table 1). However, unlike LPS, stimulation through hOSCAR did not induce secretion of significant amounts of IL-1β, IL-6, IL-10, IL-12 p70, TNF, and GM-CSF (Table 1). Significant amounts of MCP-1/CCL2 and MDC/CCL22, but not IP10/CXCL10 were detected in the supernatants of hOSCAR-stimulated mono-DC.

In order to determine the type of chemokines induced upon hOSCAR cross-linking, we assayed cDNA prepared from mono-DC incubated for 2 h with MOPC21, anti-hOSCAR and LPS using realtime semi-quantitative RTPCR (Fig. 5B). Cross-linking of hOSCAR triggered
high expression levels of TARC/CCL17 and MDC/CCL22. These chemokines attract Th2 effectors and regulatory T cells \(^{35,36}\). In contrast to LPS, hOSCAR ligation did not induce significant transcription of MIG/CXCL9, IP10/CXCL10 and RANTES/CCL5, chemokines able to recruit Th1 T cells \(^{35,37}\).

**hOSCAR ligation modulates cytokine production by mono-DC exposed to TLR-ligands**

The diversity and quantity of cytokines and chemokines produced by hOSCAR-stimulated mono-DC was more restricted than that of LPS-stimulated DC (Table 1). As some ITAM-signalling receptors, such as TREM-1 \(^{19,20,38}\), synergize with other activators, we investigated the modulation of TLR-mediated cytokine secretion upon hOSCAR engagement. Co-stimulation of the cells with anti-hOSCAR and any of the TLR-ligands used strongly augmented the secretion of IL-10 (Fig. 6). This effect was dependent on PI3K signalling, as shown by its inhibition when cells were pre-treated with LY294002. IL-12 p40 production were slightly up-regulated with poly(I:C) and R-848, while no modification was observed in combination with LPS. hOSCAR co-stimulation decreased the secretion of IL-12 p70 induced by LPS and upregulated that induced by R-848. The increase of IL-12 p70 production was dependent on the PI3K pathway, while the down-regulation observed with LPS was not altered by PI3K inhibitors. In addition, the secretion of IP10/CXCL10 upon LPS and R-848 stimulation was strongly suppressed by the ligation of hOSCAR in a PI3K-independent mechanism. Overall, only the increase of IL-10 and IL-12 p70 production induced by hOSCAR engagement was clearly PI3K-dependent, suggesting that another pathway is involved in the inhibitory activity of hOSCAR on cytokine release.

**Co-stimulation by hOSCAR ligation and TLR ligands enhances the ability of mono-DC to induce allogeneic CD45RA\(^+\) T cell proliferation**
We tested whether anti-hOSCAR stimulation alone or in combination with other activators, triggered or enhanced the ability of mono-DC to induce allogeneic CD45RA⁺ T cell proliferation. Anti-hOSCAR-treated mono-DC did not promote a significant CD45RA⁺ naive T cell proliferation (Fig. 7A). When the mono-DC were stimulated by both anti-hOSCAR and TLR ligands (LPS, poly(I:C) and R-848), T cell proliferation was enhanced compared to that induced by mono-DC treated with the TLR-ligands only (Fig. 7B). This effect was particularly strong with LPS and R-848. No synergy was observed with TNF-stimulation.

We next investigated the intracellular cytokine profile of T cells stimulated for 6 h with PMA/ionomycin after having been cultured for 14 days with mono-DC stimulated by anti-hOSCAR and/or TLR ligands. Anti-hOSCAR-stimulation of mono-DC did not enhance their ability to prime T cells for IFN-γ production, as compared with no stimulation or the use of isotype controls (Fig. 7C). LPS-, poly(I:C)- and R-848-stimulated mono-DC polarized a proportion of CD45RA⁺ naive T cells into IFN-γ-producing cells. As shown in Fig. 5C, simultaneous stimulation by hOSCAR significantly neutralised the ability of LPS to endow mono-DC with the ability to prime T cells for IFN-γ production. In a series of six experiments with different donors, an average decrease of 71% in the percentage of IFN-γ-producing T cells primed by mono-DC stimulated with both anti-hOSCAR and LPS as compared to LPS alone was observed (p=0.002, two-tailed student's t tests with pair-wise comparison). Modulation of IFN-γ production when hOSCAR-stimulation was combined with poly(I:C) or R-848 was not consistent (p=0.49 and p=0.46, respectively). The absence of IL-4 and IL-5 production and the effect on IFN-γ production, in addition to intra-cellular cytokine staining, were confirmed by measuring cytokine secretion by ELISA (Fig. 7D).
DISCUSSION

Recently we and others 22,23,25 have shown that OSCAR is an FcRγ-associated molecule, implicated in the functional development and maturation of cells of the myeloid lineage. In mouse the expression and function of OSCAR is restricted to osteoclasts 23, cells of the macrophage lineage. In humans, OSCAR is more widely expressed and it is present on peripheral blood monocytes and myeloid DC, as well as on in vitro-derived macrophages, granulocytes, and mono-DC 22. The expression of hOSCAR at all stages of DC development and maturation provides a unique tool to examine FcRγ-induced activation on these cells, as other receptors associated with this or other ITAM-bearing signaling chains are present uniquely on immature DC or on particular sub-populations. The outcome of signalling through FcRγ activated by engagement of the associated Fc receptors on human DC in terms of cell maturation is controversial 39-41 and a possible effect on DC survival has not previously been investigated.

In the absence of growth factors or upon serum starvation, most cells enter into a state of programmed cell death. hOSCAR ligation prevented the entry of mono-DC into apoptosis in the absence of survival factors. The survival of hOSCAR-stimulated mono-DC was accompanied by maintenance of the mitochondrial membrane potential and expression of the anti-apoptotic proteins Bcl-2 and Bcl-xL, two molecules that prevent mitochondrial pore formation 33. These observations demonstrate that hOSCAR ligation triggers an intracellular anti-apoptotic pathway. Among the receptors signalling through FcRγ, FcReRI was shown to block apoptosis in human monocytes after survival factor withdrawal, by directly inducing Bcl-2, Bcl-xL expression 42. We have shown that the anti-apoptotic effects of hOSCAR-stimulation in mono-DC are dependent on both PI3K and ERK pathways. In other cell types growth factor-induced cell survival has been shown to be due to PI3K that through
Akt/PKB activation allows the expression of the anti-apoptotic protein Bcl-2 and Bcl-x. PI3K and ERK have been shown to be involved in LPS-induced mono-DC survival. Bouchon et al. recently demonstrated that cross-linking of TREM-2, a receptor signalling through the ITAM-bearing chain DAP12, promotes survival of mono-DC by an ERK-dependent, but PI3K-independent pathway. The difference in the involvement of ERK and PI3K in the survival effect, as well as the capacity of hOSCAR to induce cytokine secretion, may indicate clear differences in signalling pathways of the two ITAM-bearing chains, FcRγ for hOSCAR and DAP12 for TREM-2.

We had previously shown that mono-DC stimulated by anti-hOSCAR secrete IL-8 and IL-12 p40. However, engagement of hOSCAR alone on DC does not induce secretion of large amounts of pro-inflammatory cytokines such as TNF and IL-12 p70, and induces less IL-12 p40 than TLR ligands. This suggests that the principal role of hOSCAR in DC is not pro-inflammatory. Chemokines secreted by DC are also important in the amplification and orientation of the adaptive immune response. hOSCAR ligation on DC resulted in the secretion of significant amounts of IL-8/CXCL8, MCP-1/CCL2 and MDC/CCL22 chemokines that recruit immune effector cells, including neutrophils, monocytes, DC and natural killer cells. Of interest, hOSCAR stimulated mono-DC produced the CCR4-ligands MDC/CCL22 and TARC/CCL17, chemokines known to be involved in the recruitment of Th2 effector cells and regulatory T cells, whereas IP10/CXCL10, RANTES/CCL5 and MIG/CXCL9, chemokines targeting CXCR3 and CCR5 expressed on Th1 cells, are not produced. These data suggest that hOSCAR engagement alone preferentially triggers the production of chemokines recruiting Th2 and regulatory cells.

hOSCAR ligation, coupled to the ITAM signalling pathway, clearly induces phenotypic and functional maturation of human mono-DC and ex vivo blood CD11c DC, as shown by the up-regulation of specific maturation markers and loss of antigen uptake capacity. Compared
with the classical, strong DC activation triggered by TLR4 targeting by LPS, the phenotypic maturation induced by anti-hOSCAR is moderate but consistently observed using several markers, demonstrating that hOSCAR is an activating receptor on DC. Although hOSCAR stimulation leads to up-regulation of molecules involved in co-stimulation, anti-hOSCAR-stimulated mono-DC are unable to trigger naive T cell proliferation.

Since OSCAR by up-regulating CCR7 expression also mediates a cellular maturation programme that potentially leads to the migration of DC to the lymph nodes, and interactions with T and B cells, the induction of cellular survival may be essential to maintain viability during this critical stage in the initiation of the adaptive response. Interestingly, other receptors that promote immune cell maturation and migration, such as FcR and members of the TREM family also promote cellular survival \(^{26,42}\), indicating that the two pathways may be essential in the immune response. The positive effect of hOSCAR ligation on DC survival allows the continuation of long-term DC activity, especially during and after migration.

Migration of DC is observed under healthy steady state conditions, particularly “veiled” cells, which represent DC migrating to the lymph node. Some degree of maturation has been shown for steady-state migrating DC: modulation of adhesion molecules and chemokine receptors, upregulation of MHC and costimulatory molecules. In the absence of microbial or inflammatory stimulation these DC are not presumed to produce pro-inflammatory cytokines. This semi-mature stage of DC are thus proposed to induce tolerance \(^{6}\), and may correspond to this one induced upon hOSCAR engagement. The inability of hOSCAR engagement to endow DC with the ability to stimulate proliferation of allogeneic naive T cells suggests that in absence of other maturation stimuli, hOSCAR-stimulated DC, after migration into lymph nodes, may preferentially induce tolerance, and this effect may be augmented by the secretion of chemokines able to recruit Th2 and regulatory T cells that negatively regulate Th1 polarized responses.
The finding that transmembrane RANKL and secreted IFN-γ, expressed by T cells, are able to activate and to inhibit the development and functions of bone-resorbing cells, respectively, underscores the dynamic relationship between bone and the immune system. Increasingly, cell surface receptors are demonstrated to be expressed by both bone-remodeling cells and immune cells: the inhibiting NK receptors NKRP1 recognizing Clr/OCIL molecules expressed by DC, macrophages, osteoblasts and involved in the inhibition of osteoclast differentiation; TREM-2 expressed by both osteoclasts and DC and having potential ligands on NK cells and in the bone environment. We can now add hOSCAR to the known receptors playing a role in both bone homeostasis and the activation of the immune response. The presence of a hOSCAR potential ligand in the immune system environment can be postulated, allowing regulation of the precarious balance between immune activation and suppression, to favour elimination of pathogens without deleterious effects against the host.

Large differences in maturation status of DC were previously shown for different activators, such as TNF and LPS, and were postulated to determine the tolerogenic or immunogenic ability of DC. Here we show that hOSCAR engagement induces a semi-mature status with expression of costimulatory molecules but absence of pro-inflammatory cytokines. Partial maturation, without up-regulation of CD83 and CD54 or cytokine release, has been previously described in mono-DC stimulated with anti-TREM-2. In this study, Bouchon et al. showed that ITAM-signalling induced upregulation of DC maturation markers by an ERK-PTK-dependant and NF-kB and SAPK-independent pathways, whereas LPS maturation involved NF-kB and SAPK, and moderately ERK. hOSCAR engagement triggers signal through the FcRγ-ITAM and recruit ERK and PI3K, as demonstrated in survival assay. Thus the partial maturation status might be due to the lack of NF-kB and SAPK activation, in contrast to the stimulation with strong activator, such as TLR ligands. This underlines the fine tuning of DC activation depending on the stimulator used and the signalling pathway.
triggered. This partial maturation can be reverted to a fully mature state by simultaneous PAMP-mediated activation of DC through TLR and the NF-κB pathway. This is consistent with our observation that the activation of DC with both TLR ligands and anti-OSCAR increased the expression of markers such as CD25 and CD86. In addition, when DC were co-stimulated by hOSCAR and the TLR-ligands LPS, poly(I:C) and R-848, they became more potent accessory cells able to sustain an enhanced CD45RA+ T cell proliferation. However, DC stimulated by both hOSCAR and LPS were less efficient in the polarization of Th1 effector cells and the induction of IFN-γ secretion than DC incubated with LPS alone. The LPS-induced ability of DC to sustain Th1 polarization is decreased by the engagement of hOSCAR probably due to the concomitant increase in secretion of IL-10 and decrease of IL-12 p70 production, an effect seen only with LPS and not with other TLR-ligands. The hOSCAR-mediated increase in IL-10 production induced by all of the tested TLR-ligands was mediated by PI3K. The involvement of PI3K in IL-10 induction is in agreement with previous description of its role on IL-10 production upon stimulation of CD40, TLR2, or CD28. Recent studies indicate that PI3K is also an endogenous suppressor of IL-12 and TNF production triggered by LPS. This may act to limit excessive Th1 polarization causing undesirable immune responses, however we did not observe a role of PI3K on hOSCAR-mediated IL-12 inhibition in response to LPS. In contrast, the increase of secretion of IL-12 observed with anti-hOSCAR and R-848 was PI3K-dependant. Differences in the Toll-interleukin 1 receptor (TIR) domain adapters used by each TLR, linked to alternative downstream activation programmes probably account for the observed differential DC stimulation capacities, as well as for the different modulation of cytokine release when hOSCAR is engaged. In combination with LPS, signals induced by hOSCAR engagement might contribute to control inflammation and to down-regulate Th1 polarized responses.
Many receptors are known to be involved in the first line of defence, and the initiation of the innate immune response \(^{62-64}\). Extensive studies on TLR using mice deficient for specific TLR or for TLR signalling mediators have demonstrated an almost complete requirement for these molecules in a broad range of innate immune responses \(^{63}\). Emerging data from the literature \(^{19-21,65,66}\) suggest that one important role of ITAM-linked receptors expressed by monocytes, macrophages, and neutrophils is to enhance TLR-mediated signalling and subsequently the pro-inflammatory responses. The data presented here address the question of ITAM-linked receptors and TLR cooperation in DC and indicate that FcR\(\gamma\)-signalling through hOSCAR ligation generates intracellular signals, which can modulate the acquired immune responses induced by DC upon TLR-mediated activation. This effect is specific to the targeted TLR, and probably also to the signalling pathway induced, as shown by the differences in cytokine and T cell functional modulation observed with LPS, poly(I:C) and R-848. Interestingly this effect is present at sub-optimal doses of TLR ligands, as would most probably be encountered in physiological conditions. The fact that the response differs between TLR shows that hOSCAR may help to discriminate a need to limit inflammation (in the case of LPS triggering) once events leading to adaptive immunity are initiated, or a need to continue an anti-viral response, recruiting NK cells and DC when only TLR3 or TLR8 are engaged. In the absence of a pathogen-derived signal hOSCAR engagement promotes a semi-mature state, without the ability to induce T cell proliferation. Thus we have shown that both innate and adaptive immune responses by DC can be characterised by a complex series of events, dependent on receptor engagement and the initial signals received by the cells.
ACKNOWLEDGEMENTS

We thank I. Durand for cell sorting; C. Massacrier and I. Perrot for technical help on T cell experiments; B. Salaun for invaluable advice on survival experiments; M. Vatan and D. Lepot for editorial assistance; and colleagues from EFS-Lyon who provided us with blood samples.
15. Tomasello E, Bler M, Vely F, Vivier E, Djeu JY, Jiang K, Wei S. Signaling pathways engaged by NK cell receptors: double concerto for activating receptors, inhibitory receptors and NK cells
FIGURE LEGENDS

Figure 1. Stimulation of mono-DC through hOSCAR promotes cell survival in the absence of survival factors by a PI3K-, ERK-dependent pathway.

Mono-DC were washed four times to remove GM-CSF and IL-4, before stimulation of the cells with plastic-coated mAb (MOPC21, anti-CD13, anti-hOSCAR), 200 ng/mL GM-CSF or 20 ng/ml TNF. Data shown are representative of three independent experiments.

(A) Mono-DC were stimulated as described above and photographed after 3 days. Original magnification: x200.

(B) After stimulation for the indicated time, cells were harvested and counted by exclusion of dead cells with blue trypan. Cell recovery is expressed as percentage of cells put into culture at day 0. Survival curve displays the mean and standard deviation of three independent cell counts from one representative experiment.

(C) After 3 days of stimulation as described above, cells were analysed for annexin V-binding, DiOC$_6$(3) incorporation and intracellular staining by anti-Bcl-2, and Bcl-x. Numbers in the corners correspond to the percentage of positive cells for annexin V and DiOC$_6$(3) analysis, and indicate specific mean fluorescence intensity for Bcl-2 and Bcl-x staining (shaded histogram). The dotted line shows the binding of an isotype control mAb to the cells.

(D) Lysates of mono-DC stimulated 3 days, as indicated, were analysed for expression of Bcl-x isoforms by Western blot. The anti-apoptotic long isoform of BcL-x corresponds to the band of 28 kDa.

(E) Mono-DC were stimulated for 3 days, as described above, in the presence of PI3K inhibitor (LY294002) or ERK-pathway inhibitor (PD98059). The percentage of apoptotic cells and expression of Bcl-2 were analysed by annexin V-FITC binding and anti-Bcl-2
labelling. The data were expressed as percentage for annexin V-FITC and ΔMFI, the mean fluorescence intensity minus the fluorescence detected with isotype control.

Figure 2. hOSCAR ligation induces expression of maturation markers on mono-DC.

Immature mono-DC were stimulated by coated control IgG (MOPC21, anti-CD13), anti-hOSCAR whole mAb or F(ab’)2, or soluble anti-hOSCAR, or 10 ng/mL LPS. Polymixin B was added to some of the cells cultured in the presence of anti-hOSCAR mAb or LPS. After 24-h incubation, cells were analysed by flow cytometry for CD86 expression (A) or for the indicated markers (B). Numerical values indicate the specific mean fluorescence intensity of the staining (for shaded histograms). The dotted line shows the binding of an isotype control mAb to the cells. Data shown are representative of three experiments.

(C) Freshly isolated blood CD11c+ DC were cultured in the presence of coated MOPC21, anti-hOSCAR or 10 ng/mL LPS. After 24-h culture, cells were analysed by flow cytometry for the indicated markers. The dotted line shows the binding of an isotype control mAb to the cells. Data shown are representative of six experiments.

Figure 3. Stimulation through hOSCAR synergizes with TLR ligands to increase the expression of maturation markers expressed by mono-DC.

Mono-DC were stimulated with coated MOPC21, anti-hOSCAR and/or LPS, R-848 and poly(I:C) at the indicated dose levels. After 24-h culture, cells were analyzed by flow cytometry for the indicated markers. The data are expressed as ΔMFI, the mean fluorescence intensity minus the fluorescence detected with isotype control. The results presented are representative of three independent experiments.
Figure 4. Stimulation through hOSCAR decreases the macropinocytic/endocytic activity of mono-DC.

After 48-h stimulation with coated antibodies (isotype controls, MOPC21 and anti-CD13 or anti-hOSCAR) or with 10 ng/mL LPS, and 20ng/mL TNF, mono-DC were incubated at 37°C for 30 min in presence of FITC-dextran. Then the cells were harvested, stained with anti-CD86-PE and immediately analyzed by flow cytometry. Numbers in the corners correspond to the percentage of cells in each panel. Data shown are representative of 4 independent experiments.

Figure 5. Cytokine and chemokine secretion by mono-DC upon stimulation through hOSCAR.

(A) Immature mono-DC were stimulated with plastic-coated isotype controls (MOPC21, anti-CD13), anti-hOSCAR F(ab’)2 and whole mAb, soluble anti-hOSCAR, and LPS. As stated, polymixin B was added to the culture to block LPS activity. After 24 h, supernatants were tested by ELISA for IL-8/CXCL8 secretion. Data are mean ± S.D. of triplicate samples from one representative experiment out of 3 performed with similar results. Statistical significances of * p < 0.001; are given by comparison to values obtained with the negative control anti-CD13.

(B) Mono-DC were stimulated with plastic-coated isotype control MOPC21, anti-hOSCAR, and LPS. After 2-h stimulation, cells were harvested and lysed. The transcripts coding for chemokines were analysed by real-time PCR using SYBR-Green. Data are given after normalisation of Ct (Cycle Threshold) value of one gene with the expression of GAPDH transcripts. Data are mean±S.D. of triplicate samples from one representative experiment out of 3 performed with similar results. * p < 0.01; are given by comparison to values obtained with the negative control MOPC21.
Figure 6. hOSCAR ligation modulates the pattern of cytokines/chemokines secreted by mono-DC upon TLR stimulation. Mono-DC were stimulated for 24 h with plastic-coated MOPC21, anti-hOSCAR in combination with 10 ng/ml LPS, 25 µg/ml poly(I:C), or 10 µM R-848. Supernatant fluids were tested by ELISA for IL-10, IL-12 p40, IL-12 p70, and IP10/CXCL10 secretion. When indicated, cells were pre-treated with the PI3K inhibitor LY294002. Data are mean±S.D. of triplicate samples from one representative experiment out of 4 performed with similar results. Values are shown in ng/ml/million cells.

Figure 7. hOSCAR ligation, in combination with TLR ligands, increases the ability of mono-DC to stimulate MLR and modulates Th1 polarization.

(A) Proliferative response of CD45RA⁺ naive T cells cultured for 5 days with DC treated with coated MOPC21, anti-CD13, anti-hOSCAR and TNF was estimated by incorporation of [³H]-thymidine.

(B) Mono-DC were stimulated with coated anti-hOSCAR and/or activators (20 ng/ml TNF, 10 ng/ml LPS, 25 µg/ml poly(I:C), 10 µM R-848). After 5-d culture with CD45RA⁺ T cells, the lymphocyte proliferation was measured. Data are mean±S.D. of quadruplicate samples from one representative experiment out of 4 performed with similar results.

(C) Polarization of CD45RA⁺ naive T cells cultured with mono-DC stimulated with anti-hOSCAR and/or TLR ligands. After 6-h PMA/ionomycin stimulation and 4-h of culture with GolgiPlug, T cells were intracellularly stained with FITC-anti-IFN-γ and PE-anti-IL-4, and analyzed by flow cytometer. Data shown are representative of six experiments.

(D) Secretion of IFN-γ by T cells cultured with mono-DC stimulated with anti-hOSCAR and/or TLR ligands. Supernantant of T cells restimulated by anti-CD3 and ant-CD28 were
analysed by ELISA for the presence of IFN-γ, IL-4 and IL-5. No IL-4 and IL-5 were detected.
Data are mean ± S.D. of triplicate samples from one representative experiment out of 3 performed with similar results. * p < 0.01; are given by comparison to values obtained with T cells cultured in the presence of MOPC21-stimulated DC.
Figure 1
Figure 2

A

medium MOPC21 anti-CD13 soluble
anti-hOSCAR anti-hOSCAR F(ab')2 anti-hOSCAR polymixin B LPS

CD86

B

MOPC21 anti-hOSCAR LPS

CD25 CD40 CD54 CD80 CD83 HLA-DR DC-LAMP

C

MOPC21 anti-hOSCAR LPS

CCR7 CD80 CD83 CD86
Figure 3

CD25 (ΔMFI)

medium
LPS 10 ng/mL
LPS 0.1 ng/mL
R-848 10 µM
R-848 0.1 µM
poly(I:C) 25 µg/mL
poly(I:C) 0.25 µg/mL

CD80 (ΔMFI)

medium
LPS 10 ng/mL
LPS 0.1 ng/mL
R-848 10 µM
R-848 0.1 µM
poly(I:C) 25 µg/mL
poly(I:C) 0.25 µg/mL

CD83 (ΔMFI)

medium
LPS 10 ng/mL
LPS 0.1 ng/mL
R-848 10 µM
R-848 0.1 µM
poly(I:C) 25 µg/mL
poly(I:C) 0.25 µg/mL

CD86 (ΔMFI)

medium
LPS 10 ng/mL
LPS 0.1 ng/mL
R-848 10 µM
R-848 0.1 µM
poly(I:C) 25 µg/mL
poly(I:C) 0.25 µg/mL

MOPC21
anti-hOSCAR
Figure 4
Figure 5
Figure 6
Figure 7
Table I: Panel of cytokine and chemokine secretion upon 24-h stimulation by anti-CD13, anti-OSCAR, and LPS.

<table>
<thead>
<tr>
<th></th>
<th>medium</th>
<th>anti-CD13</th>
<th>anti-hOSCAR</th>
<th>LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>0.594 ± 0.084</td>
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<td>IL-10</td>
<td>nd</td>
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<td>nd</td>
<td>2.50 ± 0.04</td>
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<td>IL-12p40</td>
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<td>nd</td>
<td>9.44 ± 1.36</td>
<td>152.78 ± 25.57</td>
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<tr>
<td>IL-12p70</td>
<td>0.394 ± 0.029</td>
<td>0.483 ± 0.034</td>
<td>0.423 ± 0.032</td>
<td>4.30 ± 0.19</td>
</tr>
<tr>
<td>TNF</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>11.26 ± 1.64</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.171 ± 0.067</td>
<td>0.383 ± 0.131</td>
<td>0.312 ± 0.129</td>
<td>16.39 ± 2.34</td>
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<tr>
<td>IL-8/CXCL8</td>
<td>14.36 ± 3.20</td>
<td>26.56 ± 4.28</td>
<td>312.50 ± 2.88</td>
<td>678.59 ± 28.61</td>
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<tr>
<td>MCP-1/CCL2</td>
<td>0.301 ± 0.173</td>
<td>0.758 ± 0.152</td>
<td>2.55 ± 0.30</td>
<td>16.53 ± 1.29</td>
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<tr>
<td>MDC/CCL22</td>
<td>6.86 ± 0.95</td>
<td>7.13 ± 0.57</td>
<td>21.80 ± 0.18</td>
<td>37.53 ± 0.37</td>
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<tr>
<td>IP-10/CXCL10</td>
<td>0.557 ± 0.027</td>
<td>0.312 ± 0.027</td>
<td>0.222 ± 0.023</td>
<td>176 ± 9.46</td>
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<tr>
<td>GM-CSF</td>
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<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>M-CSF</td>
<td>nd</td>
<td>nd</td>
<td>0.606 ± 0.061</td>
<td>1.2 ± 0.08</td>
</tr>
</tbody>
</table>

ND: not detectable. Data are mean±S.D. of triplicate samples from one representative experiment out of 3 performed with similar results. Values are shown in ng/ml/million cells.
Fc receptor γ-chain activation via hOSCAR induces survival and maturation of dendritic cells and modulates Toll-like receptor responses

Estelle Merck, Blandine de Saint-Vis, Mathieu Scuiller, Claude Gaillard, Christophe Caux, Giorgio Trinchieri and Elizabeth E Bates