Production and function of activin A in human dendritic cells

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ABSTRACT. Activin A, a member of the transforming growth factor-β superfamily, has a role in tissue repair and inflammation. In our previous studies, we identified by immunohistochemistry DC-SIGN(+) dendritic cells as a source of activin A *in vivo*. The present study was aimed at investigating activin A production by dendritic cells (DC) *in vitro* and its function. Here we demonstrate that monocyte-derived DC (Mo-DC) released abundant levels of activin A during the maturation process induced by TLR agonists, bacteria (*B. henselae, S. thyphimurium*), TNF and CD40L. Activin A was also induced in monocyte-derived Langerhans cells (LC) and in blood myeloid DC by LPS and/or CD40L stimulation, but not in blood plasmacytoid DC following stimulation with influenza virus. Activin A production by DC was selectively down-regulated by anti-inflammatory molecules such as dexamethasone or IL-10. Neutralization of endogenous activin A using its inhibitor follistatin, or the addition of exogenous activin A during LPS maturation did not affect Mo-DC maturation marker expression, cytokine release or allostimulatory function. However, Mo-DC matured with LPS in the presence of exogenous activin A displayed a higher FITC-dextran uptake, similar to that of immature DC. Moreover, activin A promoted monocyte differentiation to DC and reversed the inhibitory effects of IL-6 on DC differentiation of monocytes. These findings demonstrate that different subsets of DC release activin A, a cytokine that promotes DC generation, and affects the ability of mature DC to take up antigens (Ags).

Keywords: activin A, follistatin, dendritic cells, Langerhans cells

Activin A, initially isolated as an inducer of follicle-stimulating hormone secretion, is a growth factor composed of two βA subunits belonging to the transforming growth factor β (TGF-β) superfamily of dimeric proteins [1]. Its biological activity is mediated by two different receptors, the type I (ACTRIA and ACTRIB) and the type II receptors (ACTRIIA and ACTRIIB), and is modulated by two proteins, follistatin and follistatin-related protein, which bind to activin A and thereby inhibit its biological effects [2, 3]. Activin signals are involved in embryogenesis, hematopoiesis (especially the enhancement of erythropoiesis), neuroprotection, apoptosis and tissue repair [4, 5].

Accumulating evidence from clinical and animal model studies suggests that the alteration of activin A expression and/or signalling contributes to pathological conditions such as inflammation and fibrosis [6-12]. Elevated concentrations of activin A were observed in cerebrospinal fluid during bacterial meningitis and in the systemic circulation during sepsis, likely due to activin A release by microglial cells and macrophages [13-15]. Increased expression of activin A was also shown in chronic inflammatory lesions, such as ulcerative colitis, Crohn's disease, and in inflammatory arthropathies, where activin expression is regulated by inflammation-associated cytokines secreted by synoviocytes, and induces proliferation of fibroblast-like synoviocytes [9, 10].

Increased activin A production could contribute to the overproduction of α-smooth muscle actin, fibronectin and extracellular matrix in liver, pancreas, lung, cornea and kidney fibrosis [5, 7, 8, 11, 12]. A role for activin A in skin fibrosis has also been demonstrated. In a mouse model of skin repair, strong activin A expression was observed in the granulation tissue within the first days after skin injury, probably induced by serum growth factors released upon haemorrhage and by macrophage-derived cytokine [16]. Activin expression then declined, returning to basal levels after completion of repair. Transgenic mice overexpressing activin A, show greatly hyperthickened epidermis, accelerated wound healing and enhanced scarring [17]. Conversely, in follistatin-transgenic mice, wound closure is delayed and scar formation reduced. Thus, sustained

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persistence of activin A might influence scar formation and wound healing quality [18]. We, and others, recently observed increased activin expression in hypertrophic scar and keloid scar compared to normal skin [19, 20]. According to our immunohistochemistry data, in active-phase, hypertrophic scars, activin A is produced by α-SMA$^+$ myofibroblasts and by fibroblast-surrounding dendritic cells (DC) (20).

DC are bone marrow-, haematopoietic-derived, professional antigen-presenting cells (APCs) with the unique ability to induce both primary and secondary T- and B-cell responses as well as immune tolerance [21-24]. Immature DC reside in peripheral tissues where they exert a sentinel function for incoming antigens. On microbial contact and stimulation by inflammatory cytokines, DC ingest antigens, undergo a process of maturation and migrate through the afferent lymphatics into the T-cell area of the draining lymph nodes where they initiate immune responses. DC control Th1-Th2 polarization and the state of tolerance to self-antigens and allergens. DC have an extraordinary capacity to produce bioactive molecules that act in an autocrine and/or paracrine manner. Indeed, although the primary biological function of DC is the initiation of specific immune responses, DC regulate inflammatory responses and angiogenesis through their ability to release cytokines and chemokines [21, 24, 25]. Our findings that cells coexpressing DC-SIGN$^+$ and activin A are present in hypertrophic scar [20], prompted us to investigate the regulation of activin A production at both the mRNA and protein levels in different human DC types and at different stages of maturation. In addition, the effects of activin A on DC maturation and differentiation were investigated. The results reported here demonstrate that activin A is abundantly produced by monocyte-derived DC (Mo-DC), Langerhans cells (LC) and myeloid circulating DC following maturating stimuli, and that the production is strictly regulated by anti-inflammatory cytokines and immunosuppressive agents. Moreover, we provide evidence that activin A affects the ability of LPS-matured Mo-DC to take up antigens (Ags) and promote the differentiation of monocytes towards DC.

METHODS AND MATERIALS

DC preparation and culture

PBMC were obtained from buffy coats (through the courtesy of the Centro Trasfusionale, Spedali Civili di Brescia, Italy) by Ficoll (Biochrom, Berlin, Germany) gradient (Pharmacia Fine Chemicals, Uppsala, Sweden). CD14$^+$ cells were isolated from PBMCs by positive magnetic separation using CD14 immunomagnetic beads and magnetic separation columns (magnetic cell sorter [MACS]; Miltenyi Biotec, Auburn, CA, USA), according to the manufacturer's instructions. The purity of the CD14$^+$ cells exceeded 95%, as determined by a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA). To generate Mo-DC or LC, monocytes were cultured for 6 days at 1 x 10^6/mL in 6-well tissue culture plates (Falcon; BD Biosciences, Franklin Park, NJ, USA) in RPMI 1640 supplemented with 100 U/mL penicillin, 100 µg/mL streptomycin, 2 mM L-glutamine, and 10% heat-inactivated FCS, with 100 ng/mL GM-CSF and 10 ng/mL IL-4 (Mo-DC) or 100 ng/mL GM-CSF, 10 ng/mL IL-4 and 10 ng/mL TGF-β1 (LC). Half of the culture medium was replaced with fresh medium containing cytokines on days 2 and 4 [26]. Peripheral blood DC were obtained from PBMC by magnetic sorting with blood DC Ag 1 and blood DC Ag 4 kits (Miltenyi Biotec) [27]. DC maturation (10^6 DC/mL) was induced by incubation with 100 ng/mL LPS (*Escherichia coli* 055:B5; Sigma-Aldrich, St. Louis, MO, USA), 1/5 000 dilution of *Staphylococcus aureus* Cowan I (SAC) (Sigma-Aldrich), 20 ng/mL TNF-α (Peprotech, Rocky Hill, NJ, USA), or CD40L-transfected J558 cells (1:4 ratio) for 24 h. *B. henselae* and *S. thyphimurium* were added at a 10:1 ratio. Where indicated, DC were treated with 50 ng/mL human IL-10, 50 ng/mL TGF-β1, 1 000 U/mL IFN-γ, 100 ng/mL IL-6 (PeproTech), 10^{-5} M PGE_2, or 10^{-6} M calcitriol (1,25-dihydroxyvitamin D_3) (Sigma-Aldrich).

RNA purification and real time RT-PCR analysis

Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). To exclude amplification of genomic DNA, RNA samples were treated with DNase (Invitrogen). Single-stranded complementary DNA (cDNA) was synthesized by reverse transcription of 2 µg total RNA using random hexamers and the Superscript First-Strand Synthesis System for RT-PCR (Invitrogen). The cDNAs were then amplified in duplicate by real-time PCR using the Platinum SYBR Green (Invitrogen) in a final volume of 25 µL. Following an initial denaturing step at 95°C for 2 min to activate 0.75 units of Platinum TaqDNA polymerase (Invitrogen), the cDNAs were amplified for 30 cycles (95°C for 1 min, 60°C for 1 min and 72°C for 1 min). The sequences of primers were as follows: activin A (sense 5'-GCA GAA ATG AAT GAA CTT ATG GA-3'; antisense 5'-GTC TTC CTG GCT GTT CCT GAC T-3'), ACTRIB (sense 5'-CCC TCG GGC TTG TAT ATT GG-3'; antisense 5'-ATG GAA GGG TCA GAG GGC AC-3'), ACTRIIA (sense 5'-ATA TGT ATG CCA TGG GAT TAG TCC TAT-3'; antisense 5'-TCA AAT GGC AAC ATG TAT TCA TCT AC-3'), follistatin (sense 5'-GTT TTC TGT CCA GGC AGC TCC AC-3'; antisense 5'-GCA AGA TCC GGA GTG CTT TAC T-3'), β-actin (sense, 5'-GTT GCT ATC CAG GCT GTG-3'; antisense, 5'-TGT CCA CGT CAC ACT TCA-3').

Flow cytometry

DCs were preincubated for 30 minutes at 4°C in PBS containing 2% goat serum plus 0.2% sodium azide, washed twice with 1% bovine serum albumin (BSA) in PBS, and incubated for 30 minutes at 4°C with anti-CD14 FITC, anti-CD1a PE, anti–major histocompatibility complex (MHC) PE, anti-CXCR4 PE, anti-CCR7 FITC, anti-CD83 PE or isotype control IgG PE or FITC. After washing with PBS, cells were analyzed on a FACSCalibur (Becton Dickinson).

Antigen uptake assay

After 24 h of stimulation with LPS, Activin A, or LPS and Activin A, DC were incubated at 1 x 10^6 cells/ml in RPMI 10% FCS for 15 min at 4 or 37°C. FITC-labeled dextran

and BSA (Sigma-Aldrich) were added at the final concentration of 1 mg/mL, and the cells were incubated for 30 min to allow capture of Ag. After thorough washing with cold PBS, fluorescence was measured by FACSCalibur CellQuest analysis to reveal uptake [28].

ELISA

Human activin A, follistatin, IL-8, IL-6, IL-10 protein levels in the DC culture supernatants were measured by sandwich ELISA (R&D Systems).

STATISTICAL ANALYSIS

Comparison among treatments was performed by Student's *t*-test or by analysis of variance, as appropriate.

RESULTS

Activin A production during DC maturation

In the first set of experiments, activin A production during monocyte-derived DC (Mo-DC) maturation was investigated. Different protocols for inducing Mo-DC maturation *in vitro* were employed. Mo-DC were stimulated in the presence of 100 ng/mL LPS (pathogen-derived agonist), 20 ng/mL TNF-α (proinflammatory cytokine) or CD40L-transfected cells (a T cell-derived signal). After 48 h of culture, Mo-DC maturation was assessed as the percentage of CD83+ cells (always > 80%). *Figure 1* shows that low levels of activin A were released by immature Mo-DC (iMo-DC). Induction of activin A production was easily detectable in the presence of LPS, CD40L and TNF-α, with the latter being the weakest agonist. Induction of activin A release was also observed when other pathogen-derived maturing agents such as SAC, Poly:IC were used or when Mo-DC were infected with *B. henselae* and *S. thyphimurium*. The highest secretion of activin A was observed in the presence of the whole, Gram- bacterium *B. henselae*. Activin A became detectable after 3 h incubation with LPS, and its production increased linearly up to 48 h *(figure 1)*. Similar kinetics were observed in the presence of the other stimuli (data not shown). Activin βA-subunit mRNA levels, as evaluated by real-time PCR, paralleled the protein expression. As shown in *figure 2*, significantly higher values were observed in matured Mo-DC (mMo-DC) compared to iMo-DC.

The biological activity of activin is mediated by specific receptors and by the secreted protein follistatin. Therefore, we analyzed mRNA expression of follistatin and the receptors ACTRIIA and ACTRIB. Follistatin mRNA expression was very low in all conditions, but minor increases were observed following Mo-DC stimulation by maturating stimuli. Follistatin concentrations in the supernatants from iMo-DC and mMo-DC were below the ELISA detection range (data not shown). ACTRIIA and ACTRIB mRNAs were expressed in DC, but while ACTRIB mRNA was equally expressed in quiescent or stimulated DC, ACTRIIA mRNA was upregulated by maturating stimuli *(figure 2)*.

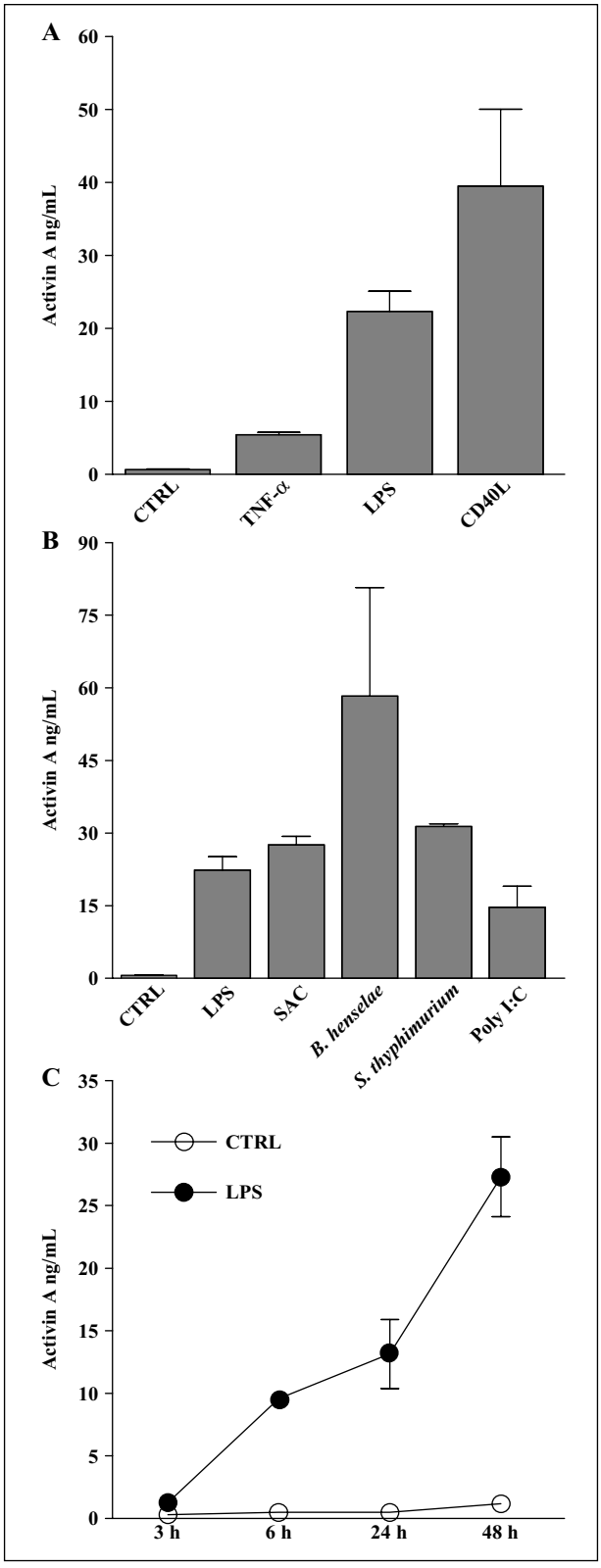

Figure 1

Induction of activin A during maturation. Mo-DC (10^6/mL) were incubated in the presence of TNF-α (20 ng/mL), LPS (100 ng/mL), CD40L-transfected J558 cells (1:4 ratio) (**A**) and in the presence of SAC (1/5 000 dilution), *B. henselae* (ratio 10:1 *B. henselae*/DCs), *S. thyphimurium* (ratio 10:1 *S. thyphimurium*/DCs) or poly I:C (10 μg/mL) (**B**) for 24 h. Mo-DC were cultured in the absence (control) or in the presence of LPS for the indicated time (**C**). Supernatants were collected and tested for activin A production by ELISA. Data are expressed as the mean ± SD of three separate experiments.

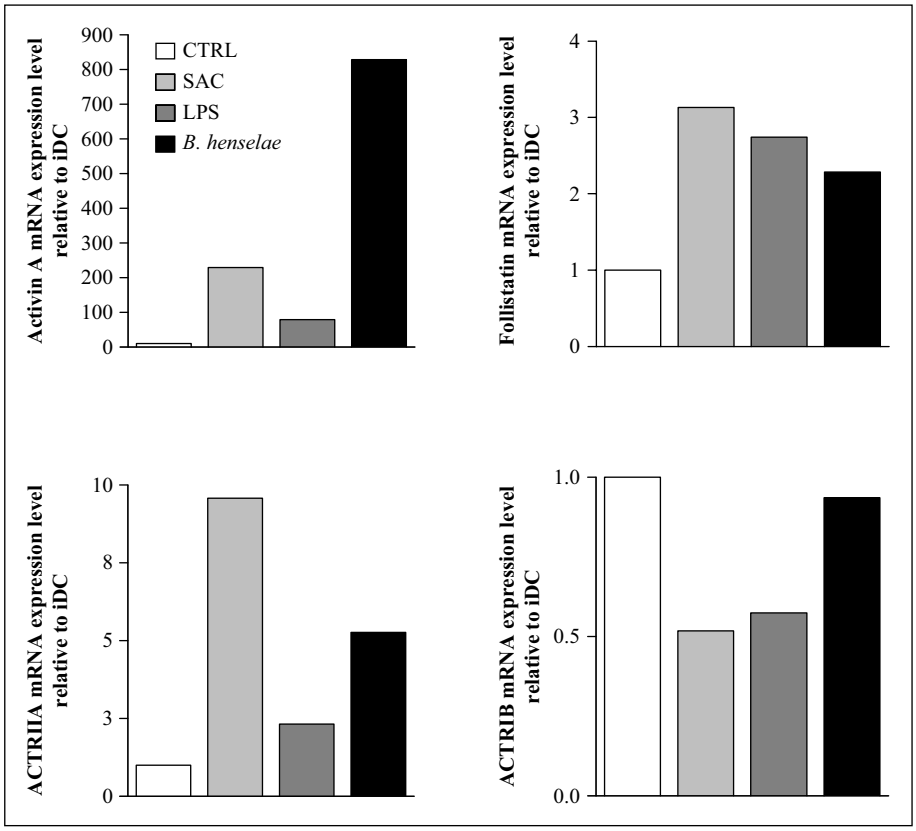

Figure 2

Activin A, follistatin and activin A receptor gene expression by Mo-DC. Mo-DC were incubated for 6 h in the presence of the indicated stimuli. Activin A, follistatin, ACTRIIA and ACTRIB mRNA was determined by real-time PCR relative to GAPDH mRNA used as internal control. The expression level of iMo-DC was assumed as the 1.0 value. Similar results were obtained for three different donors.

Activin A production by different DC subsets

DC may be divided into distinct subsets whose heterogeneity is reflected at different levels, such as the precursor cell type, anatomical localization, and immune response activation [29, 30]. The studies on activin A production were extended to monocyte-derived Langerhans cells (LC) and to the circulating DC subsets, myeloid and plasmacytoid DC. Mo-DC and LC were obtained from CD14+ cells cultured in the presence of GM-CSF and IL-4 or GM-CSF, IL-4 and TGF-β. Both Mo-DC and LC released significant amounts of activin A following stimulation with LPS or CD40L trasfected cells for 24 h *(figure 3)*. Blood-purified myeloid and plasmacytoid DC were stimulated with LPS or influenza virus respectively. Weakly detectable activin A was found in both unstimulated myeloid and plasmacytoid DC; activin A induction was observed in myeloid DC stimulated with LPS, although at levels that were lower than those observed with Mo-DC, but not in virus influenza-stimulated plasmacytoid DC. Thus, the production of activin A is apparently restricted to the myeloid DC subset.

Regulation of activin A production

The maturation and function of DC are controlled by pro- and anti-inflammatory cytokines. For instance, IFNγ is known to promote IL-12 production by mature DC,

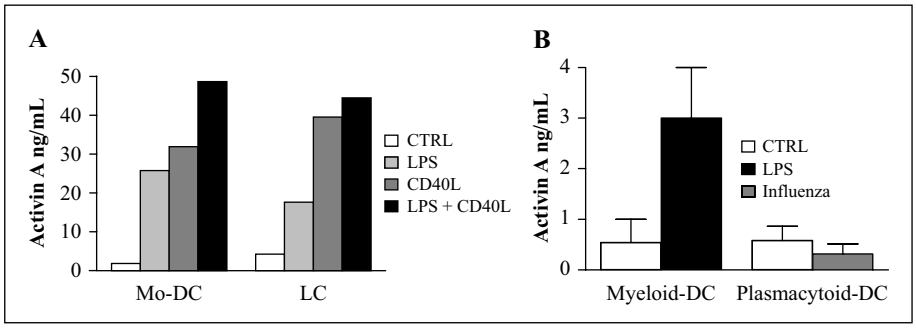

Figure 3

Activin A production by different DC subsets. Mo-DC and LC were obtained by blood monocytes cultured respectively in the presence of GM-CSF and IL-4 or GM-CSF, IL-4 and TGF-β (**A**). Myeloid and plasmacytoid DC were sorted with BDCA-1 and BDCA-4 magnetic beads (**B**). Cells were incubated for 24 h with the indicated stimuli. Supernatants were collected and tested for activin A. Data shown are one representative experiment out of three (**A**) or the mean ± SD of three separate donors (**B**).

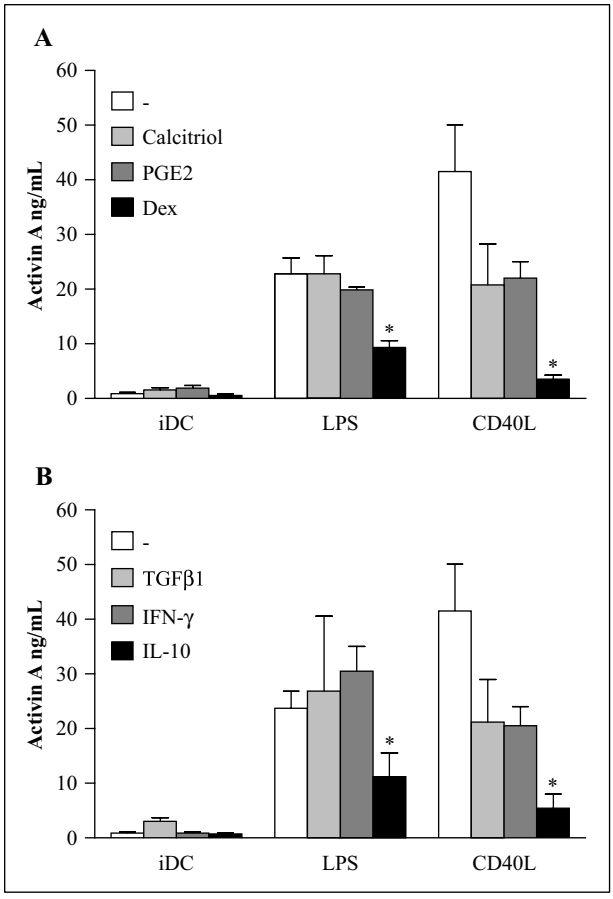

Figure 4
Regulation of activin A production by drugs and cytokines affecting DC functions. **A**) DC were exposed to calcitriol (1 uM), PGE2 (10 uM), or dexamethasone (Dex; 10 uM), for 3 h before stimulation with LPS or CD40L. **B**) DC were cultured with LPS or CD40L in the presence or absence of TGFβ1, IFN-γ, or IL-10. Supernatants were collected after 24 h and activin A was measured by ELISA. Results are the average determination ± SD of three to five experiments.
* $p < 0.05$ *versus* LPS and CD40L stimulated DC.

whereas IL-10 and TGFβ inhibit DC maturation and IL-12 production [31-35]. The effects of these cytokines on activin A production were investigated. *Figure 4* shows that IL-10, but not TGF or IFN-γ, reduced the levels of activin A released by LPS- or CD40L-matured Mo-DC. The studies were then extended to agents known to interfere with DC maturation and cytokine production such as dexamethasone, calcitriol and PGE2 [36, 37]. Calcitriol and dexamethasone block DC maturation and inhibit chemokine production. On the other hand, PGE2 does not affect the acquisition of a mature phenotype but inhibits IL-12 production [38]. *Figure 4* shows that dexamethasone decreased activin A production induced by either LPS or CD40L. Conversely, calcitriol and PGE2 did not show a statistically significant effect.

Activin A effects on DC maturation and cytokine release

To investigate whether activin A mediates DC maturation in an autocrine manner, Mo-DC were stimulated with LPS for 48 h in the presence or absence of follistatin *(figure 5)*. LPS increased expression levels of CD83, CCR7 and MHC class II indicating efficient maturation. Activin A neutralization by follistatin did not significantly alter the

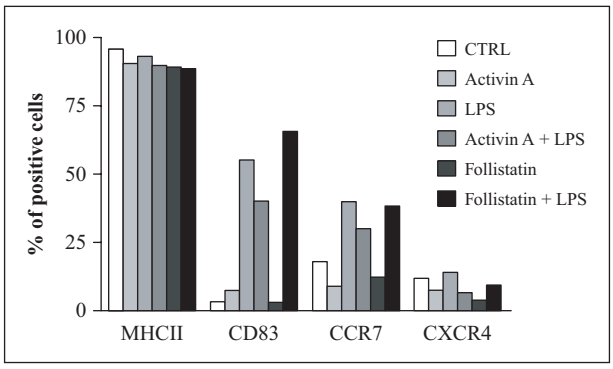

Figure 5
Activin A does not regulate LPS-induced Mo-DC maturation. Mo-DC were matured with LPS (100 ng/mL) in the absence or presence of follistatin (400 ng/mL) for 40 h and stained with anti-CD83, MHCII, CCR7, CXCR4. Results are expressed as the percentage of positive cells for MHCII, CD83, CCR7 and CXCR4.

expression of these cell surface markers, either in unstimulated or in LPS-matured Mo-DC, suggesting that DC maturation is not affected by deprivation of endogenous activin A. To evaluate the effect of exogenous activin A, Mo-DC were matured with LPS alone or in combination with 100 ng/mL of activin A. Addition of activin A neither matured Mo-DC nor affected LPS-induced Mo-DC maturation evaluated on the basis of CD83, CCR7, and HLA DR expression. Next, cytokine production and the allostimulatory function of Mo-DC matured with LPS in the presence or in the absence of activin A were compared. Activin A was not a sufficiently potent stimulus to induce the secretion of IL-6, IL-8, IL-10, and the secretion of these cytokines was not modified by activin A. Similarly, DC matured in the presence of activin A performed comparably to LPS-matured Mo-DC in allogenic MLR (data not shown).

Immature DCs have an extraordinary ability to sample the surrounding environment by endocytosis. They use two main mechanisms for Ag capture: macropinocytosis, which occurs in a receptor-independent fashion in DC and allows continuous internalization of Ags present in the fluid phase; receptor-mediated endocytosis, which involves the internalization of soluble Ags after clustering of receptors in clathrin-coated pits [39, 40]. We analyzed whether activin A was able to modulate the endocytic capacity of immature and mature Mo-DC. To this aim, we used two endocytic markers, FITC-Dex, thought to be internalized via a receptor-mediated pathway, and FITC-BSA internalized via receptor-independent macropinocytosis in DC.

Immature Mo-DC untreated or treated with activin A, exhibited a similar capacity to internalize both FITC-dextran and FITC-BSA. LPS-matured Mo-DC, as expected, hardly internalized either fluorescein-conjugated substrate at all; activin A increased FITC-dextran uptake by mature Mo-DC, but did not exert a significant influence on FITC-albumin uptake *(figure 6)*.

Activin A effects on monocyte differentiation into DC

Human monocytes express activin A receptors (our unpublished observation) and respond to the cytokine [41]. We therefore examined whether activin A might affect the

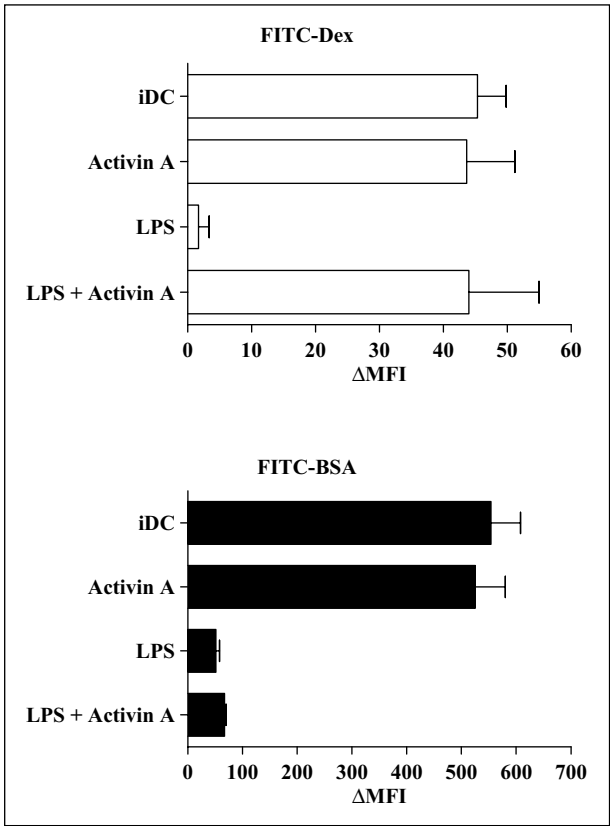

Figure 6
Activin A increases the endocytosis of FITC-Dex by LPS-matured Mo-DC. Mo-DC matured with LPS (100 ng/mL) in the absence or presence or activin A (100 ng/mL) for 24 h were incubated with FITC dextran (1 mg/mL) or FITC-albumin (2 mg/mL) for 30 min at 37°C and endocytosis was measured by flow cytometry. Results, expressed as the difference between the MFI of the sample incubated at 37°C and the MFI of that maintained at 4°C, represent the mean ± SD of three experiments.

differentiation of monocytes into DC. Monocytes were cultured with GM-CSF and IL-4 in the presence or absence of activin A, and the expression of the differentiation marker was assessed. In a series of experiments, we consistently found that a higher percentage of CD1a+ cells was obtained in monocytes cultured in the presence of activin A than in monocytes cultured in the presence of GM-CSF and IL-4 only *(figure 7)*. These results indicate that activin A promotes monocyte differentiation into DC. We next determined whether the addition of activin A could counteract the effect of cytokines known to switch the differentiation of monocytes towards macrophages at the expenses of DC [42, 43]. Chomarat *et al*. showed that the addition of IL-6 to monocytes cultured with GM-CSF and IL-4 yield a high percentage of CD1a-CD14+ cells [43]. As shown in *figure 7*, adding activin A to the GM-CSF/IL-4/IL-6 culture resulted in an increased percentage of CD1a+ cells and therefore in a repolarization of monocyte differentiation toward DC.

DISCUSSION

In this study, we analyzed activin A production by DC, its regulation, and the ability of activin A to modulate DC differentiation and functions. We found that immature Mo-DC and LC produced low levels of activin A, whereas abundant activin A secretion was induced by a number of maturation stimuli. High levels of activin A were induced by inflammatory and immune stimuli (TNF and CD40L), TLR agonists (LPS, Poly I:C, SAC) and whole bacteria *(S. typhimurium* and *B. henselae)*. The highest activin A secretion was observed in the presence of *B. henselae,* a Gram negative bacterium recently shown to induce DC maturation through engagement of TLR2 and TLR6 [44]. Two subsets of human circulating blood DC have been defined based on the expression of CD11c, namely $CD11c^+$ myeloid DC and $CD11c^-$ plasmacytoid DC [30]. These two DC subsets have distinct roles in the induction and regulation of the immune response. Our data show that activin A production is restricted to mature myeloid DC.
We have demonstrated that activin A is abundantly released by DC following stimulation with TLR agonists, and suggest that DC represent an important source of this cytokine during inflammation and sepsis. These data are in agreement with the observations that the activin A serum levels are elevated in patients with sepsis and that activin A is systemically released following LPS injection in mice [13-15]. In our *in vitro* model, we checked the bioavailability of activin A in culture supernatants by concurrently analyzing the concentrations of follistatin, a secreted protein that inhibits activin A by sequestration. Indeed, activin A measurement by ELISA does not allow differentiation between free and follistatin-bound activin, the latter being biologically inactive. We found that maturation stimuli that up-modulated activin A did not affect follistatin expression, and in all cases, follistatin concentrations in culture supernatants were below the ELISA detection

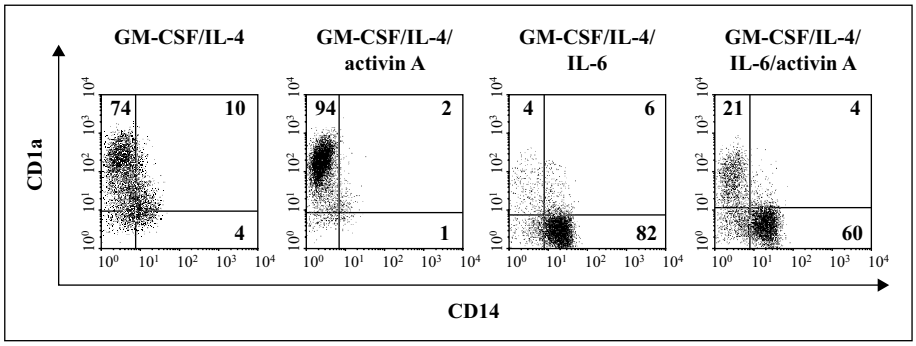

Figure 7
Activin A promotes the development of CD1a+ DCs from human monocytes. Monocytes were cultured with GM-CSF (100 ng/mL) and IL-4 (10 ng/mL) or GM-CSF, IL-4 and IL-6 (100 ng/mL) for 6 days in the absence or presence of activin A (100 ng/mL). CD1a and CD14 expression was measured by flow cytometry.

limit. As a consequence, we believe that mature DC represent a relevant source of free and bio-available activin A.

DC functions are modulated by factors that interfere with their maturation and/or cytokine production. For instance, calcitriol and dexamethasone block DC maturation and inhibit IL-12 and chemokine production. On the other hand, PGE2 does not affect the DC maturation process, assessed as the acquisition of the mature phenotype, but does inhibit the ability of DC to produce IL-12 [36-38]. We have shown that activin A production by LPS-stimulated DC is selectively inhibited in the presence of dexamethasone, whereas it is not affected by PGE2 and calcitriol. In line with these findings, it has been shown that glucocorticoids inhibit both the constitutive and cytokine-stimulated production of activin A by human marrow stromal cells and monocytes [45], and that dexamethasone induces a follistatin-dominant microenvironment favouring the differentiation of mesenchymal progenitor cell lines in adipocytes [46].

DC functions are also controlled by pro- and anti-inflammatory cytokines. IFN-γ is considered a costimulator of DC, as it enhances their ability to release IL-12 [32-35]. However, we observed that the addition of IFN-γ to LPS or CD40L did not enhance activin A production by DC. IFN-γ has been reported to inhibit the secretion of activin A by stromal fibroblast [47]. Conversely, Ebert *et al*. showed that IFN-γ is essential for the release of activin A by mouse microglial cells and peritoneal macrophages stimulated with Toll-like receptor agonists [13]. The different effects of IFN-γ on activin A production may be related to the cell types and experimental systems used.

Both IL-10 and TGF-β have been involved in the suppression of DC functions [32-35]. IL-10 has been identified as a major inhibitory factor: it prevents DC differentiation from monocytes, strongly inhibits DC maturation induced by different stimuli, and blocks the up-regulation of costimulatory molecules and IL-12 production, thus impairing the DC-induced generation of Th1 responses. Similarly, TGF-β prevents DC maturation *in vitro*, reduces their Ag-presenting capacity and inhibits the up-regulation of critical costimulatory molecules on their surface [32]. Despite these similar effects of TGF-β and IL-10 on DC, we found that IL-10 inhibits activin A release by DC, whereas TGF-β had no effect. The lack of TGF-β-induced inhibition of activin expression by DC is common to other cell types such as pancreatic cells [11]; actually, the induction of activin A by TGF- β has been observed by us in fibroblasts [20].

Our results indicate that Mo-DC express activin type I and II receptors. Thus, endogenous activin A might be an important regulator of DC biology. However, neutralization of LPS-induced activin A by follistatin did not affect LPS-induced MoDC maturation and did not alter cytokine production by LPS-matured MoDC. These observations are in agreement with the data recently reported by Robson *et al*. [48]. The lack of activin A autocrine/paracrine effects on DC activity could be due to a discrepancy between the amount of activin A produced by LPS-stimulated MoDC and the amount of activin A necessary to regulate DC functions. Several reports underline the low potency of activin A compared to TGF-β due to the low mobilization of the Smad 2/3 pathway [49, 50]. Exposure to efficacious exogenous activin A combined with LPS activation did not influence MoDC maturation, cytokine production or allostimulatory function.

iDC are specialized in capturing and processing Ags to form MHC-peptide complexes. In the maturation process, their endocytic activity is down-regulated and mature DC, whose main activity is to present antigens to T cells, possess a low residual endocytic activity [39, 40]. Our findings however, reveal that DC matured with LPS in the presence of activin A displayed a high FITC-dextran uptake, similar to that of control iDC, whereas the endocytosis of FITC-albumin, which is receptor independent, was not affected. A similar prolonged ability of mDC to take up Ags has been reported for MoDC induced to mature by CCL16 [51]; in addition, CCL19 has been shown to induce rapid endocytosis in mDC [52, 53]. Further experiments are required to examine whether activin A plays a similar role in the endocytosis of other kinds of antigens including bacteria, and whether the Ag uptake by DC matured in the presence of activin A leads to efficient presentation or is rather responsible for the removal of bacteria.

Monocytes can be recruited to the site of tissue damage to differentiate into either macrophages or DC depending on the micro-environmental conditions. Chomarat *et al*. showed that IL-6 released by fibroblasts switches monocyte differentiation to macrophages rather than to DC [43]. Because activin A released by fibroblasts and DC is abundant in inflammatory sites, we surmised that activin A might influence the differentiation of DC from circulating monocytes. When activin A was added to GMCF and IL-4 in the monocyte cultures, we observed an increase in the CD14-CD1a+ DC and a reduction in the CD14+CD1a- population. Moreover, activin A reversed, at least partially, the inhibitory effects of IL-6 on DC differentiation of CD14+ monocytes. Activin A has been shown to suppress the ability of IL-6 to promote B lymphoid cell proliferation and to inhibit IL-6-mediated acute-phase protein synthesis [54]. The antagonistic effect of activin A on IL-6 activity suggests an anti-inflammatory potential of this cytokine [55], though, this issue is still a matter of debate. The proposed anti-inflammatory role of activin A is supported by its ability to antagonise other pro-inflammatory cytokines including IL-11 and IL-1β. Studies conducted on the human monocytic cell lines U-937 and THP-1 revealed that activin A inhibits the production of IL-1β, a potent pro-inflammatory cytokine, and furthermore, enhanced the production of the IL-1 receptor antagonist [56]. Recently, Robson *et al*. showed that MoDC-derived activin A attenuates CD40-mediated cytokine/chemokine production (48). On the other hand, several studies in diverse cell types suggest that activin A has a role in stimulating some inflammatory pathways. In bone marrow-derived macrophages, activin A profoundly stimulated the release of TNF-α and IL-1β [57]. Further pro-inflammatory actions include the stimulation of the prostanoids and the activation of the nitric oxide pathway by macrophages [57]. Considering these apparently contrasting results, we propose that the role of activin A may be anti- or pro-inflammatory depending on the different microenvironmental setting. Our results provide evidence that DC exposed to maturating stimuli represent an important source of activin A. Its production is tightly regulated by anti-inflammatory cytokines, such as IL-10, or by immunosuppressive agents, as in the case of corticosteroids. We also observed that activin A is involved in the commit-

ment of monocytes to the DC pathway by interfering with the action of IL-6.

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