Increased IL-23 secretion and altered chemokine production by dendritic cells upon CD46 activation in patients with multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS). In MS, myeloid dendritic cells (mDCs) secrete elevated amounts of IL-23, a potent pro-inflammatory cytokine, compared to healthy donors. Here, we examined the role of CD46, a complement binding factor, in mDCs by analyzing cytokine and chemokine production in healthy donors and patients with MS. There were striking differences between these groups with increased IL-23p19, CCL2 and CCL5 production, but decreased CCL2 levels in patients. This demonstrates major differences of DC activation upon CD46 activation, with a potential role in the pathogenesis of MS.

Keywords

Multiple sclerosis; human dendritic cells; chemokines; IL-23; CD46

1-Introduction

Multiple sclerosis is a chronic inflammatory demyelinating disease of the CNS (Hafler et al., 2005; Weiner, 2004b) in which both the release of inflammatory mediators and chemotaxis are important (Adorini, 2004; Elhofy et al., 2002). DCs are professional antigen-presenting cells (APC) which secrete cytokines and chemokines upon maturation and play a key role in the induction of immune responses by activating naïve T cells. We previously demonstrated that mDCs from patients with MS secrete elevated amounts of IL-23 compared to healthy controls (Vaknin-Dembinsky et al., 2006). IL-23 is a proinflammatory cytokine that consists of a specific p19 subunit associated with the shared IL-12p40 subunit (also called IL-12 beta1 subunit which associates with IL-12p35 to form IL-12 (p70)) (Frucht, 2002; Oppmann et al., 2000; Trinchieri et al., 2003). IL-23 promotes the expansion of a specific subset of T cells secreting IL-17, a potent inflammatory cytokine (Bettelli et al., 2007) involved in several

autoimmune diseases including experimental autoimmune encephalomyelitis (EAE), a murine model of MS (Komiyama et al., 2006). Previous MS studies have also demonstrated increased amounts of IL-12p40 in the nervous system and increased IL-12 production by PBMC (Balashov et al., 1997; Comabella et al., 1998; Soldan et al., 2004). Therefore, production of both IL-12 and IL-23 are dysregulated in patients with MS (Gran et al., 2004).

The engagement of CD46 at the surface of APC has been shown to modulate the production of IL-12 (p70) and/or p40 subunit, either increasing or decreasing their expression depending on the cell type and stimulus used (Karp et al., 1996; Kurita-Taniguchi et al., 2000; Schnorr et al., 1997; Smith et al., 2003). CD46 is a widely expressed transmembrane protein initially identified as a complement regulatory protein (Seya et al., 1986). It has then been described as a 'magnet for pathogens' (Cattaneo, 2004), acting as a receptor for several virus and bacteria. More recently, it was identified as a co-stimulatory molecule for T cell activation (Astier et al., 2000; Marie et al., 2002; Zaffran et al., 2001) and it induces a Tr1 regulatory phenotype with considerable secretion of IL-10 and granzyme B (Grossman et al., 2004; Kemper et al., 2003). This Tr1 differentiation is altered in patients with MS, characterized by a lack of IL-10 production upon CD46 activation (Astier and Hafler, 2007; Astier et al., 2006). Herein, we further investigated the role of CD46 in MS by analyzing its role on mDCs and comparing healthy donors and patients with MS. There were striking differences between these two groups with increased IL-23 production and modulation of CCL2, CCL3 and CCL5 secretion.

2-Material and Methods

Subjects

Local Ethical Committee approval has been received for this study and the informed consent of all participating subjects was obtained. All patients were seen at the Partners MS Center at the Brigham and Women's Hospital in Boston. Peripheral blood was obtained from healthy subjects (10 donors; average age = 35yrs±6.6, 6 females/4 males) and patients with MS (10 untreated patients with MS (41yrs±8; EDSS: 1.44±0.95, 9 females/1 male) in the relapsing–remitting stage. No patient was in relapse at the time of the blood draw. None of the patients had received steroids in the 2 months prior to blood drawing, and were not treated with interferon beta 1a in the 10 months prior to blood drawing or with immunosuppressive therapy in the 3 years prior to blood drawing. None of the patients were treated with glatiramer acetate prior to blood drawing.

Purification of mDCs and T cells

mDCs were directly isolated from the blood using CD1c beads (Miltenyi Biotec) and matured by adding 100ng/ml LPS with or without CD46 (5µg/ml, mAb 20.6). Forty-eight hours later, cells were collected for gene expression assays. For IL-17 expression, CD4$^+$ T cells were isolated as described before (Astier et al., 2006) and cultured at 2×10^6 cells/ml in 48-well plates coated with anti-CD3 mAb (OKT3, 2.5 µg/ml) for 48h with and without mDCs supernatants matured with LPS or LPS plus anti-CD46.

Detection of cytokines by ELISA

Secretion of cytokines was determined in cell culture supernatants by ELISA as previously described (Astier et al., 2006; Vaknin-Dembinsky et al., 2006). IL-23/12p40, IL-12p70, and IL-17 ELISA were predesigned kits from R&D Systems. According to the manufacturer's specifications, the IL-12 p70 ELISA does not cross-react with recombinant human IL-12 p40 (rhIL-12) p40 or rhIL-12 p35 monomers or homodimers.

Real time (TaqMan) RT-PCR analysis

Total RNA was extracted from $0.5*10^5$ cells using an RNeasy kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized using Applied Biosystems kit. The primers and probes for the TaqMan qPCR assays for IL-12p35, IL-23p19, CCL2, CCL3, and CCL5 were obtained from Applied Biosystems. Human GAPDH (Applied Biosystems) was used to normalize each sample.

Statistics

The groups were analyzed using the Prism software (GraphPad Software Inc.) and compared using the Wilcoxon matched pair test to assess CD46 stimulation effect or the Mann-Whitney test to assess significance between healthy donors and patients, two non-parametric tests that do not assume Gaussian variation.

3-Results

CD46 modulates LPS-mediated maturation of mDCS

We first analyzed the cytokine profiles produced by CD46 engagement on mDCs. A role of CD46 in IL-12p40 and p70 expression have been previously reported (Karp et al., 1996; Kurita-Taniguchi et al., 2000; Schnorr et al., 1997; Smith et al., 2003). Hence, we analyzed the role of CD46 in both IL-12 and IL-23 inductions. mDCs from 10 healthy donors were matured with LPS with or without anti-CD46 antibodies for 2 days. Supernatants and cells were then harvested, and cytokine production was assessed by quantitative real-time PCR (qPCR) or ELISA (Figure 1). We observed a significant increase of the specific p35 IL-12 chain by qPCR ($p < 0.05$)(Fig. 1A) upon CD46 activation. We next assessed the levels of both p40 and p70 by ELISA (Figure 1B). The levels of IL-12 p70 detected were barely detectable (average p70: 39 vs. 43 pg/ml, p=0.11). An increase in p40 could be detected (average p40: 1933 vs. 15873 pg/ml, **p=0.0078). Hence, we next examined whether CD46 stimulation could affect IL-23 secretion. An increased expression of the specific IL-23p19 subunit was observed (Fig. 1A, $p < 0.05$). IL-23 triggers the expansion and survival of a novel T cell subset characterized by the secretion of IL-17, a potent proinflammatory cytokine (Bettelli et al., 2006). Hence, to confirm the induction of IL-23 by CD46-stimulated mDCs, we next evaluated by ELISA the amounts of IL-17 secreted by activated T cells co-cultured with the supernatants of CD46-stimulated mDCs. Addition of CD46-activated DC supernatant significantly augmented IL-17 secretion (Fig. 1B; average IL-17: 477 vs. 772 pg/ml **p=0.0078), which was inhibited by the addition of sIL-23R (Fig. 1B). The addition of sIL-23R also led to significantly lower IL-23 levels than with LPS alone (p=0.0007), indicating that low amounts of IL-23 are secreted by mDCs matured by LPS alone, which are increased by concomitant stimulation with CD46.

IL-23p19 increase in patients with MS

mDCs from patients with MS secrete increased amounts of IL-23 compared to healthy donors (Vaknin-Dembinsky et al., 2006). Therefore, we next determined whether the specific chains for IL-12 and IL-23, p35 and 19 respectively, were differently modulated upon CD46 in patients with MS compared to healthy donors. mDCs obtained from a cohort of 10 patients with MS in the relapsing-remitting stage were compared to mDCS from a subset of 10 age-matching healthy donors. mDCs were cultured in presence of LPS with or without CD46 antibodies and cytokine production were determined by qPCR 48hrs later (Fig.2). The IL-12p35 specific chain was induced in both groups, and no significant difference was observed between these groups (Fig. 2A). An ELISA assessing IL-12p70 was also performed but very low levels were detected and no difference between these two groups was observed (not shown). However, a marked increase in IL-23p19 expression was detected in patients compared to healthy controls (Fig. 2B, **p=0.0043; >4.5fold induction).

Chemokine modulation by CD46 stimulation

Among the pathogenic mechanisms that contribute to the inflammation observed in MS, the production of inflammatory mediators and leukocyte chemotaxis into the CNS are both important (Sorensen et al., 1999; Zhang et al., 2000). We next examined whether CD46 could modulate chemokine secretion by mDCs, and compared patients with MS and healthy donors. mDCs were matured in presence of LPS with or without CD46 antibodies for 48hrs and the relative expression of CCL2 (MCP-1), CCL3 (MIP-1α) and CCL5 (Rantes) compared to their expression in immature mDCs was determined by qPCR (Fig. 3A). While LPS induced CCL2 secretion, CD46 engagement led to a significant decrease of CCL2 expression. Furthermore, a significant difference was observed between healthy donors and patients with MS. While ~50% of CCL2 expression was inhibited by CD46 in mDCs from healthy donors, ~90% inhibition was observed in patients. Alternatively, CD46 stimulation induced a significant increase of CCL3 and CCL5 in patients with MS. These data were further confirmed by ELISA (Figure 3B): while LPS increased CCL2 production, CD46 led to a decrease in CCL2 secretion in both healthy donors and patients with MS. However, the decrease observed in patients was much more pronounced as the levels of CCL2 were similar to those obtained with immature DC. Conversely, CD46 stimulation led to an increase of both CCL3 and CCL5, which was significantly increased in patients when compared to healthy donors.

4-Discussion

MS is a demyelinating disease with a chronic inflammation of the central nervous system (Weiner, 2004a). Dendritic cells are professional APC that initiate the immune response by activating naive T cells. Upon maturation, they then secrete cytokines such as IL-12 and IL-23 that regulate inflammation. In particular, IL-23 is a potent inflammatory cytokine which plays a crucial role in the inflammation of the brain (Cua et al., 2003). The expression of the specific IL-23p19 subunit in transgenic mice also results in systemic inflammation (Wiekowski et al., 2001). Moreover, IL-23 triggers the expansion and survival of a novel T cell subset characterized by the secretion of the proinflammatory cytokine IL-17 (Bettelli et al., 2007; Kolls and Linden, 2004). Production of IL-12p70 by APC can be modulated by cross-linking of CD46 at their surface, either increasing or decreasing IL-12 depending on the cell type and stimulus used. An inhibition of IL-12 production by monocytes is observed after CD46 ligation by antibodies or C3b binding (Karp et al., 1996), and a posttranscriptional inhibition of IL-12p70 was also observed when CD46 was triggered by HHV6 (Smith et al., 2003). On the other hand, IL-12p40 was increased by CD46 ligation on GM-CSF treated monocytes (Kurita-Taniguchi et al., 2000), and infection of dendritic cells by measles virus led to an increase in IL-12p40 (Schnorr et al., 1997). In this report, we demonstrate that activation of mDCs through CD46 increases the expression of both p35 and p19, specific chains for IL-12 and IL-23 respectively. However, no significant secretion of IL-12p70 could be detected, while the secretion of IL-23 was further confirmed by the proliferation of T cells secreting IL-17 when co-cultured with the supernatant of CD46-activated DCs. Furthermore, this was blocked by the addition of sIL-23R in the culture. The addition of sIL-23R also led to lower IL-17 levels than with LPS alone, indicating that low amounts of IL-23 are secreted by mDCs matured by LPS alone, which are increased by concomitant stimulation with CD46 (Fig. 1B). We had previously shown that increased amounts of IL-23 were secreted by DCs in patients when compared to healthy donors (Vaknin-Dembinsky et al., 2006). Herein, we show that the main increase in IL-23 detected in patients might be due to an alteration of CD46, as CD46-activated DCs from patients express much more p19 than healthy donors do (>4.5 fold induction). No significant differences in IL-12 were observed.

Among the pathogenic mechanisms that contribute to the inflammation observed in MS, the production of inflammatory mediators and leukocyte chemotaxis into the CNS are both

important. We therefore investigated the chemokine profile secreted after CD46 ligation on DCs. We demonstrated that CD46 activation modulated the profile of chemokines expressed by DCs. First, although LPS induced CCL2 expression, CD46 engagement induced a significant decrease of CCL2 expression (Fig. 2A). Of note, the differences observed on CCL2 secretion between LPS and CD46 indicated that the effects observed with CD46 were not due to endotoxins, but were specific to CD46. Furthermore, a significant difference was observed between healthy donors and patients with MS. While CD46 activation led to a ~50% inhibition in healthy donors, CCL2 production was almost totally inhibited in patients with MS (~90%). In MS, the level of CCL2 in CSF during relapses is reduced but increases during remission periods (Bartosik-Psujek and Stelmasiak, 2005;Sorensen et al., 2004). None of the patients analyzed in this study were in relapse. A systematic study by Sorensen and colleagues on the level of CCL2 in both serum and CSF showed that there is a significant decrease in CCL2 in CSF from patients compared to controls (Sorensen et al., 2004). Furthermore, CCL2 levels are higher in PBMC from untreated patients in stable phase, and are modulated by IFNβ or methylprednisolone treatments (Iarlori et al., 2002;Moreira et al., 2006). Altogether, these data argue in favor of a negative correlation between the levels of CCL2 and active MS. We show that CCL2 levels are markedly reduced in patients with MS upon CD46 stimulation, likely contributing to MS pathogenesis.

Alternatively, CD46 stimulation led to increased CCL3 and CCL5 levels compared to LPS alone, and both chemokines were significantly further increased in patients with MS. A role of CCL3 in EAE has been previously described (Karpus et al., 1995). Indeed, the blockade of CCL3 with antibodies prevents the development of disease and inhibits the infiltration of mononuclear cells into the CNS. Increased expressions of CCL5 and of its receptors, CCR1 and CCR5, have also been described in MS lesions (Balashov et al., 1999; Ubogu et al., 2006), and correlated to increased IL-12 concentrations in serum from patients (Bartosik-Psujek and Stelmasiak, 2005). Furthermore, MS is an autoimmune disease with environmental influences and genetic predisposition (Hafler et al., 2005). Genetic linkage studies in multiple sclerosis have identified the chromosome 17q11 as a susceptibility locus, which encodes a cluster of genes for beta-chemokines or CC chemokine ligands (CCLs) including CCL2 and CCL3 (Vyshkina and Kalman, 2005; Vyshkina et al., 2005). We show here that CD46 activation modulates the secretion of these chemokines with differential expressions in patients with MS. Altogether, these data suggests that CD46 activation is altered in patients with MS with decreased CCL2 production and increased CCL3 and CCL5 secretion that might contribute to the development of MS lesions.

Hence, our data illustrate novel roles of CD46 in mDC function that are altered in MS. We show that CD46 controls the release of chemokines and of both IL-12 and IL-23 by activated mDCs. IL-23 production was drastically increased in patients with MS, along with significant differences in chemokine production which likely contribute to the inflammation and subsequent development of MS lesions. Altogether with our recent data on CD46 in T cells from patients with MS, this report underlines the importance of CD46 in the control of the immune response and how its dysfunction may contribute to MS pathogenesis.

Acknowledgments

5-References

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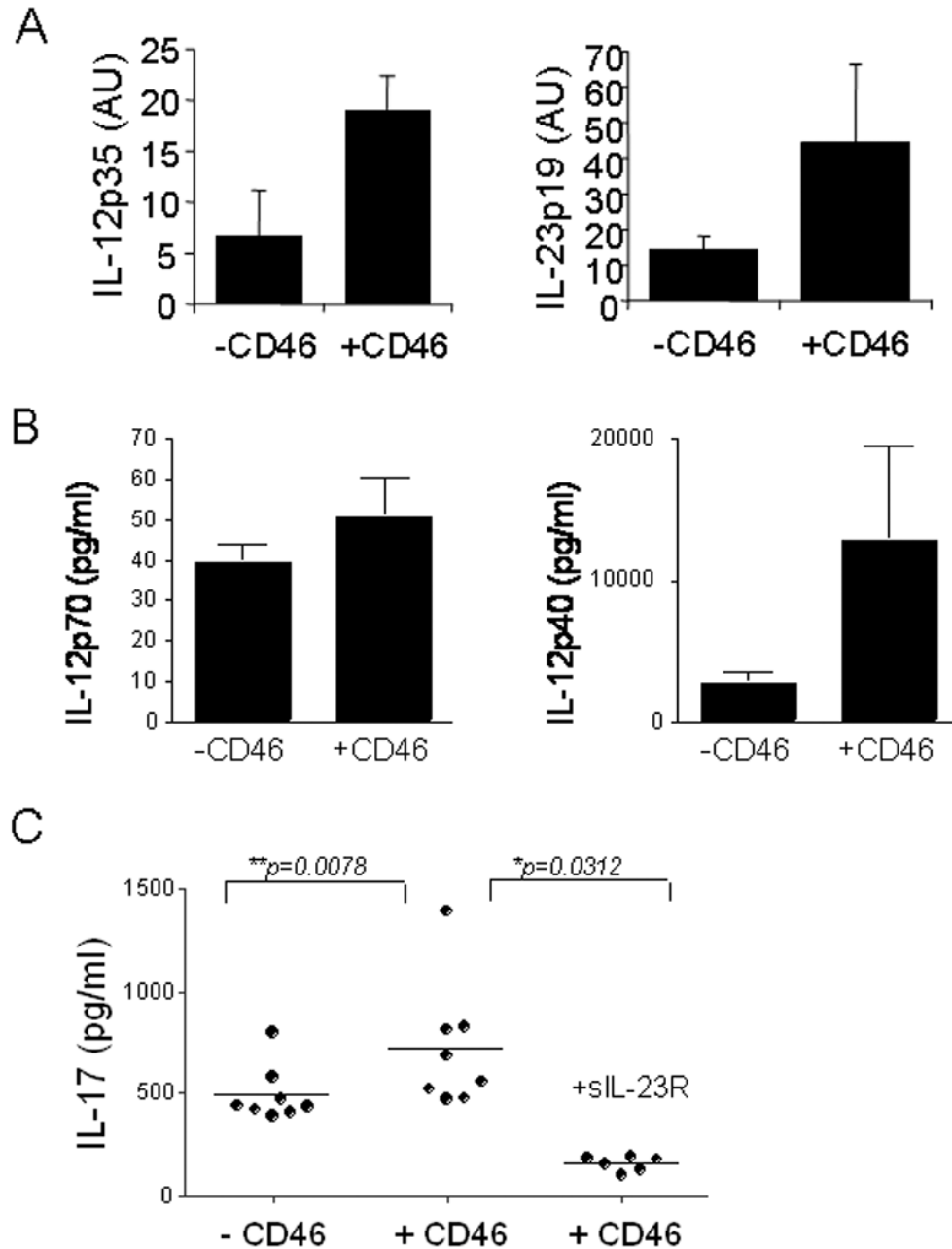
Figure 1. IL-12 and IL-23 production by CD46-activated mDCs
(A) The production of IL-12 and IL-23 by CD46-activated mDCs was quantified by qPCR.
Isolated mDCs from 10 healthy donors were matured in presence of LPS in presence or absence
of anti-CD46 antibodies. After 48h, RNA were isolated and cytokine production examined by
qPCR. (B) The amounts of IL-12p40 and IL-12 p70 secreted by mDC in presence of absence
of CD46 were also quantified by ELISA (C) The amount of IL-17 produced by activated T
cells cultured in presence of supernatants from mature mDCs with or without anti-CD46 was
determined by ELISA. The addition of sIL-23R in the T cell culture blocked IL-17 induction.
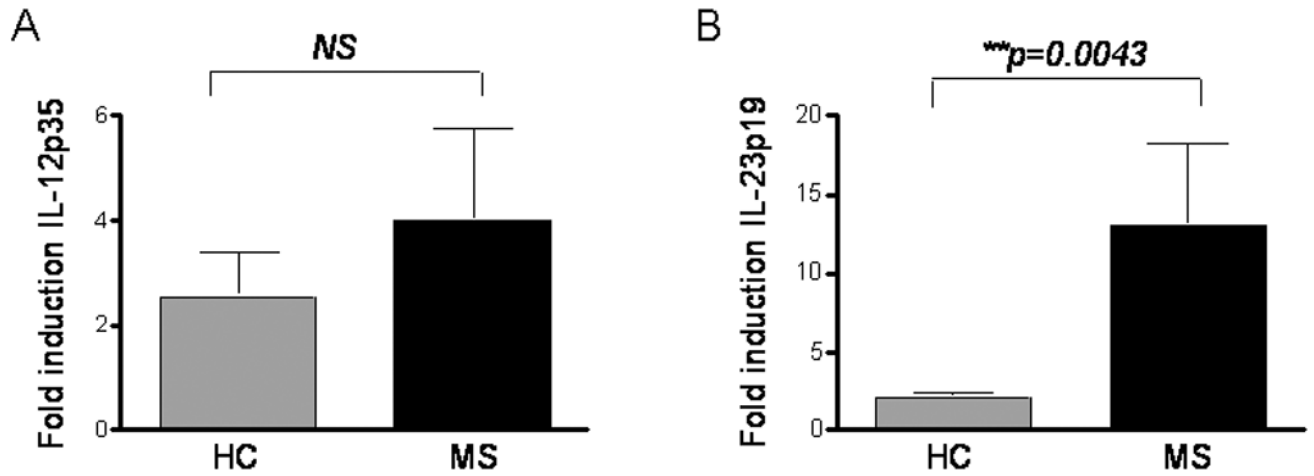
Figure 2. Increased IL-23p19 production in patients with MS
The induction of IL-12p35 (A) and IL-23p19 (B) upon CD46 activation was assessed in healthy controls (HC) and patients with MS (MS). Isolated mDCs from 10 untreated patients with MS in the relapsing-remitting phase and of 10 healthy donors were matured in presence of LPS in presence or absence of anti-CD46 antibodies. After 48h, RNA were isolated and cytokine production examined by qPCR. The induction of both IL-12 and IL-23 upon CD46 activation was calculated.
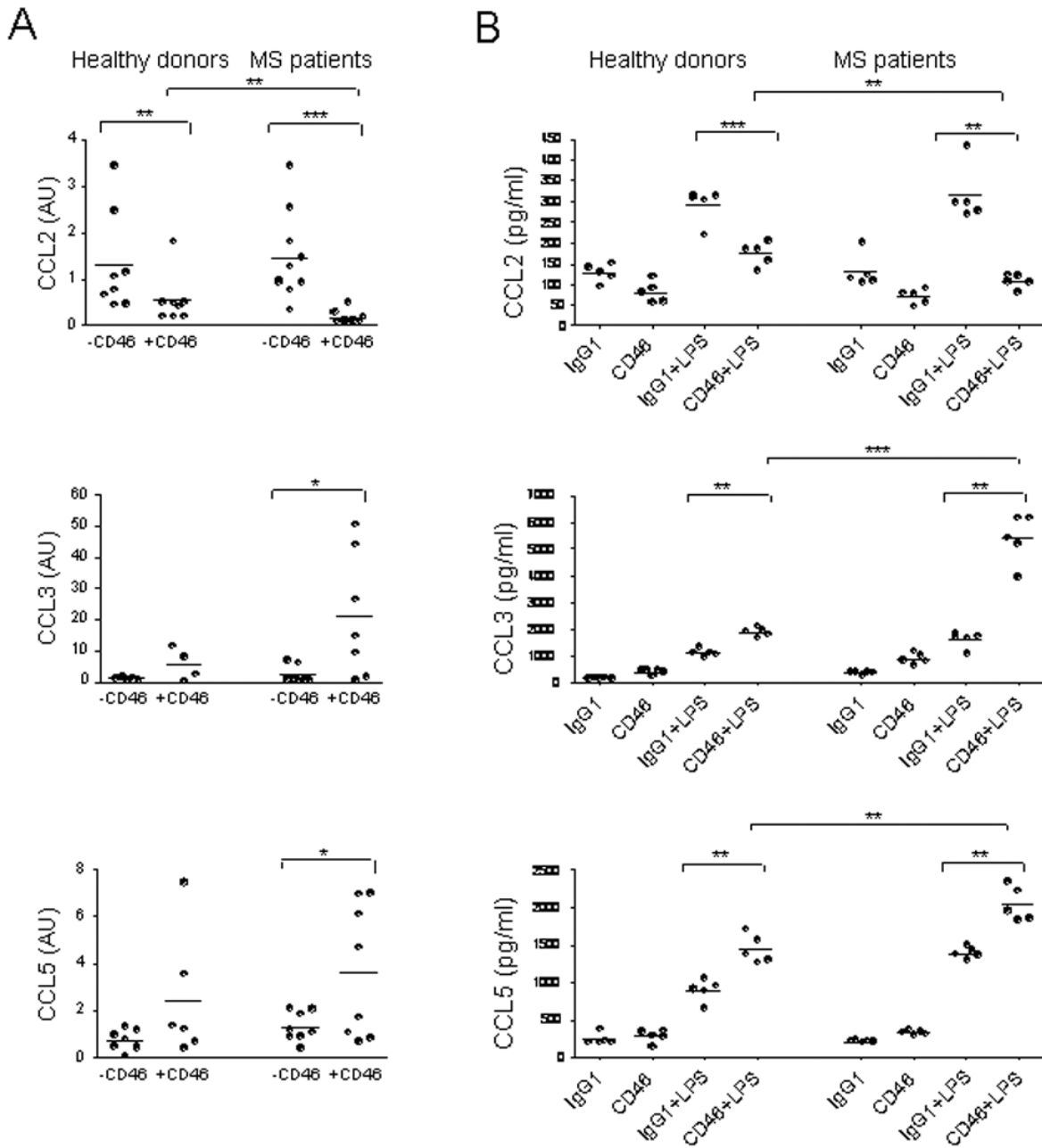
Figure 3. Chemokine production by CD46-activated mDCs
(A) The productions of CCL2, CCL3 and CCL5 by mature mDCs obtained by culture with LPS in presence or absence of CD46 antibodies were measured by qPCR (A) in a cohort of 8 healthy donors and 9 untreated patients with MS in the relapsing-remitting phase. Their relative expression compared to immature mDCs is plotted. (B) The amounts of CCL2, CCL3 and CCL5 secreted by mDC activated by CD46 (or irrelevant IgG1) were assessed by ELISA.