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Short communication

CD46 activation induces distinct CXCL-10 response in monocytes and monocyte-derived dendritic cells

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ABSTRACT

CD46 is an important immune regulatory receptor with dual functions, however, the CD46 isoform distribution and the effect of CD46 activation on the cytokine production in monocytes and monocyte-derived dendritic cells (moDCs) is unclear. Here, we show that CD46 activation of moDCs downregulates LPS-induced CXCL-10 expression, while the expression of CXCL-10 in monocytes is unaffected. Furthermore, the differentiation of moDCs induces a switch towards dominance of CYT-2 isoforms of CD46. These data indicate that CD46 activation exerts different functions in monocytes and moDCs and this correlates with a switch in CD46 isoform expression upon differentiation of moDCs.

1. Introduction

Upon infection, monocytes migrate to the site of infection where they can differentiate into monocyte-derived dendritic cells (moDCs). Through secretion of cytokines and chemokines these cells play a key role for attracting and activating naïve T cells. Accordingly, microorganisms may modulate the immune response in favor of their own survival by suppressing the cytokine production from DCs [1].

CD46 is a surface receptor with important immune regulatory functions, that is used by distinct microorganisms as a receptor for infection [2]. Alternative splicing generates several isoforms of CD46 denoted BC1, BC2, C1, and C2, with B and C indicating the alternative spliced extracellular domain, and 1 and 2 indicating which one of the two separate cytoplasmic tails (CYT-1 and CYT-2) that is expressed [3]. Both cytoplasmic tails contain signaling motifs and may be utilized by the invading microorganism to reduce the cellular immunological response [4]. In T cells, the co-stimulation of CD3 and CD46 recently emerged as essential for the induction of a T helper type 1 (Th1) response and subsequent intrinsic contraction [5–7], processes proposed to be dependent on the cytoplasmic tail of CD46 [8].

The functional significance of CD46-mediated regulation of monocytes and moDCs has been inconclusive. Engagement of CD46 on monocytes downregulates the expression of IL-12p40 and IL-12p70 [9]. In contrast, CD46 engagement may also upregulate the expression of LPS-induced IL-12p35, IL-12p40 and IL-23p19 in DCs as well as modulating the chemokine response in these cells [10]. This discrepancy in results is suggested to be explained in part by the developmental stage of the cells [4].

To further explore the regulatory function of CD46 in the innate immune system, we investigated functional consequences of CD46 activation in monocytes and moDCs and found that CD46 activation downregulated LPS-induced CXCL-10 production in moDCs, whereas the production of CXCL-10 in monocytes was unaffected. Intriguingly, differentiation of monocytes to moDCs correlated with a switch to a dominant expression of CYT-2 isoforms of CD46.

2. Materials and methods

2.1. Ethical approval

Buffy coats from healthy Caucasian donors were collected at the Blood Bank of the Department of Clinical Immunology, Aarhus University Hospital, and were provided anonymously for analysis according to the guidelines from the Danish Society for Clinical Immunology and the Ethical Committee on the use of donor samples for research purposes. All donors provided informed consent as to the use of their blood samples for scientific purposes.

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2.2. Cell isolation

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Pague PLUS density gradient centrifugation (GE Healthcare Bioscience). The PBMCs were cryopreserved for less than 3 months at −80 °C in 90% heat-inactivated fetal bovine serum (FBS) (Sigma-Aldrich) and 10% DMSO (Sigma-Aldrich). Monocytes were isolated using the EasySep™ Human Monocytes Isolation Kit (Stemcell Technology) and cultured in RPMI-1640 (Lonza) containing 2 mM glutaMAX (Gibco), 20 µg/mL gentamicin (Gibco), and 2% normal human serum type AB (Invitrogen).

2.3. Cell differentiation and stimulation

Monocytes were differentiated into moDCs as previously described [11]. In brief, 0.6×10^6 monocytes/mL were seeded in the presence of 100 ng/mL recombinant human (rh) GM-CSF (R&D system) and 20 ng/mL rhIL-4 (Immunotools). At day 6 the adherent, differentiated moDCs were harvested. Approximately 300,000 monocytes or 50,000–200,000 moDCs were stimulated in a 48-well plate pre-coated with 5 µg/mL αCD46 (clone M177, Thermo Scientific or clone J4.48, Meridian Life Science), IgG1 (clone MOPC-21, Biolegend), or PBS (Sigma-Aldrich) in the presence of 100 ng/mL LPS (*E. Coli* O26:B26, Sigma-Aldrich). Monocytes were stimulated with 20 ng/mL rhIL-4, 100 ng/mL rhGM-CSF, 100 ng/ml rhIL-10 (R&D System), or 100 ng/mL rhIL-35 (Enzo Life Science).

2.4. RT-PCR

RNA was isolated using the Nucleospin RNA Purification Kit (Macherey-Nagel) or the RNeasy Mini Isolation Kit (Qiagen) according to the manufacturers' protocol and converted to cDNA using the QuantiTect RT Kit (Qiagen). The relative expression of *IL-23p19* (fw AGTGTGGAGATG, rev GGGACTGAGGCT), *IL-12p40* (fw CATCAAACC TGACCCACCC, rev TTTCTCTCTTGCTCTTGCCCT), and *CXCL-10* (fw ACCTGCATCAGCATTAGTAATC, rev CCTTTCCTTGCTAACTGCTT) mRNA was analyzed by RT-PCR using Brilliant II SYBRgreen (Agilent Technology) and normalized to *PPIB* (fw TGTGGTGTTTGGCAAAGT, rev TGGAATGTGAGGGGAGTG) using the $2^{-\Delta CT}$ method. The mRNA expression of the four common CD46 isoforms was analyzed using a CD46 isoform-specific RT-PCR assay as previously described [12].

2.5. ELISA

The amount of CXCL-10 in the supernatants was assessed by ELISA using Human CXCL-10 Duoset ELISA Kit (R&D system). Each sample was performed in duplicates and the data analyzed by VersaMax using 4-parameter fit for the standard curve.

2.6. Flow cytometry

MoDCs were stained with αHLA-DR-FITC, αCD40-APC, αCD80-PE-Cy7, αCD83-Brilliant Violet 421, αCD86 PE-Cy5 (all from BD Biosciences), and LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit (Life Technologies). Data were acquired on LSR Fortessa (BD) and processed in FlowJo (Tree Star).

2.7. Imaging flow cytometry

Monocytes and moDCs were stained with αCD46-PE (Sigma-Aldrich) and LIVE/DEAD Fixable Near-IR Dead Cell Stain Kit and analyzed using ImageStream$^{®X}$ Mark II (Amnis). Brightfield images were captured in channel 1 and 9, PE in channel 3, and Near-IR in channel 12. All images were captured with a 60X objective with lasers adjusted to maximum values without saturating the brightest cells. Imaging flow cytometry data analyses were performed in IDEAS® 6.2 software (Amnis). Following exclusion of dead cells, cell area was determined using a M01 mask and the PE signal was normalized by dividing PE intensity with cell area.

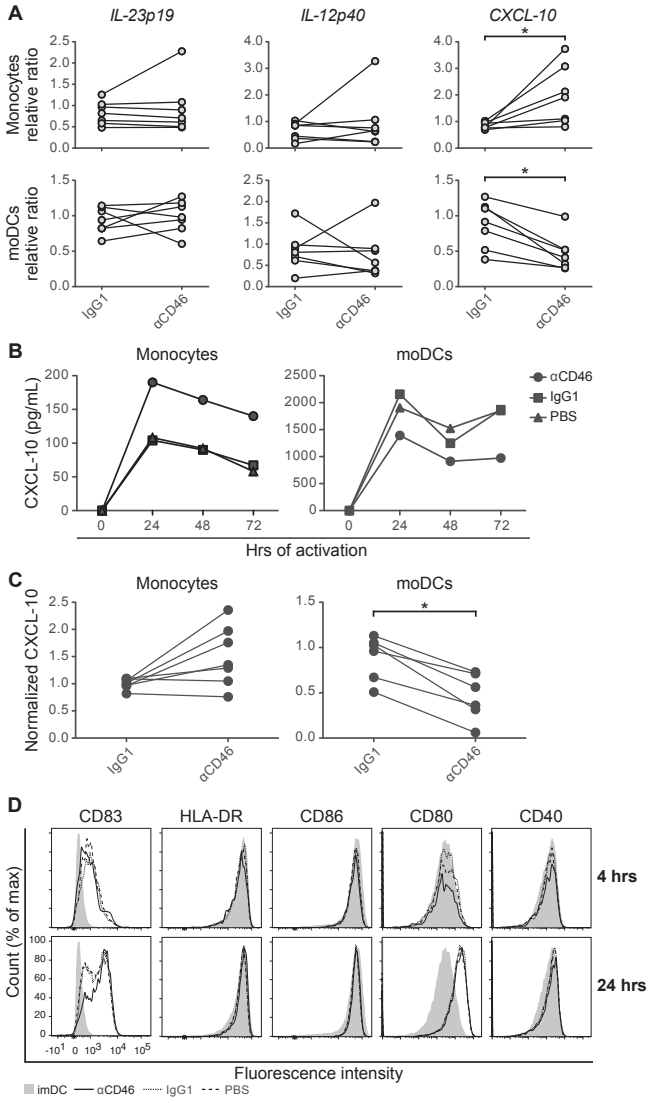

Fig. 1. CD46 activation reduces LPS-induced CXCL-10 in moDCs. Monocytes and moDCs were stimulated with 100 ng/ml LPS in presence of 5 µg/ml αCD46 (clone M177 (A–C) and clone J4.48 (D)) or IgG1 isotype control. (A) Relative mRNA expression of cytokine genes in monocytes and moDC. RT-PCR data are represented as a relative ratio compared to the expression level in control samples with no antibody stimulation. N = 7 for both monocytes and moDCs. *$p < 0.05$. (B) Secretion of CXCL-10. CXCL-10 in the cell culture supernatants was measured at the indicated time-points by ELISA. Data represents monocytes and moDCs from 1 donor. (C) Concentration of CXCL-10 in cell culture supernatants 24 h after stimulation. CXCL-10 protein levels are normalized to control samples with no antibody stimulation. N = 7 for monocytes, N = 6 for moDCs. *$p < 0.05$. (D) Expression of cell surface molecules. MoDCs were stimulated with LPS in the presence of αCD46 or IgG1 isotype control for 4 or 24 h. Flow cytometry histograms show data from one of two donors examined. The two donors were analyzed in independent experiments and had similar expression of surface molecules. imDC = immature dendritic cells.

2.8. Statistical analysis

The data were analyzed using the Wilcoxon signed rank test and $p < 0.05$ was considered a significant difference.

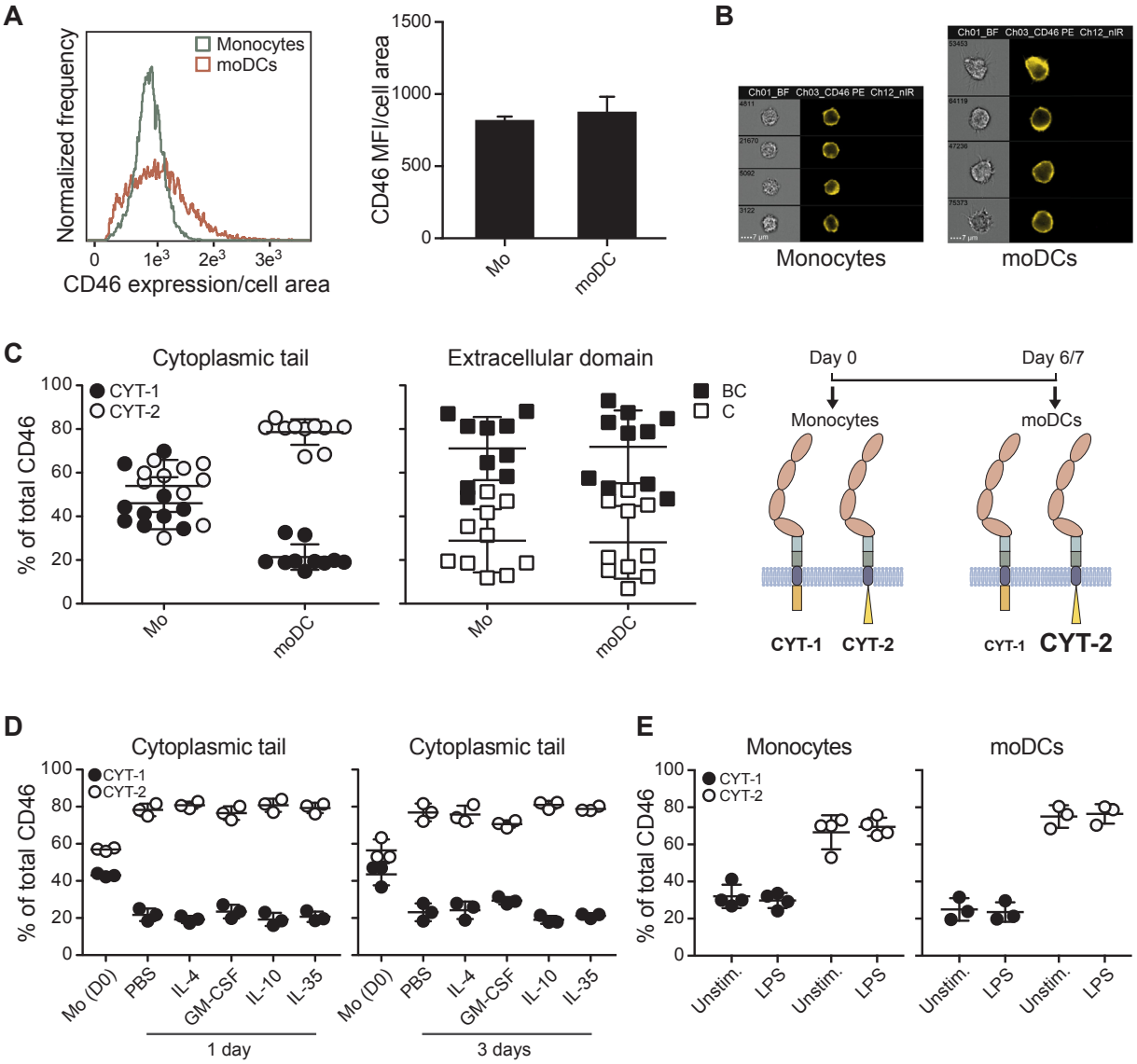

Fig. 2. Differentiation of moDCs induces a switch in the expression of the cytoplasmic tails of CD46. (A) Relative surface expression of CD46 on monocytes and moDCs. Monocytes and moDCs were stained with an antibody recognizing CD46 and analysed using imaging flow cytometry (IFC). The expression of CD46 was normalized to the cell area. Left panel illustrates flow histograms from 1 representative donor and right panel illustrates mean + SD of the median fluorescence intensity (MFI) normalized to the cell area of 3 different donors. (B) Images from IFC of 4 selected monocytes and 4 selected moDCs from the same representative donor as presented in (A). (C) Expression of CD46 isoforms in monocytes and moDC. Monocytes were differentiated into moDCs according to the culture scheme. Arrows indicate the time-points when mRNA was extracted from the cells and analyzed for expression of the CD46 isoforms BC1, BC2, C1, and C2 by RT-PCR. The relative frequency of CYT-1 and CYT-2 was calculated as the summarized percentage of (BC1 + C1) or (BC2 + C2) of the relative expression level of all isoforms. Similar, the relative frequency of BC and C was calculated as the summarized percentage of (BC1 + BC2) or (C1 + C2). Data show mean ± SD of 10 different donors. (D) Monocytes were cultured in the presence of the indicated cytokines for 1 or 3 days and the expression of the CD46 isoforms BC1, BC2, C1, and C2 was determined by RT-PCR as described in (C). The relative frequency of CYT-1 or CYT-2 is illustrated and the data show mean ± SD of 3 different donors. Mo = monocytes. (E) Monocytes or moDCs were either left unstimulated or stimulated with 100 ng/ml LPS for 24 h. The expression of the CD46 isoforms BC1, BC2, C1, and C2 was determined by RT-PCR as described in (B). Data represents the relative frequency of CYT-1 and CYT-2 and shows mean ± SD of 4 different donors (monocytes) or 3 different donors (moDCs). Unstim = unstimulated.

3. Results & discussion

To investigate the immune regulatory function of CD46 in innate immune cells, we examined the expression by RT-PCR of *IL-23p19*, *IL-12p40,* and *CXCL-10* following LPS stimulation of monocytes and moDCs with and without CD46 activation using plate-bound antibody. At 4 h post stimulation CD46 activation enhanced the LPS-induced mRNA expression of *CXCL-10* in monocytes, whereas it significantly reduced the *CXCL-10* expression in moDCs (Fig. 1A). The mRNA expression of *IL-23p19* and *IL-12p40* was unaffected in both monocytes and moDCs suggesting that CD46 activation specifically modulates *CXCL-10*.

To address whether CD46 activation also modulated secretion of CXCL-10, we analyzed the CXCL-10 concentration in the cell-culture supernatant. The secretion kinetics upon CD46 activation followed the same pattern as the control samples for both monocytes and moDCs (Fig. 1B) with a peak in secretion after 24 h of stimulation. For this time-point, the LPS-induced CXCL-10 secretion from moDCs was significantly reduced upon CD46 activation, whereas CXCL-10 secretion from monocytes was unaffected or slightly increased (Fig. 1C).

Upon infection, CXCL-10 attracts macrophages, DCs, natural killer cells, and activated T cells to the site of infection and is thereby

important for initiating an inflammatory response. Hence, reduced CXCL-10 expression from moDCs mediated by CD46 activation might impair the recruitment of important immune cells and thereby reduce the inflammatory response. It is feasible that this mechanism could be exploited by *Neisseria meningitidis,* which uses CD46 as a receptor [14].

In contrast to previous findings, we did not observe modulation of the *IL-12p40* subunit by CD46 activation in neither monocytes nor moDCs. This discrepancy might be due to the use of distinct DC-subtypes or could be caused by the use of different CD46 antibodies that may bind to different epitopes of CD46 and thereby initiate a different cellular response. In our study, we used the antibody clone M177, which binds to the SCR2 domain of CD46. However, we have observed a similar impaired CXCL-10 production in moDCs following CD46 stimulation with an antibody clone (J4.48) recognizing SCR1 (data not shown), suggesting that this function might not be mediated by anti-CD46 binding to a specific epitope.

To determine whether the reduced CXCL-10 response in moDCs was caused by a delay in maturation, moDC markers was examined by flow cytometry (Fig. 1D). CD83 was upregulated at 4 h of LPS-stimulation and both CD83 and CD80 was upregulated after 24 h of stimulation, and importantly, this was unaffected by concomitant CD46 activation. This indicates that CD46 activation does not modulate the maturation of moDCs and further suggest that CD46 activation directly modulates CXCL-10 expression.

The differential effect of CD46 stimulation might be caused by different expression level of CD46 on the surface of monocytes and moDCs. We therefore examined the initial amount of CD46 expression on the surface of the cells by imaging flow cytometry (Fig. 2A and B). Monocytes and moDCs differ in size and morphology (Fig. 2B) and when adjusted for these differences in cell area, we found a similar expression level of CD46 at the surface of monocytes and moDCs (Fig. 2A). This indicates a similar distribution of CD46 in the membrane of both monocytes and moDCs.

In T cells, the different cytoplasmic tails of CD46 may influence the response. We therefore speculated whether a change in CD46 isoform expression would occur during differentiation of monocytes to moDC. The relative expression of the four common isoforms of CD46 was examined as previously described [12]. Intriguingly, a switch into high dominance of CYT-2 containing isoforms was observed upon differentiation of moDCs regardless of the CD46-isoform phenotype in the monocytes (Fig. 2C). In contrast, the frequency in expression of the extracellular domains BC and C was not changed. A switch into a high frequency of CYT-2 containing isoforms was observed after only 1 day of culture, regardless of the presence of different polarizing cytokines in the cell-culture media (Fig. 2D). This switch was also observed by culturing the monocytes without any cytokines, suggesting that the activation triggered by putting the monocytes into culture induces the switch in CD46-isoform expression. This is in accordance with our previous results from activation of T cells, where we have demonstrated a similar switch into high dominance of CYT-2 [12], suggesting a general mechanism for activation-induced switch into CYT-2 expression in immune cells that may function to dampen the immune response.

We further determined whether stimulation of monocytes and moDCs with LPS altered the expression level of the cytoplasmic tails of CD46. Importantly, we found no significant change in the frequency of CYT-1 and CYT-2 in either monocytes or moDCs following LPS stimulation for 24 h (Fig. 2E) demonstrating that LPS does not further change the expression of CD46 cytoplasmic tails. Importantly, the difference between monocytes and moDC in expression of CYT-2 and secretion of CXCL-10 is observed when comparing freshly isolated monocytes with differentiated moDC. Although monocytes change their expression of intracytoplasmic tail from CYT-1 to CYT-2, this occurs concomitantly with being on plastic, i.e. when a differentiation is initiated. Together, these data suggest the possibility that a change in the expression of CD46 isoforms following differentiation may have consequences for the functionality of the moDC, although we cannot rule out that other differentiation-related changes are responsible for the observed differences.

In conclusion, CD46 activation reduces the LPS-induced CXCL-10 response in moDCs, whereas it might enhance the CXCL-10 response in monocytes. The reduction in CXCL-10 occurs concomitantly with a differentiation of moDCs and a switch into high dominance of CYT-2 expressing isoforms of CD46. A shifted balance between the CYT-1 and CYT-2 tail of CD46 may contribute to the distinct function of CD46 activation and it remains to be determined whether microorganisms engaging CD46 on moDCs uses this mechanism to suppress the inflammatory immune response e.g. by reducing CXCL-10 production during infection.

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Conflict of interest

The authors declare no financial or commercial conflict of interests.

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