Journal of Neuroimmunology 301 (2016) 53–60

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Myeloid dendritic cells exhibit defects in activation and function in patients with multiple sclerosis

Jürgen Haas, Alexander Schwarz, Mirjam Korporal-Kuhnke, Sven Jarius, Brigitte Wildemann *

Department of Neurology, University Hospital of Heidelberg, Germany

ARTICLE INFO

Article history:
Received 7 September 2016
Received in revised form 23 October 2016
Accepted 30 October 2016

Keywords:
Multiple sclerosis
Myeloid dendritic cells
Thymic stromal lymphopoietin receptor
Regulatory T cells
Immunomodulatory therapy

ABSTRACT

Background: Regulatory T cells (Tregs) are functionally defective in patients with multiple sclerosis (MS) and this dysfunction is related to an imbalanced composition of naïve and memory Treg subtypes. Several lines of evidence indicate that these abnormalities might result from a premature decline in thymic-dependent Treg neogenesis. Myeloid dendritic cells (mDCs) critically determine Treg differentiation in the thymus, and thymic stromal lymphopoietin receptor (TSLPR) expressed on mDCs is a key component of the signaling pathways involved in this process. TSLPR-expression on mDCs was previously shown to be decreased in MS. We hypothesized that functional alterations in mDCs contribute to aberrant Treg neogenesis and, in turn, to altered Treg homeostasis and function in MS.
Methods: We recruited blood samples from 20 MS patients and 20 healthy controls to assess TSLPR expression on mDCs *ex vivo* by flow cytometry and by activating mDCs induced by recombinant TSLP (rhTSLP) *in vitro*. As previous studies documented normalization of both function and homeostasis of Tregs under immunomodulatory (IM) therapy with interferon-beta (IFN-beta) and glatiramer acetate (GA), we also tested phenotypes and function of mDCs obtained from IM-treated patients (IFN-beta: $n = 20$, GA: $n = 20$).
Results: We found that TSLP-induced mDC activation and effector function *in vitro* was reduced in MS and correlated with TSLPR-expression levels on mDCs. IM treatment prompted upregulation of TSLPR on mDCs and an increase in TSLP-induced activation of mDCs together with a normalization of Treg homeostasis.
Conclusion: The decreased TSLP-induced activation of MS-derived mDCs *in vitro*, together with the reduced density of TSLPR on the cell surface of mDCs corroborates the hypothesis of mDCs being critically involved in impairing Treg development in MS.

1. Introduction

MS is considered to be an autoimmune disorder in which autoreactive T cells drive an inflammatory process, leading to myelin/oligodendrocyte and axonal destruction. Regulatory T cells (Tregs) are critically involved in controlling autoimmunity and inflammation, and defects in either the number or function of Tregs were previously shown to contribute to human autoimmunity (Brusko et al., 2008). We and others have shown that Tregs derived from patients with MS are functionally impaired as their potential to inhibit myelin-specific and antigen-nonspecific T-cell proliferation is diminished compared to healthy individuals (Viglietta et al., 2014; Haas et al., 2005; Venken et al., 2006, 2008a, 2008b; Frisullo et al., 2009). This Treg defect appears to be related to altered homeostasis of circulating Tregs possibly prompted by decreased thymic neogenesis of Tregs (recent thymic emigrants, RTE) and a compensatory increase in more short-lived memory Tregs (Haas et al., 2007, 2011; Venken et al., 2008b; Schwarz et al., 2013; Balint et al., 2013). Thymic generation of Tregs requires cytokine signaling *via* receptors for IL-7 and thymic stromal TSLP, which share the IL-7 receptor alpha chain (IL-7Rα) (Bayer et al., 2008; Vang et al., 2008; Yates et al., 2007; Watanabe et al., 2005; Mazzucchelli et al., 2008). In particular, the secretion of TSLP by Hassall's corpuscles, structures composed of epithelial cells in the thymic medulla, has been demonstrated to condition both mDCs and plasmacytoid dendritic cells (pDCs) and thus induce the differentiation of thymocytes into Tregs (Watanabe et al., 2005; Hanabuchi et al., 2010). A key role for mDCs and TSLP for Treg development is further underlined by a recent study demonstrating that peripheral blood DCs can induce *ex vivo* differentiation of Tregs from $CD4^+CD8^-CD25^-$-naive thymocytes following activation by TSLP (Watanabe et al., 2005; Hanabuchi et al., 2010). We recently demonstrated that $CD4^+$ T cells derived from peripheral blood of MS patients express markedly lower levels of IL-7Rα on their cell surface than those from healthy persons of the same age thereby affecting frequencies of naive and RTE Tregs and, consequently, Treg function (Haas et al., 2011, 2015). We also found a correlation between impaired Treg homeostasis and a reduced surface expression of TSLPR on

* Corresponding author at: Department of Neurology, University of Heidelberg, Im Neuenheimer Feld 350, D-69120 Heidelberg, Germany.
E-mail address: brigitte.wildemann@med.uni-heidelberg.de (B. Wildemann).

http://dx.doi.org/10.1016/j.jneuroim.2016.10.007
0165-5728/

peripheral mDCs, suggesting that, in the thymic environment, dysfunctional mDCs might potentially account for disturbances in Treg neogenesis in patients with MS. To better elucidate the role of mDCs in Treg development and their influence on Treg homeostasis and function in MS, we determined TSLPR surface expression levels *ex vivo* and functional properties of mDCs *in vitro* by using blood specimens obtained from patients with relapsing-remitting MS (RRMS) and healthy control donors (HC). As we previously demonstrated that Tregs normalize under treatment with interferon-beta (IFN-beta) and glatiramer acetate (GA) (Korporal et al., 2008; Haas et al., 2009), we additionally tested the effects of prolonged therapy with these two drugs on phenotype and function of patient-derived mDCs.

2. Material and methods

2.1. Study participants

Our study enrolled a total of 50 patients with RRMS according to the revised McDonald criteria (Polman et al., 2011). Subjects were recruited from the outpatient clinic of the Department of Neurology, University of Heidelberg, Germany. Twenty patients were treatment-naïve and 40 patients had received disease modifying treatment with INF-beta ($n = 20$, median treatment duration: 11.5 months (3–18)) or GA ($n = 20$, median treatment duration: 12.0 months (3–18)). None of the patients had been treated with corticosteroids in the last month before study entry. All patients were clinically stable at the time of blood sampling and at least for two months before recruitment. Control cohorts included 20 healthy donors (matched for age and sex). Demographic and clinical data of the study cohort are given in Table 1. The study was approved by the Ethics Committee of the University Hospital Heidelberg. Written informed consent was obtained from all patients.

2.2. Sampling

Samples of 10–50 ml EDTA blood were withdrawn from all study participants to analyze cells by flow cytometry and by *in vitro* assays.

2.3. Cell separation

Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by density-gradient centrifugation using Ficoll-Hypaque (Biochrom AG, Berlin, Germany). $CD11c^+$ mDCs were isolated from PBMCs with the Myeloid Dendritic Cell Isolation Kit (Miltenyi Biotec, Bergisch-Gladbach, Germany). $CD4^+CD45RA^+$ naïve T cells were separated from PBMCs by employing a Naive $CD4^+$ T Cell Isolation Kit II (Miltenyi). All phenotyping and cell cultures experiments were performed with freshly isolated cells.

2.4. Cell culture

For the study 2×10^5 freshly isolated mDCs obtained from MS patients or control donors (MS-mDCs, HC-mDCs) were cultured in 96-well tissue culture test plates (TPP Techno Plastic Products AG, Trasadingen, Switzerland) in 200 μl cell culture medium (RPMI, 10% FCS, 1% pyruvate, 1% HEPES, penicillin-streptomycin) in the presence or absence of 25 ng/ml recombinant TSLP (rhTSLP, R&D Systems, Minneapolis, USA). To test for rhTSLP-mediated activation, rhTSLP-activated mDCs were collected after 24 h of cell culture. After a washing step, resuspended mDCs were stained with FITC- or PE-conjugated mAbs specific for CD40, CD80, CD86, and HLA-DR and analyzed by flow cytometry. In parallel, mDC culture supernatants were collected, frozen at −80 °C, and analyzed for IL-1β and TNFα by ELISA (R&D Systems). To assess their effect on $CD4^+CD45RA^+$-naïve T cells, rhTSLP-activated mDCs were collected after 24 h of cell culture, washed, and then co-cultured with 5×10^4 allogeneic $CD4^+CD45RA^+$-naïve T cells obtained from control donors (1:5 ratio) in round-bottomed, 96-well plates. After 5 d, cells were pulsed for another 16 h with 0.5 μCi $^3[H]$thymidine per well. After harvesting, T-cell proliferation was measured with a scintillation counter. To induce T-cell cytokine secretion, mDC-primed $CD4^+CD45RA^+$-naïve T cells were restimulated for 24 h by adding soluble *anti*-CD3/CD28 (1 μg/ml) mAbs. Supernatants were collected and subjected to ELISA measurement.

2.5. Flow cytometry

For flow cytometric detection of mDCs, 10^6 PBMCs were stained with fluorescent dye-labeled mAbs specific for CD11c, CD123, HLA-DR, TSLPR, and the lineage cocktail (Lin, mAbs specific for CD3 (T cells), CD14 (monocytes), CD16, CD56 (natural killer cells), and CD19, CD20 (B cells) Stained cells were first gated for $HLA\text{-}DR^+Lin^-$ and further analyzed for expression of CD11c and CD123, identifying $CD11c^+CD123^-$ mDCs. Finally, TSLPR-MFIs (mean fluorescence intensities) were determined on gated mDCs. All antibodies were purchased from BD Pharmingen (Heidelberg, Germany). FACS acquisition was performed with a FACS Calibur™ cytometer and analyzed with CellQuestTM software (BD Biosciences, Heidelberg, Germany). For quantitation of RTE-Treg, PBMCs were stained with fluorescence-conjugated mAbs specific for CD4, CD25, CD45RO, CD31, and FOXP3. Stained PBMCs were gated for CD4 and analyzed for FOXP3/CD25 expression to identify $CD4^+$-$CD25^{high}FOXP3^+$ Treg cells. CD31 co-expression on $CD45RO^-$ naive Treg cells identified $CD4^+CD25^{high}FOXP3^+CD45RO^-CD31^+$ RTE-Treg. Gating strategies are illustrated in Fig. 1.

2.6. ELISA

Cytokine levels of IL-5, IL-13, and TNFα in cell culture supernatants were determined by using commercial ELISA (R&D Systems, Wiesbaden-Nordenstadt, Germany) according to the manufacturer's instructions. All samples were measured in duplicate.

2.7. Statistical analysis

For normally distributed samples, two-sided paired t-tests (paired samples) or two-sided t-tests (unpaired samples) were used to

Table 1
Demographic and clinical data of study participants.

	Healthy donors	MS, untreated	MS, IFN-beta[a]	MS, GA[b]
n	20	20	20	20
Number of females (%)	10 (50%)	14 (70%)	14 (70%)	15 (75%)
Median age in yrs	30.5 (17–60)	32.5 (17–54)	36.0 (18–56)	37.0 (19–58)
Median of previous relapses [n]	–	2.0 (1–4)	3.5 (1–5)	3.5 (1–6)
Median EDSS[c]	–	2.5 (1–3)	3.5 (1–4)	3.5 (1–5)
Median disease duration [yrs]	–	3.5 (1–9)	6.2 (1–14)	6.3 (1−11)
Median treatment duration [months]	–	–	11.5 (3–18)	12.0 (3–18)

[a] Interferon beta.
[b] Glatiramer acetate.
[c] Expanded Disability Status Score.