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Comparison of dendritic cells obtained from autoimmunity-prone and resistant rats

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List of abbreviations

AO – Albino Oxford; CD – cluster of differentiation; CNS - central nervous system; DA – Dark Agouti; DC – dendritic cells; EAE - experimental autoimmune encephalomyelitis; FCS - fetal calf serum; GM-CSF - granulocyte-macrophage colony-stimulating factor; iDC – immature DC; IFN - interferon; IL - interleukin; LNC - lymph node cells; LPS – lipopolysaccharide; mDC – mature DC; PBS - phosphate buffer saline; SD - standard deviation; Th – T helper; TNF – tumor necrosis factor; tolDC – tolerogenic DC.

Abstract

Dendritic cells (DC) are responsible for the initiation and shaping of the adaptive immune response and are in the focus of autoimmunity research. We were interested in comparison of DC obtained from autoimmunity-prone Dark Agouti (DA) rats and autoimmunity-resistant Albino Oxford (AO) rats. DC were generated from bone marrow precursors and matured (mDC) by lipopolysaccharide. Tolerogenic DC (tolDC) obtained by vitamin D3 treatment were studied in parallel. Profile of cytokine production was different in AO and DA mDC and tolDC. Expression of MHC class II molecules and CD86 were higher in DA DC, while vitamin D3 reduced their expression in dendritic cells of both strains. Allogeneic proliferation of CD4⁺ T cells was reduced by AO tolDC, but not with DA tolDC in comparison to respective mDC. Finally, expression of various genes identified as differentially expressed in human mDC and tolDC was also analyzed in AO and DA DC. Again, AO and DA DC differed in the expression of the analyzed genes. To conclude, AO and DA DC differ in production of cytokines, expression of antigen presentation-related molecules and in regulation of CD4⁺ T proliferation. The difference is valuable for understanding the divergence of the strains in their susceptibility to autoimmunity.

Keywords: dendritic cells; autoimmunity; Dark Agouti rats; Albino Oxford rats; allogeneic proliferation.

Introduction

Dendritic cells (DC) are the professional antigen-presenting cells able to activate and differentiate naïve CD4⁺ T cells into T helper cells (Th), and are, hence, the major initiators and propagators of the adaptive immune response [1, 2]. Besides stimulatory activity, DC also possess the ability to restrain T cell activity, i.e. they have tolerogenic effects [3]. Thus, proper functions of DC are needed for efficient adaptive immunity against infectious agents and for prevention of autoimmunity [4]. Having in mind that interferon (IFN)- γ -producing Th1 cells and interleukin (IL)-17-producing Th17 cells are the major pathogenic populations in multiple sclerosis and in its animal model, the experimental autoimmune encephalomyelitis (EAE) [5], their activation and propagation by DC has a significant role in the pathogenesis of the central nervous system (CNS) autoimmunity observed in these diseases. Indeed, inappropriate functions of DC have been reported in the CNS autoimmunity [6]. For instance, DC obtained from peripheral circulation of multiple sclerosis patients had greater production of Th1-promoting IL-12 and expression of chemokine receptors CCR5 and CCR7 in response to activation through toll-like receptors (TLR) [7]. Also, they were shown to produce more tumor necrosis factor (TNF), to induce higher expression of T cell co-stimulatory molecules CD80 and CD40 and lower expression of regulatory programmed cell death protein 1 (PD-1) [8]. Interestingly, these cells also had decreased steady-state release of IL-12 and type 1 IFN [9]. Importantly, lack of endogenous IFN- β could be a predisposing factor towards development of multiple sclerosis, as it was shown that IFN- β treatment corrects abnormal expression of various genes in DC [10].

Albino Oxford (AO) and Dark Agouti (DA) rats are at the opposite poles of the susceptibility to the induction of EAE. It is really difficult to induce EAE in AO rats. Actually, our single successful attempt in the induction was based on the harsh and prolonged treatment of neonatal rats with antibiotics, resulting in marked changes in gut microbiota and gut-associated immune cells [11]. Even then, the severity of the disease was mild and short-lasting. On the contrary, DA rats develop EAE after immunization without adjuvants [12]. We have compared the two strains in relation to their divergence to EAE induction at multiple systemic, cellular and molecular levels. We demonstrated that these strains differ in their response to encephalitogenic immunization in lymph nodes draining the site of immunization [13, 14], in the gut-associated lymphoid tissue [15], and within the CNS [16, 17]. Generally, DA rats have dominant Th1 and Th17 reactivity in comparison to AO rats. Therefore, we have been interested in investigating the differences in DC between these strains.

In the present study, mature DC (mDC) and tolerogenic DC (tolDC) generated from AO and DA rats were compared by analyzing their phenotype, cytokine secretion profile, gene expression and functionality. As general observation, DA DC were more stimulatory and pro-inflammatory in comparison to AO DC.

Material and Methods

Experimental animals

Females of AO and DA rats, 2–3 months of age, were used in all experiments. Animal manipulation and experimental procedures were approved by the local Ethics Committee (Institute for Biological Research “Siniša Stanković”, N° 03-01/17).

Cells and cell cultures

DC were obtained from bone marrow progenitors of AO and DA rats by two different protocols. According to the first protocol, cells (5×10^6 /mL/well in 6-well plate) were cultured in RPMI 1640 (Biological Industries, Kibbutz Beit-Haemek, Israel) supplemented with 10% fetal calf serum (FCS) (PAA Laboratories), in the presence of GM-CSF, IL-4 and Flt3L (25ng/ml each, all from Peprotech, Rocky Hill, NJ). The medium was refreshed on days 3 and 6. Cell maturation was performed in the presence of 100 ng/mL lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (PolyI:C) (both from Sigma-Aldrich, St. Louis, IL) for the last 48h of cultivation. In the second protocol, cells (1×10^6 /mL/well in 24-well plate) were cultured in RPMI 1640 supplemented with 10% FCS, 2mM glutamine and 1 mM sodium pyruvate (both from Sigma-Aldrich), in the presence of 20 ng/ml GM-CSF during 8 days. For the last 24h of cultivation, cells were stimulated with 100 ng/mL LPS. These cells are considered as mDC. In order to obtain tolDC) an additional treatment with 2 nM vitamin D3 (Calbiochem, San Diego, CA) was performed every 2 days, starting from day 0.

Cervical lymph node cells (LNC) were obtained by mechanical disruption of the lymph nodes from DA and AO rats and stained with carboxyfluorescein succinimidyl ester (CFSE) (2 μ M, from Invitrogen, Carlsbad, CA) prior to cultivation with DC.

Allogeneic cultivation

DC from AO rats were co-cultured with CFSE-labeled DA LNC and the other way around at a 1:20 ratio (2.5 x 10⁴ DC: 5 x 10⁵ lymph node cells) in 96-well round-bottom plates (Sarstedt, Nümbrecht, Germany). Co-cultivation lasted for 5 days. Cell proliferation was determined by the sequential loss of CFSE fluorescence, as detected by cytofluorimetry. The cells were stained for CD4 expression (PE-coupled anti-CD4, eBioscience, San Diego, CA) and CFSE was detected in CD4 cells. Samples were gated using forward (FSC) and side (SSC) scatter to exclude dead cells and debris (R1), as well as using FSC and FSC-W to exclude cell doublets (R2), CFSE+ population (R3) and CD4⁺ population (CD4).

ELISA

Cytokine concentration in cell culture supernatants was determined by sandwich ELISA using MaxiSorp plates (Nunc, Rochild, Denmark) and anti-cytokine paired antibodies according to the manufacturer's instructions. Samples were analyzed in duplicates for rat IL-1 β , IL-6, IL-10 (R&DSystems, Minneapolis, MN), TNF (eBioscience), IL-12, p40 and transforming growth factor β (TGF- β) (BioSource, Camarrilo, CA). The results were calculated using standard curves made on the basis of known concentrations of the appropriate recombinant cytokines.

Flow cytometry

The phenotype of DC was assessed by flow cytometry using anti-rat antibodies specific for MHCII and CD86. Cells were washed, re-suspended in 100 µl of PBS supplemented with 2% of rat serum and incubated with PE-labeled MHCII and FITC-labeled CD86 antibodies (eBioscience) for 30 minutes at 4°C. After washing, stained cells were detected using CyFlow Space flow cytometer (Partec, Munster, Germany). Samples were gated using forward (FSC) and side (SSC) scatter to exclude dead cells and debris, as well as using FSC and FSC-W to exclude cell doublets.

RT-PCR

Total RNA was isolated from cells using a TRI Reagent Solution (Ambion, Foster City, CA) and reverse transcribed using random hexamer primers and MMLV (Moloney Murine Leukemia Virus) reverse transcriptase, according to the manufacturer's instructions (Fermentas, Vilnius, Lithuania). Prepared cDNA were amplified by using Maxima SYBR Green/ROX qPCR Master Mix (Fermentas) according to the recommendations of the manufacturer in an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Thermocycler conditions comprised an initial step at 50 °C for 5 min, followed by a step at 95 °C for 10 min and a subsequent 2-step PCR program at 95 °C for 15 s and 60 °C for 60 s for 40 cycles. The PCR primers (Metabion) were as follows: *Gapdh*: 5'-TGG ACC TCA TGG CCT ACA T-3'; 5'-GGA TGG AAT TGT GAG GGA GA-3'; *Cyp24a1*: 5'-AAC GGT TCT GGGTGA ATA CG-3'; 5'-AGC GAA GGG GTT GAT CTT TT-3'; *Muc11*: 5'-CCT CTG ATG GA CCA ACG AT-3'; 5'-

CGT CAT CAT CAT CCC CTT CT-3'; *Map7*: 5'-GCG ACA TGT TCA AAG GAG TT-3'; 5'-TTG GCT GCT TCT CCA AAT CT-3'; *Sparc*: 5'-AAA CAT GGC AAG GTG TGT GA-3'; 5'-AAG TGG CAG GAA GAG TCG AA-3'; *Camp*: 5'-TCT ACC GTC TCC TGG ACC TG-3'; 5'-TCA CTA CCC CCT GTT CCT TG-3'; *Atp6v0d2*: 5'-GAG CTC TTC AAG GCT GTG CT-3'; 5'-CCG TGA TCC TTG CAG AAT TT-3'; Accumulation of PCR products was detected in real time and the results were analysed with 7500 System Software (AB). The relative RNA expression was calculated as 2^{-dCt} , where dCt is the difference between Ct values of a gene of interest and the endogenous control (*Gapdh*).

Statistics

Two way ANOVA followed by Tukey's multiple comparisons test and Student's t test were applied, as appropriate, using Graphpad Prism 7.

Results

Comparison of cytokine production in AO and DA DC

The ability of DC obtained from AO and DA rats to produce various pro- and anti-inflammatory cytokines was determined. DC were generated from bone marrow precursors under the influence of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-4 and Fms-related tyrosine kinase 3 ligand (Flt3L). They were cultivated in the absence or presence of LPS or PolyI:C stimulation. In the absence of stimulation, production of IL-6, IL-10, IL-12, p40 and TGF- β was relatively low, and there were no statistically significant differences between AO and DA DC for all of the tested cytokines (Fig 1). TGF- β levels were not statistically significantly changed with either of the stimuli applied. LPS stimulation increased IL-6, IL-10 and p40 levels in both strains, and IL-12 levels only in DA DC. PolyI:C increased IL-6 and IL-10 levels in AO DC, and IL-12 and p40 levels in DA DC. As for the difference between the strains, IL-6 and IL-10 levels were higher in AO DC, while IL-12 and p40 were higher in DA DC, irrespectively of the stimulus used (Fig 1).

Further, DC were generated from bone marrow precursors under the treatment of GM-CSF only, and matured in the presence of LPS (mature DC – mDC). This protocol for DC generation was applied as our aim was to generate tolDC in parallel. TolDC generated in the presence of GM-CSF and vitamin D3 [18] were compared with mDC in both strains. IL-10 levels were higher in tolDC in comparison to mDC in both strains (Fig 2). Moreover, AO mDC and tolDC had

significantly higher level of IL-10 in comparison to their respective DA DC (Fig 2). IL-6 was higher in AO tolDC in comparison to DA tolDC, while there was no difference between the strains in the level of IL-1 β and TNF (Fig 2). Finally, TNF was higher in DA mDC in comparison to DA tolDC (Fig 2).

Taken together these results demonstrate differences in cytokine production between both mDCe and tolDC obtained from AO and DA rats with the dominance of pro-inflammatory cytokines in the latter DC.

Comparison of dendritic cell phenotype

Next, mDC and tolDC from AO and DA rats were compared for the expression of co-stimulatory and antigen presenting molecules. The proportion of cells expressing MHC class II molecules and their level of expression were higher in DA than in AO DC, irrespectively if mDC or tolDC were compared (Fig 3A, B). In addition, there were less tolDC expressing MHC class II and also its expression was lower on tolDC in comparison to mDC in both rat strains. Similar results were observed with CD86 molecule, although there was no statistically significant difference between AO and DA tolDC (Fig 3C, D).

Allogeneic proliferation

AO DC were co-cultured with DA LNC, while DA DC were co-cultured with AO LNC. Higher proliferation of responding AO LNC was observed in co-cultures of DA DC in comparison to the proliferation of DA LNC co-cultured with AO DC, irrespectively if mDC or tolDC were compared (Fig 4A,B). Proliferation of DA LNC co-cultured with AO tolDC was lower when compared to that observed in co-culture with AO mDC. However, such difference was not observed when allostimulatory capacities of DA mDC and DA tolDC were compared.

Comparison of gene expression in human and rat DC

Our next goal was to compare tolDC of AO and DA rats to human tolDC. In a recent study, a comparative transcriptomic profiling of human DC differentiated with vitamin D3. *CYP24A1*, *MUCL1* and *MAP7* were identified as genes whose expression is differentially expressed in human vitamin D3-induced tolDC *versus* both mDC and immature DC (iDC). Additionally, *ATP6VOD2*, *CAMP* and *SPARC* were identified as differentially expressed genes in vitamin D3-induced tolDC in comparison to mDC [19]. Therefore, an analysis of the expression of these six genes was performed in rat DC (Fig 5). Statistically significant differences between tolDC and mDC or iDC were observed for *Cyp24a1* in both strains (Fig 5A). Also, there was a statistically significant decrease of *Sparc* expression in AO tolDC in comparison to AO mDC (Fig 5 D). The difference between AO and DA rats reached statistical significance for *Cyp24a1* expression in tolDC, and for *Sparc* expression in mDC (Fig 5 A,D). No statistically significant differences neither among iDC, mDC and tolDC, nor between rat strains, were found in the expression of the other genes. Comparison of the expression differences between tolDC and iDC or mDC was also

performed in rat and human DC in parallel (Fig 6). Despite the lack of statistical significance, a similar trend of expression was observed in both rat strains and human cells for *Cyp24a1*, *Muc11* and *Map7*. Also, similar pattern of expression to human cells was observed for *Sparc* and *Camp* in DA, but not in AO DC. Expression of *Atp6vod2* was opposite in human and rat DC.

Discussion

DC obtained from AO and DA rats differ significantly in their cytokine response to maturation stimuli, in the level of antigen presentation-related molecules and in their allostimulatory capacities. Also they differ in expression pattern of genes potentially relevant for tolerogenic phenotype of human DC. We could describe DA rat DC as pro-inflammatory in comparison to AO rat counterparts.

DC have the central role in the activation of naïve T cells towards pathogenic Th1 and Th17 effector subpopulations [4, 6]. DA DC express higher levels of MHC class II molecules and co-stimulatory CD86. This implies that they are more efficient in antigen-presentation and activation of T cells. This assumption is in accordance with earlier data from our group and others that draining lymph node cellularity increases faster and to the greater extent in DA rats in response to encephalitogenic immunization [13, 20]. Similar results were observed in the contact hypersensitivity reaction [21]. The ability of DA rat DC to produce more IL-12 than AO rat DC implies that Th1 response is dominant in DA rats in comparison to AO rats. This fits well with our previous observation that DA rat lymph node cells produce more IFN- γ [22, 14]. Higher IL-10 release from AO rat DC is also in accordance with our previous report on the increased IL-10

expression and production in AO rat lymph nodes and within the CNS of the immunized rats [17]. Interestingly, IL-6 generation is also higher in AO rat DC, while the clear dominance of DA rat lymph node cells in production of IL-6 was observed in previous reports [14, 20]. Since there are multiple cellular sources of IL-6 within unseparated population of lymph node cells, cell-specific regulation of IL-6 production in relation to the strain specificity is worthy of further studies.

Higher allogeneic proliferation of AO CD4⁺ T cells observed in co-cultivation with DC is in agreement with stronger alloreactivity in AO rats in comparison to DA rats [23]. Importantly, the decrease in allogeneic proliferation was observed under the influence of AO, but not DA tolDC. This implies that vitamin D3 treatment does not diminish allostimulatory properties of DA DC, at least *in vitro*. It will be important to determine if the discrepancy in the activity of tolDC between the two strains observed in alloreactive system also exists in the antigen specific system *in vitro*, as well as *in vivo*. Interestingly, DA tolDC were as efficient as mDC in allo-proliferative stimulation of AO LNC, despite the reduction in the expression of antigen-presenting molecules MHC class II and CD86 and increase of IL-10 generation that were observed in tolDC in comparison to mDC. This could be explained by low threshold of activation of AO allo-reactive T cells and/or by the inability of DA tolDC to act in tolerogenic manner. These options have to be studied in more details in the future, especially *in vivo*.

Comparison of the selected gene expression in DC obtained from EAE-resistant AO rats and EAE-prone DA rats with human DC obtained from healthy individuals revealed various differences in the expression patterns. *Cyp24a1* expression was higher in tolDC than in iDC and

mDC in both rat strains and humans. *Cyp24a1* codes for the key catabolic vitamin D3 enzyme 25-hydroxyvitamin D3-24-hydroxylase, that is up-regulated in response to elevated levels of vitamin D3, and can be considered as the positive control for the response of cells to vitamin D3. In accordance with our results, it was previously shown that human monocyte-derived DC increased expression of *Cyp24a1* in response to vitamin D3 [24]. *Muc11* has previously been identified as breast cancer-specific gene whose product Mucin-like protein 1 controls proliferation in breast cancer cells [25]. The protein is present intracellularly or secreted from the cancer cells. FAK/JNK pathway was identified as the downstream effector of MUCL1 signaling [25]. High expression of MUCL1 in human and rat tolDC implies that this protein might play an important role in tolerogenic activity of DC. The same holds true for microtubule-associated protein 7 (MAP7 also known as ensconsin) coded by *Map7*. MAP7 is an exceptional protein that interacts with both microtubules and motor protein kinesin-1 [26, 27]. Kinesin-1 is a main microtubule motor that pushes transport of several cellular cargoes towards the plus ends of microtubules [26]. MAP7 has been shown important for neuronal cell morphogenesis [27], while its role in tolDC is yet to be explored. SPARC (Secreted protein acidic and rich in cysteine, also known as osteonectin) encoded by *Sparc*, is a matricellular protein that organizes collagen deposition in tissue stroma [28]. Interestingly, accelerated migration of DC and T cell priming were observed in *Sparc*-deficient mice [28]. Therefore, increased expression of *Sparc* in tolDC might be involved in deceleration of T cell activation, a possibility that is surely worthy of further studies. Cathelicidin antimicrobial peptide (CAMP) exhibits direct antimicrobial effects, but it also acts as an oligonucleotide carrier of nucleic acids towards endosomal compartment of DC [29], thus facilitating recognition of oligonucleotides by intracellular pattern-recognition

receptors [30]. Therefore, it is involved in efficient recognition of pathogens. However, it can also contribute to the breakdown of immune tolerance to self-DNA as observed in psoriasis [31]. An increase of CAMP expression under the influence of vitamin D3 was previously observed in DC [32]. Elevated CAMP expression was also previously shown in lamina propria mononuclear cells of healthy subjects receiving high-dose vitamin D3 supplementation [33]. In our study, the increased CAMP expression was detected in human and DA, but not in AO tolDC. The possibility that limitation of CAMP expression in AO tolDC contributes to the resistance of AO rats to autoimmunity is worthy of further investigation. Interestingly, expression of ATP6V0D2 (ATPase, H⁺ transporting, lysosomal 38kDa, V0 subunit d2) was increased in human tolDC, but decreased in rat tolDC in our study. ATP6V0D2 was identified as an important element in DC transdifferentiation into osteoclasts [34]. Its immunomodulatory effects have not been identified, so far.

To conclude, AO and DA DC differed in their ability to produce cytokines, express antigen-presentation molecules and regulate T cell alloproliferation. DA DC were more inflammatory and stimulatory in comparison to AO DC. Moreover, the expression of genes, demonstrated to be associated with a vitamin D3-induced tolerogenic phenotype in human DC, was different between AO and DA tolDC. Interestingly, regarding the expression of these genes, DA tolDC were more similar to human tolDC than AO tolDC. This study contributes to the view that the difference in susceptibility of AO and DA rats towards autoimmunity might be associated with a variability in the intrinsic regulation of the immune response within the strains.

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Conflict of Interest Statement

The Authors declare that there is no conflict of interest.

References

1. **Marland G, Bakker AB, Adema GJ, Figdor CG.** Dendritic cells in immune response induction. *Stem Cells*. 1996 Sep;**14(5)**:501-7.
2. **Hivroz C, Chemin K, Tourret M, Bohineust A.** Crosstalk between T lymphocytes and dendritic cells. *Crit Rev Immunol*. 2012;**32(2)**:139-55.
3. **Rutella S, Danese S, Leone G.** Tolerogenic dendritic cells: cytokine modulation comes of age. *Blood*. 2006 Sep 1;**108(5)**:1435-40.

4. **Sozzani S, Del Prete A, Bosisio D.** Dendritic cell recruitment and activation in autoimmunity. *J Autoimmun.* 2017 Dec;**85**:126-140. doi: 10.1016/j.jaut.2017.07.012.
5. **Petermann F, Korn T.** Cytokines and effector T cell subsets causing autoimmune CNS disease. *FEBS Lett.* 2011 Dec 1;**585(23)**:3747-57. doi: 10.1016/j.febslet.2011.03.064.
6. **Nuyts AH, Lee WP, Bashir-Dar R, Berneman ZN, Cools N.** Dendritic cells in multiple sclerosis: key players in the immunopathogenesis, key players for new cellular immunotherapies? *Mult Scler.* 2013 Jul;**19(8)**:995-1002. doi: 10.1177/1352458512473189.
7. **Thewissen K, Nuyts AH, Deckx N, Van Wijmeersch B, Nagels G, D'hooghe M, Willekens B et al.** Circulating dendritic cells of multiple sclerosis patients are proinflammatory and their frequency is correlated with MS-associated genetic risk factors. *Mult Scler.* 2014 Apr;**20(5)**:548-57. doi: 10.1177/1352458513505352.
8. **Karni A, Abraham M, Monsonogo A, Cai G, Freeman GJ, Hafler D, Khoury SJ, Weiner HL.** Innate immunity in multiple sclerosis: myeloid dendritic cells in secondary progressive multiple sclerosis are activated and drive a proinflammatory immune response. *J Immunol.* 2006 Sep 15;**177(6)**:4196-202.
9. **Deckx N, Willekens B, Wens I, Eijnde BO, Goossens H, Van Damme P, Berneman ZN, Cools N.** Altered molecular expression of TLR-signaling pathways affects the steady-state release of IL-12p70 and IFN- α in patients with relapsing-remitting multiple sclerosis. *Innate Immun.* 2016 May;**22(4)**:266-73. doi: 10.1177/1753425916642615.
10. **Aung LL, Brooks A, Greenberg SA, Rosenberg ML, Dhib-Jalbut S, Balashov KE.** Multiple sclerosis-linked and interferon-beta-regulated gene expression in plasmacytoid dendritic cells. *J Neuroimmunol.* 2012 Sep 15;**250(1-2)**:99-105. doi: 10.1016/j.jneuroim.2012.05.013.
11. **Stanisavljević S, Dinić M, Jevtić B, Đedović N, Momčilović M, Đokić J, Golić N et al.** Gut Microbiota Confers Resistance of Albino Oxford Rats to the Induction of Experimental Autoimmune Encephalomyelitis. *Front Immunol.* 2018 May **2;9**:942. doi: 10.3389/fimmu.2018.00942.

12. **Stosic-Grujicic S, Ramic Z, Bumbasirevic V, Harhaji L, Mostarica-Stojkovic M.** Induction of experimental autoimmune encephalomyelitis in Dark Agouti rats without adjuvant. *Clin Exp Immunol.* 2004 Apr;**136(1)**:49-55.
13. **Miljkovic D, Stosic-Grujicic S, Markovic M, Momcilovic M, Ramic Z, Maksimovic-Ivanic D, Mijatovic S et al.** Strain difference in susceptibility to experimental autoimmune encephalomyelitis between Albino Oxford and Dark Agouti rats correlates with disparity in production of IL-17, but not nitric oxide. *J Neurosci Res.* 2006 Aug 1;**84(2)**:379-88.
14. **Markovic M, Miljkovic D, Momcilovic M, Popadic D, Miljkovic Z, Savic E, Ramic Z, Mostarica Stojkovic M.** Strain difference in susceptibility to experimental autoimmune encephalomyelitis in rats correlates with T(H)1 and T(H)17-inducing cytokine profiles. *Mol Immunol.* 2009 Nov;**47(1)**:141-6. doi: 10.1016/j.molimm.2009.01.012.
15. **Stanisavljević S, Lukić J, Momčilović M, Miljković M, Jevtić B, Kojić M, Golić N et al.** Gut-associated lymphoid tissue, gut microbes and susceptibility to experimental autoimmune encephalomyelitis. *Benef Microbes.* 2016 Jun;**7(3)**:363-73. doi: 10.3920/BM2015.0159.
16. **Petković F, Blaževski J, Momčilović M, Mostarica Stojkovic M, Miljković D.** Nitric oxide inhibits CXCL12 expression in neuroinflammation. *Immunol Cell Biol.* 2013 Jul;**91(6)**:427-34. doi: 10.1038/icb.2013.23.
17. **Blaževski J, Petković F, Momčilović M, Jevtic B, Miljković D, Mostarica Stojković M.** High interleukin-10 expression within the central nervous system may be important for initiation of recovery of Dark Agouti rats from experimental autoimmune encephalomyelitis. *Immunobiology.* 2013 Sep;**218(9)**:1192-9. doi: 10.1016/j.imbio.2013.04.004.
18. **Mansilla MJ, Sellès-Moreno C, Fàbregas-Puig S, Amoedo J, Navarro-Barriuso J, Teniente-Serra A, Grau-López L et al.** Beneficial effect of tolerogenic dendritic cells pulsed with MOG autoantigen in experimental autoimmune encephalomyelitis. *CNS Neurosci Ther.* 2015 Mar;**21(3)**:222-30. doi: 10.1111/cns.12342.
19. **Navarro-Barriuso J, Mansilla MJ, Naranjo-Gómez M, Sánchez-Pla A, Quirant-Sánchez B, Teniente-Serra A, Ramo-Tello C, Martínez-Cáceres EM.** Comparative

transcriptomic profile of tolerogenic dendritic cells differentiated with vitamin D3, dexamethasone and rapamycin. *Sci Rep.* 2018 Oct 8;**8(1)**:14985. doi: 10.1038/s41598-018-33248-7.

20. **Stojić-Vukanić Z, Pilipović I, Vujnović I, Nacka-Aleksić M, Petrović R, Arsenović-Ranin N, Dimitrijević M, Leposavić G.** GM-CSF-Producing Th Cells in Rats Sensitive and Resistant to Experimental Autoimmune Encephalomyelitis. *PLoS One.* 2016 Nov 10;**11(11)**:e0166498. doi: 10.1371/journal.pone.0166498.
21. **Popov Aleksandrov A, Mirkov I, Demenesku J, Ninkov M, Zolotarevski L, Kataranovski D, Kataranovski M.** Strain differences in contact hypersensitivity reaction to dinitrochlorobenzene (DNCB) in rats. *Food Chem Toxicol.* 2015 Jan;**75**:94-103. doi: 10.1016/j.fct.2014.11.010.
22. **Arsov I, Pravica V, Ejodus L, Badovinac V, Mostarica M, Lukic ML.** Selection for susceptibility to experimental allergic encephalomyelitis also selects for high IFN-gamma production. *Transplant Proc.* 1995 Apr;**27(2)**:1537-8.
23. **Lukic ML, Mostarica M, Ejodus L, Bonaci B.** Strain differences in alloreactivity in rats: the roles of T-cell subsets. *Transplant Proc.* 1990 Dec;**22(6)**:2549-50.
24. **Kundu R, Chain BM, Coussens AK, Khoo B, Noursadeghi M.** Regulation of CYP27B1 and CYP24A1 hydroxylases limits cell-autonomous activation of vitamin D in dendritic cells. *Eur J Immunol.* 2014 Jun;**44(6)**:1781-90. doi: 10.1002/eji.201344157.
25. **Conley SJ, Bosco EE, Tice DA, Hollingsworth RE, Herbst R, Xiao Z.** HER2 drives Mucin-like 1 to control proliferation in breast cancer cells. *Oncogene.* 2016 Aug 11;**35(32)**:4225-34. doi: 10.1038/onc.2015.487.
26. **Barlan K, Lu W, Gelfand VI.** The microtubule-binding protein ensconsin is an essential cofactor of kinesin-1. *Curr Biol.* 2013 Feb 18;**23(4)**:317-22. doi: 10.1016/j.cub.2013.01.008.
27. **Tymanskyj SR, Yang BH, Verhey KJ, Ma L.** MAP7 regulates axon morphogenesis by recruiting kinesin-1 to microtubules and modulating organelle transport. *Elife.* 2018 Aug 22;**7**. pii: e36374. doi: 10.7554/eLife.36374.

28. **Sangaletti S, Gioiosa L, Guiducci C, Rotta G, Rescigno M, Stoppacciaro A, Chiodoni C, Colombo MP.** Accelerated dendritic-cell migration and T-cell priming in SPARC-deficient mice. *J Cell Sci.* 2005 Aug 15;118(Pt 16):3685-94.
29. **Zhang X, Oglęcka K, Sandgren S, Belting M, Esbjörner EK, Nordén B, Gräslund A.** Dual functions of the human antimicrobial peptide LL-37-target membrane perturbation and host cell cargo delivery. *Biochim Biophys Acta.* 2010 Dec;1798(12):2201-8. doi: 10.1016/j.bbamem.2009.12.011.
30. **Nakagawa Y, Gallo RL.** Endogenous intracellular cathelicidin enhances TLR9 activation in dendritic cells and macrophages. *J Immunol.* 2015 Feb 1;194(3):1274-84. doi: 10.4049/jimmunol.1402388.
31. **Lande R, Gregorio J, Fachinetti V, Chatterjee B, Wang YH, Homey B, Cao W et al.** Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide. *Nature.* 2007 Oct 4;449(7162):564-9.
32. **Lowry MB, Guo C, Borregaard N, Gombart AF.** Regulation of the human cathelicidin antimicrobial peptide gene by $1\alpha,25$ -dihydroxyvitamin D3 in primary immune cells. *J Steroid Biochem Mol Biol.* 2014 Sep;143:183-91. doi: 10.1016/j.jsbmb.2014.02.004.
33. **Bak NF, Bendix M, Hald S, Reinert L, Magnusson MK, Agnholt J.** High-dose vitamin D3 supplementation decreases the number of colonic CD103+ dendritic cells in healthy subjects. *Eur J Nutr.* 2017 Sep 14. doi: 10.1007/s00394-017-1531-y.
34. **Zhu X, Zeng Z, Qiu D, Chen J.** $V\gamma 9V\delta 2$ T cells inhibit immature dendritic cell transdifferentiation into osteoclasts through downregulation of RANK, c-Fos and ATP6V0D2. *Int J Mol Med.* 2018 Oct;42(4):2071-2079. doi: 10.3892/ijmm.2018.3791.

Figure legends

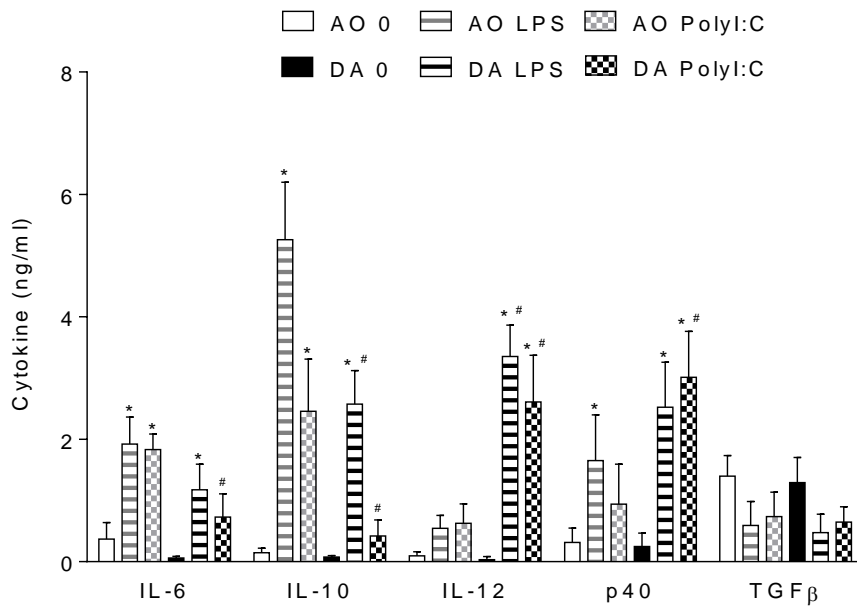


Fig 1 Cytokine profile in AO and DA DC. DC were propagated from rat bone marrow cells in the presence of GM-CSF, IL-4 and Flt3L. They were untreated (0), stimulated with LPS or with Poly I:C for 48 hours. Subsequently, cytokines were determined in cell culture supernatants by ELISA for IL-6, IL-10, IL-12, p40 and TGF-β. Data are presented as mean + SD of values obtained in 4 independent experiments. * $p < 0.05$, vs. 0 within the strain, # $p < 0.05$, AO vs. DA in the same treatment group; Tukey's multiple comparisons test.

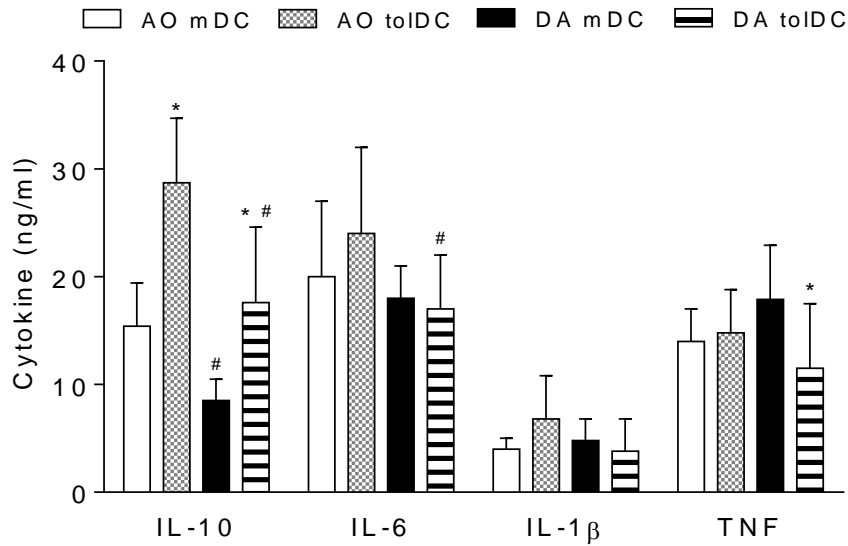


Fig 2 Cytokine profile in AO and DA toIDC. DC were propagated from rat bone marrow cells in the presence of GM-CSF and in the absence (mDC) or presence of vitamin D3 (toIDC). Cells were treated with LPS for 24h. Subsequently, cytokines were determined in cell culture supernatants by ELISA for IL-10, IL-6, IL-1 β and TNF. Data are presented as mean + SD of values obtained in 7 independent experiments. * $p < 0.05$, mDC vs. toIDC, # $p < 0.05$, AO vs. DA; Tukey's multiple comparisons test.

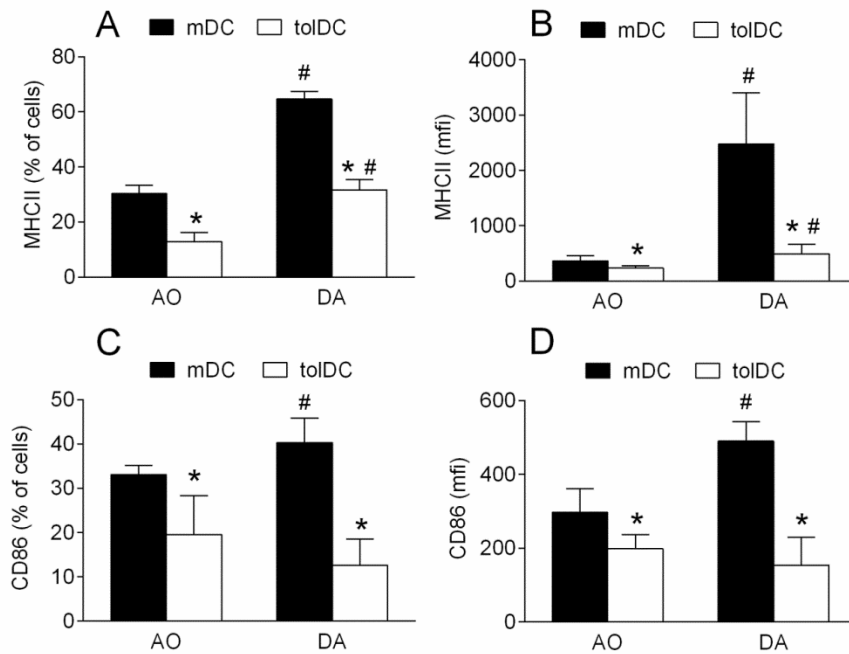


Fig 3 Phenotype of AO and DA DC. DC were propagated from rat bone marrow cells in the presence of GM-CSF, and in the absence (mDC) or presence of vitamin D3 (tolDC). Cells were treated with LPS for 24h. Subsequently, expression of MHC class II molecules (A, B) and CD86 (C, D) was determined by cytofluorimetry. Data are presented as mean + SD of values obtained in 3 independent experiments. * $p < 0.05$, mDC vs. tolDC, # $p < 0.05$, AO vs. DA; Tukey's multiple comparisons test.

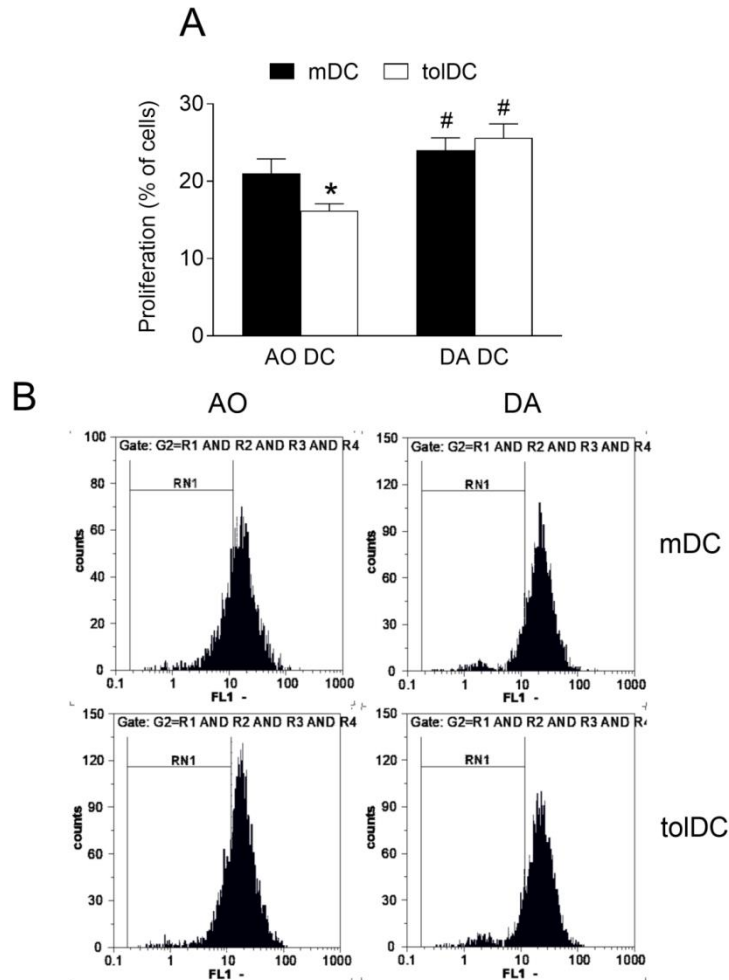


Fig 4 Alloproliferative response. DC were propagated from rat bone marrow cells in the presence of GM-CSF, and in the absence (mDC) or presence of vitamin D3 (toIDC). They were treated with LPS for 24h. AO DC were co-cultured with DA lymph node cells and the other way around. Lymph node cells were stained with CFSE. Co-cultivation lasted for five days. After co-culture, cells were stained for CD4 expression and CFSE was detected in CD4 cells by cytofluorimetry. Data are presented as mean + SD of values obtained in 2 independent

experiments (A). Representative plots are also presented (B). * $p < 0.05$, mDC vs. tolDC, # $p < 0.05$, AO vs. DA; Tukey's multiple comparisons test.

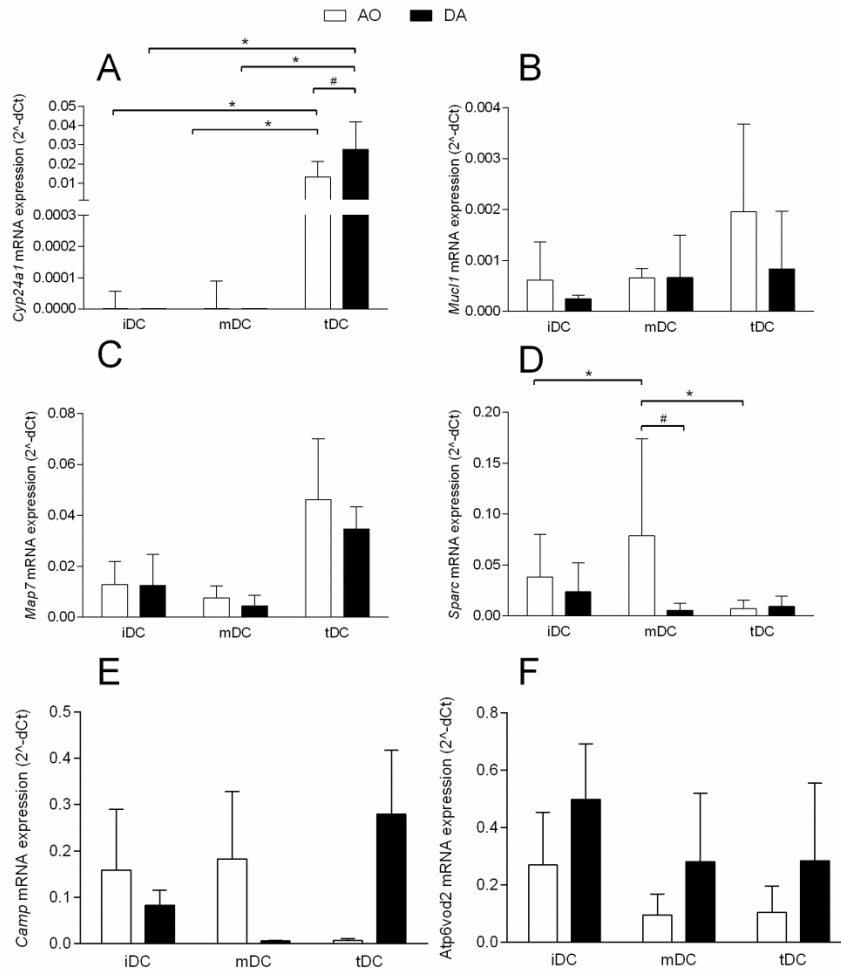


Fig 5 Gene expression profiles in AO and DA DC. DC were propagated from bone marrow cells. DC were grown in the presence of GM-CSF only, without additional stimuli (iDC) or stimulated with LPS (mDC), or they were grown in the presence of GM-CSF and vitamin D3 and stimulated with LPS (tolDC). Subsequently, “real-time” RT-PCR for *Cyp24a1* (A), *Mucl1* (B), *Map7* (C), *Sparc* (D), *Camp* (E), and *Atp6vod2* (F) genes was performed. Data are presented

as mean + SD of values obtained from 5 samples. * $p < 0.05$, within the strain; # $p < 0.05$, AO vs. DA; Tukey's multiple comparisons test.

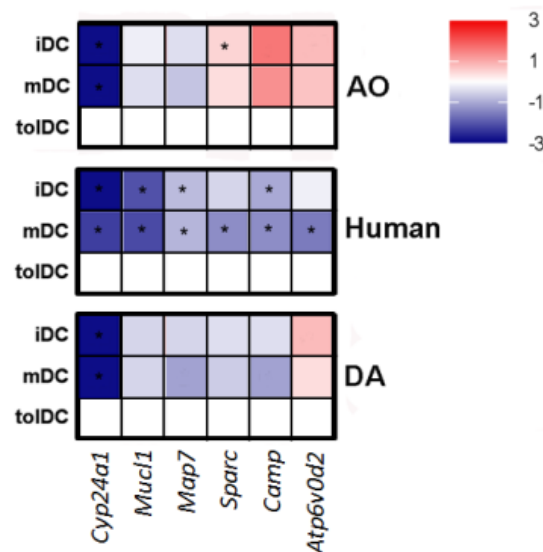


Fig 6 Gene expression profile in rat and human DC. Data from Fig 5 were compared to data obtained from human cells [19]. * $p < 0.05$, in comparison to toDC; Tukey's multiple comparisons test.