Thymic Stromal Lymphopoietin is Expressed in the Intact Central Nervous System and Upregulated in the Myelin-Degenerative Central Nervous System

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Thymic stromal lymphopoietin (TSLP) is an epithelial cytokine expressed at barrier surfaces of the skin, gut, nose, lung, and the maternal/fetal interphase. At these sites, it is important for the generation and maintenance of non-inflammatory, tissue-resident dendritic cell responses. We show here that TSLP is also expressed in the central nervous system (CNS) where it is produced by choroid plexus epithelial cells and astrocytes in the spinal cord. Under conditions of low-grade myelin degeneration, the numbers of TSLP-expressing astrocytes increase, and microglia express transcripts for the functional TSLP receptor dimer indicating that these cells are targets for TSLP in the myelin-degenerative CNS.

Key words: thymic stromal lymphopoietin, astrocytes, microglia, myelin degeneration

Introduction

Epithelial cells at barrier surfaces like skin, gut, nose, and lung express the cytokine thymic stromal lymphopoietin (TSLP) (Kamekura et al., 2009; Reche et al., 2001). Changes in the tissue-specific expression of TSLP are associated with a number of diseases. For example, in patients with atopic dermatitis, asthma, or allergic rhinitis, TSLP expression is dramatically upregulated in the skin, lung, or nose (Kamekura et al., 2009; Ziegler and Artis, 2010), and is associated with type 2 inflammatory reactions (Ziegler and Artis, 2010). In patients with Crohn's disease, this molecule shows lower expression levels in colonic epithelial cells (Rimoldi et al., 2005), coinciding with the development of type 1 and type 17 inflammatory reactions (Ziegler and Artis, 2010). TSLP is a key molecule for the generation and maintenance of non-inflammatory, tissue-resident dendritic cell (DC) responses (Ziegler and Artis, 2010).

Since under pathological conditions, also microglial cells can differentiate to non-inflammatory, tissue-resident DCs (Fischer et al., 2000; Reichmann et al., 2002), we investigated whether TSLP is also expressed in the central nervous system (CNS), whether the expression of TSLP at this site changes under pathological conditions, and whether microglial cells express the appropriate receptors for TSLP.

Materials and Methods

Animals

For cell culture and isolation of microglial cells by FACSorting, proteolipid protein (PLP)-overexpressing transgenic and wildtype Lewis rats were obtained from the Decentral Facilities of the Institute for Biomedical Research, Medical University, Vienna. The genotype of
the transgenic animals was determined by PCR analysis as described (Bradl et al., 1999).

For histology, archival CNS material from 10 to 12 months old PLP-transgenic Lewis rats and age-matched wildtype Lewis rats (Bradl et al., 2005) was used. In all these cases, the animals had been sacrificed by inhalation of an overdose of CO₂, and perfused with 4% paraformaldehyde (PFA) prior to the dissection of brains and spinal cords. The tissue was post-fixed in 4% PFA for additional 18–24 hours and subsequently embedded in paraffin.

**Histological Analysis**

For the analysis of TSLP expression in tissue slices, 2–4 µm thick sections were used without further pre-treatment (anti-mouse/rat TSLP; anti-Iba1), or after antigen retrieval for 1 hour in a commercial food steamer in 10 mM EDTA buffer pH 8.5 (anti-GFAP). The sections were rinsed in 0.05 M Tris-buffered saline (TBS) and incubated in Dako buffer (DakoCyromation, Vienna, Austria) containing 10% fetal calf serum (DB/FCS) for 20 min. The slides were incubated in blocking buffer at 4°C overnight with the following primary antibodies: polyclonal rabbit anti-mouse/rat TSLP (ab3, Sigma Aldrich; 1:50), polyclonal rabbit anti-Iba1 (Wako Chemicals GmbH, Neuss, Germany; 1:50), or mouse anti-rat glial fibrillary acidic protein (GFAP; Neomarkers, Fremont, CA; 1:200). The sections were then washed three times in TBS and incubated with biotinylated secondary antibodies (donkey anti-rabbit, 1:2,000, or sheep anti-mouse, 1:500; both antibodies from Jackson Immunoresearch) in DB/FCS for 1 hour at room temperature, followed by three times washing in TBS and incubation with avidin–peroxidase complex (1:100 in DB/FCS; Sigma) for 1 hour. After another washing step, labeling was visualized with 3,3′-diaminobenzidine-tetra-hydrochloride (DAB, Sigma) containing 0.01% hydrogen peroxide. All sections were counterstained with 4% paraformaldehyde in PBS and mounted in Eukitt (Sigma).

**Choroid Plexus Epithelial Cultures**

Adult Lewis rats were sacrificed by an overdose of CO₂, their brains dissected and sagitally sliced, and the choroid plexuses excised from the two lateral and from the fourth ventricle. This tissue was first incubated with 1 mg/mL collagenase in PBS (20 min, 37°C) and centrifuged (800g, 5 min). Afterward, the cell pellet was further subjected to five rounds of brief trypsin/DNase (0.025% Trypsin–EDTA and 12.5 µg/mL DNase I in PBS) treatment, followed by centrifugation (800g, 5 min). Finally, the cells were resuspended in Ham’s F-12/DMEM (1:1) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL penicillin/streptomycin, 5 µg/mL insulin, 5 µg/mL transferrin, 5 µg/mL sodium selenite, 10 ng/mL EGF, 2 µg/mL hydrocortisone, 5 ng/mL basic fibroblast growth factor, 500 µM hypoxanthine, and 250 µg/mL hydroxy-l-proline, and plated on 35 mm culture dishes coated first with 0.01% collagen and then with 10 µg/mL laminin. Cells were cultured further in the presence of 20 µM cytarabine for the first 3 days in vitro. Purity of cultures, as estimated by an immunofluorescent staining of aquaporin-1 (AQP-1, marker of choroidal epithelial cells), morphology, and expression of transthyretin (Ttr) was approximately 98%.

**Microglia Isolation and Characterization**

**Isolation.** About 5- to 12-month-old PLP-transgenic and wildtype Lewis rats were sacrificed with an overdose of CO₂, transcardially perfused with PBS, and their spinal cords were carefully dissected, stripped from meninges, and passed through cell strainers. Microglia cells and macrophages were then purified from this suspension using Percoll (density 1.0489 g/mL) gradient centrifugation (Nycomed-Pharma AS, Oslo, Norway). Afterward, a cell surface staining of the cells was made, using FITC-labeled anti-CD45 (recognizing leucocyte common antigen, from AbD Serotec, Düsseldorf, Germany). Since microglia cells express substantially less cell surface CD45 than macrophages (Ford et al., 1995), CD45<sup>low</sup> microglia cells could be clearly distinguished from CD45<sup>high</sup> macrophages, and therefore be isolated by FACS sorting, using a FACS Aria sorter with a 70-µm nozzle and DiVa software, or the FACS caliber system using the Cellquest Pro-software (all Becton Dickinson, San Jose, CA).

**Characterization by Flow Cytometry.** Microglial cells from wildtype and PLP-transgenic rats were simultaneously stained with FITC-labeled anti-CD45 and biotinylated anti-CD80 (B7.1), anti-CD86 (B7.2), and isotype control antibodies (all from AbD Serotec). The biotinylated antibodies were finally detected using APC-conjugated anti-biotin antibodies (Miltenyi Biotec, Bergisch Gladbach, Germany). For flow cytometry, gates were set on CD45<sup>low</sup> cells, and the expression profile of the gated cells was analyzed using the FACScalibur system and Cellquest Pro-software.

**Gene Expression Studies.** Microglial cells of seven wildtype and eight PLP-transgenic rats were used. The RNA of these cells was isolated as described above and sent to Imagenes (Berlin, Germany)
TABLE 1: Sequence of the Nucleotide Probes Used for Gene Expression Studies

| Gene   | Accession number | Probe sequence (5’->3’)          |
|--------|-----------------|----------------------------------|
| Cd68   | NM_001031638    | ATCCCTCATTTCTTCCAGCTGCAATTGTGACGTACAGGTTATTCTCCCTCTCTGTCTTTAAAA |
| RT1-B^a| NM_001004084    | CAGCTTGTGACACCTTGGAAATACCGTCTTCTGGATAACAGAGTGAGTACTTCTTCTCAGGTTATA |
| Cd86   | NM_0200881      | TAAGCAAGGTAACCCCAAAAGACGATGTGAAAGGCGCTACGACACCTGAAAAAACCAAGA |
| Cd80   | NM_012926       | TGAATCTGACAGCTTGACACATTTAGTAGTTAGCGAACAGGCTAGACCATCTCTCTCTCAGGTTATA |
| Ccl17  | NM_057151       | CTGCACAGAGGCAACACAGCTGACATGAAAGGCCTACGACACCTGAAAAAACCAAGA |
| Ccl22  | NM_057203       | GATATCTGTGACCCAGAGTCTGCTGGTGAAGAAGAATCTACCAAGATTTGGGCTAGA |
| Ccl22  | NM_057203       | AAATATATACTTTTGAAGAGGCAACACAGGCTAGACCATCTCTCTCTCAGGTTATA |
| II12a  | NM_053390       | ACTTCAGACGCCAATATCAGCTACGACATGAAAGGAGCTGATTGCTAGAGATTAGATA |
| II12b  | NM_022611       | TTGGCTCAGCCAGATTTTAAAAATTTTCAAATAGACTTTCTGAGGCTGAAGACACCA |
| C40    | NM_134360       | ATGAGGAAAAAGCTTTGGCCGTAGGCTCAGCTAATCTACAGATGGCCAGAAAGT|
| C40    | NM_134360       | ACTTCAGACGCCAATATCAGCTACGACATGAAAGGAGCTGATTGCTAGAGATTAGATA |
| Oxl0L  | NM_053552       | TTGTGTTGACATCAGTGTTCTTGAACCTCCAGAACGGCTAGATGAGATTAGATA |

Isolation of Meningeal Antigen Presenting Cells

Meningeal antigen presenting cells were isolated according to modified protocols (Hochmeister et al., 2008; Kivasakk et al., 2009). Briefly, rats were perfused with 50 mL PBS and meninges were carefully dissected from the brain and spinal cord tissue. This was followed by a 1 hour incubation in ice-cold HBSS (without Ca^2+ and Mg^2+; BioWhittaker) supplemented with 2 mM EDTA (Serva) and 5 mM HEPES (Sigma). Cells were further mechanically dissociated from meningeal tissue by passage through a 70-μm cell strainer. After centrifugation (300 g, 1 min/mL), cells were either re-suspended in stain buffer (PBS/0.2% BSA) alone, or in stain buffer containing mouse anti-rat MHC class II RT1B antibody (OX-6 clone, AbD Serotec, 1:100) or IgG1 isotype control (Dako Cytomation, 1:100) and incubated for 30 min at 4°C.

Rat Brain Endothelial Cultures

These cultures were established and characterized as recently published (Kitic et al., 2013).

Characterization of Cell Cultures

RNA Isolation, cDNA Synthesis, and Polymerase Chain Reactions. Cellular RNA was isolated using the RNeasy Mini Kit (Qiagen, Vienna, Austria) according to the manufacturer's instructions. To avoid contamination of the RNA sample with genomic DNA, the following procedures were carried out: 50 μL RNA sample was mixed with 7 μL 10× Dnase buffer, 2 μL RiboLock RNase inhibitor, 7 μL DNase 1 (all from Fermentas), and 4 μL RNase-free water (GIBCO, Invitrogen) and incubated for 30 min at 37°C, then 7 μL 25 mM EDTA (Fermentas) were added, and the samples were further incubated for 10 min at 65°C. After RNA clean-up, the RNA was eluted in 30 μL RNase-free water and subsequently annealed with a T7-T7 primer (5'<CCAAGCTTTCTAATACGACTCACTATAGG-GAGANNNNNN-3'; incubation at 70°C for 5 min), supplemented with 10 μL 5× M-MLV RT Buffer (Promega), 3 μL dTTPs (Roche), 3 μL RiboLock RNase Inhibitor (Fermentas), and 5 μL RNase/DNase-free H2O (GIBCO, Invitrogen), and incubated at 40°C for 2 min. Reverse-transcriptase [M-MLV Reverse Transcriptase, RNaseH, point mutant, 200 U/μL (Promega)] was added to each sample. The samples were incubated first at RT for 10 min, then at 40°C for 50 min, and finally at 70°C for 15 min.

Quantitative real-time polymerase chain reaction (qPCR) was performed using the QuantiFast™ SYBR® Green PCR Kit (Qiagen) in a StepOnePlus RT-PCR system (Applied Biosystems), according to the manufacturer's instructions. The following QuantiTect Primer Assays (Qiagen) were used for gene amplification: Rn_Crfl2_1_SG for Crfl2 (Tslpr), Rn_Gadph_1_SG for Gpdh, Rn_Gfap_1_SG for Gfap, Rn_Il7r_2_SG for Il7r, and Rn_Ttr_1_SG for Ttr. For the detection of Tslp, transcript self-designed primers (Tslp-forward: 5‘-TCCCTGAAAATCGACTCATGACTATATAGG-GAGANNNNNN-3‘; incubation at 70°C for 5 min), supplemented with 10 μL 5× M-MLV RT Buffer (Promega), 3 μL dTTPs (Roche), 3 μL RiboLock RNase Inhibitor (Fermentas), and 5 μL RNase/DNase-free H2O (GIBCO, Invitrogen), and incubated at 40°C for 2 min. Reverse-transcriptase [M-MLV Reverse Transcriptase, RNaseH, point mutant, 200 U/μL (Promega)] was added to each sample. The samples were incubated first at RT for 10 min, then at 40°C for 50 min, and finally at 70°C for 15 min.
FIGURE 1: Expression of TSLP in the central nervous system of Lewis rats. Shown here are choroid plexus (a–c) and spinal cord (d–h). Consecutive sections of choroid plexus were reacted with anti-TSLP (a, brown reaction product), anti-iba-1 (b, brown reaction product), or without first antibody (c), and were counterstained with hematoxylin to reveal nuclei in blue color. In the spinal cord, TSLP is expressed at the subpial (d, e, h) and perivascular (g) glia limitans. For further control, consecutive sections of the spinal cord were reacted with anti-TSLP to reveal staining of astrocytes (e), and with anti-iba-1 to reveal the absence of astrocytic and the presence of microglial staining specific for this antibody (f). Double staining of sections with anti-TSLP (blue) and anti-GFAP (brown) antibodies confirmed TSLP expression by astrocytes (h).

equation: \( 2^{-\Delta C_T} = 2^{-(\Delta C_{(GOI)} - \Delta C_{(HKG)})} \) (GOI, Gene of interest; HKG, house-keeping gene).

Further PCR analyses were done using the FastStart Taq Polymerase kit (Roche Applied Science, Vienna, Austria). One reaction consisted of: 5 μL 10× PCR buffer (200 mM Tris–HCl, pH 8.4, 20 mM MgCl₂), 1 μL 10 mM dNTP mix, 1 μL forward primer (100 pmol/μL), 1 μL reverse primer (100 pmol/μL), 0.4 μL polymerase (5 U/μL), 1 μL cDNA, and 40.6 μL H₂O. The following primer pairs were used: Gfap (forward: 5’-TTGTTTAGCT-3’; reverse: 5’-GCCCAATTC-3’; product size: 356 bp); GAPDH (forward: 5’-GGCATGCTCTCAATGACACC-3’; reverse: 5’-TGAGGCGTCAAGCGAAC TTAT-3’; product size: 311 bp); TSLP (forward: 5’-TTCCTGAA AATCGACACCAC-3’; reverse: 5’-AAATGGAAAGCC ACAATC-3’; product size: 230 bp); and IL-7Rα (forward: 5’-GCCACTGGACGCTCTGAGCTC-3’; reverse: 5’-AATGCCAACGCTCCAG-3’; product size: 204 bp). The reaction mixture was subjected to an initial denaturation step (10 min, 95°C), and then to 30, 35, and 40 cycles of denaturation (30 s, 95°C), annealing (30 s, 50°C for TSLP, 51°C for GFAP, 53°C for GAPDH, 55°C for TSLPα, and IL-7Rα, 57°C for TTR), and elongation (30 s, 72°C). The reaction was terminated with final extension for 10 min at 72°C, and PCR products were detected by agarose gel electrophoresis. Sequencing of the PCR products (VBC Biotech, Vienna, Austria) confirmed their identity.

**Statistical Analysis.** Statistical analyses were conducted using Kruskal–Wallis test, followed by pair-wise Mann–Whitney U-tests and Bonferroni correction. The P-values are results of two-sided tests. Data are represented in boxplots that show the range of numbers of TSLP⁺ cells (whiskers), with 50% scores (interquartile range, boxes) centered around median values (horizontal lines within boxes). Analyses were performed using PASW Statistics 18 software (SPSS Inc.).

**Results**

**TSLP Expression and Recognition in the Intact CNS**

In the spinal cord of healthy Lewis rats, GFAP⁺ astrocytes at the superficial glia limitans displayed cytoplasmatic and often also nuclear reactivity for TSLP. The numbers of TSLP⁺ astrocytes were highly variable, independent of the location of the spinal cord sections along the neuraxis (Fig. 1). Weaker staining was seen in astrocytes and astrocytic foot processes at the perivascular glia limitans of the white matter (Fig. 1). Throughout spinal cord gray matter, diffuse TSLP reactivity was noted, and only few cells showed TSLP expression levels above this background reactivity.

In the brain, TSLP-reactive astrocytes were essentially absent from the perivascular or subpial glia limitans (data not shown). Instead, choroid plexus cells were positive for TSLP (Fig. 1). We also detected nuclear and cytoplasmatic TSLP expression in some brain neurons. The most prominent reactivity was found in neurons located in the substantia nigra, lemniscus medialis, nucleus ruber, and decussatio tegmenti ventralis, while TSLP expression was essentially absent in neurons located within the adjacent interpeduncular nucleus. Also hippocampal neurons, Purkinje cells, and other neuronal cells throughout the brain parenchyma showed nuclear staining for TSLP (Supp. Info. Fig. 1). This finding was surprising, since...
neither TSLP knock-out animals (Reardon et al., 2011) nor TSLP-overexpressing transgenic mice (Osborn et al., 2004) display any neurological abnormalities. We did not further analyze neuronal TSLP expression in more detail.

To further confirm the expression of TSLP in astrocytes and choroid plexus epithelium, we established cultures of these cells and searched for the presence of TSLP transcripts by qPCR. TSLP mRNA was detectable in both types of cells indicating that they are able to produce TSLP (Fig. 2).

It has been described that TSLP preferentially stimulates myeloid cells (Qiao et al., 2011; Reche et al., 2001), and that DCs and monocytes coexpress TSLPR and IL-7Rα (Reche et al., 2001). Since microglia and monocytes share the ability to differentiate along the macrophage and DC lineage under several different pathological conditions (Fischer and Reichmann, 2001; Reichmann et al., 2002; Santambrogio et al., 2001), we analyzed by qPCR whether DCs from the meningeal DC network or microglial cells from the spinal cord contain the molecular machinery needed for TSLP recognition, that is, a functional TSLP receptor dimer consisting of the TSLP-specific receptor chain (TSLPR) and the common IL-7 receptor alpha chain (IL-7Rα). We found transcripts for IL-7Rα, and low amounts of transcripts encoding TSLPR (Fig. 3). This finding clearly indicated that microglia in the myelin-degenerative CNS can produce TSLP receptor dimers.

**Microglial Properties in the Myelin-Degenerative CNS**

We could not detect the expression of functional TSLP receptor dimers by immunohistochemistry, probably due to expression levels below the limit of detection. Therefore, we used a different approach to study the effect of TSLP on microglia in the myelin-degenerative CNS. We isolated microglia from the spinal cords of PLP-transgenic rats and their wildtype Lewis counterparts and studied the expression of genes known to be affected by TSLP, that is, MHC class II products, the costimulatory molecules CD40, CD80 and CD86, OX40L, CCL17, and CCL22 (Zhou et al., 2005). Compared with their wildtype Lewis rat-derived counterparts, microglia...
isolated from the spinal cord of PLP-transgenic rats upregulated MHC class II products, showed enhanced expression of the costimulatory molecules CD40 and CD86 but not of CD80, and transcribed more OX40L products. mRNA coding for CCL22 and CCL17 was either unchanged or downregulated, respectively (Fig. 5). Hence, microglial cells revealed some, but not all features of TSLP-instructed cells.

Discussion

TSLP is an epithelial cell-derived cytokine that is typically expressed at barrier surfaces of the body, that is, in the skin, gut, nose, lung, and at the maternal–fetal interphase, and plays an important role in the crosstalk between epithelial cells and DCs needed for the induction of physiological or pathological immune responses (Bogiatzi et al., 2007). In light of the epithelial expression pattern of TSLP it is not unexpected that also choroid plexus epithelial cells produce TSLP possibly for the communication with DCs found under physiological conditions in the choroid plexus (McMenamin, 1999; McMenamin et al., 2003). However, it is much more surprising that astrocytes can produce TSLP as well, both in the intact and in the diseased spinal cord, and that TSLP is located both in the cytoplasm and in the nucleus.

The presence in the nucleus is not a unique feature of TSLP but has also been noted for other cytokines like high mobility group box 1 (HMGB1), interferon-γ, interleukin-16, and interleukin-1 family cytokines (for review see Luheshi et al., 2009). Interestingly, in the IL-1 system, nuclear location was most often described for pro-IL-1α, and was associated with proliferation, induction of gene expression and
migration (Luheshi et al., 2009). It is tempting to speculate that cytoplasmic/secreted and nuclear TSLP show a similar dual functionality.

TSLP reactivity of astrocytes is highly variable. This may reflect astrocyte heterogeneity (Black et al., 1993; Pfrieger and Slezak, 2012) whose effects on chemokine and cytokine production have already been described (Morga et al., 1999, Fitting et al., 2010).

In the intact CNS, TSLP expression by choroid plexus epithelial cells in the brain, and by astrocytes at the glia limitans in the spinal cord might regulate the survival and the effector functions of TSLP receptor dimer-expressing MHC class II<sup>+</sup> cells in the meningeal/perivascular DC network.

However, in the myelin-degenerative spinal cords of PLP-transgenic Lewis rats, both the extent of TSLP expression and the cellular targets of TSLP change. Then, additional astrocytes become TSLP-positive. Interestingly, these astrocytes were predominantly located in the spinal cord gray matter, a compartment characterized by low-grade myelin degeneration, oligodendrocyte stress/death and microglia activation (Aboul-Enein et al., 2004; Bradl et al., 2005; Grundtner et al., 2007). In the same animals, microglial cells in the spinal cord expressed transcripts for both subunits of the TSLP receptor, suggesting that these cells are potential TSLP targets. TSLP might play a role in the activation and proliferation of these microglial cells, since it induces the phosphorylation and activation of signal transducers and activators of transcription (STATs), most prominently of STAT5 (Roan et al., 2012), which serves as a molecular regulator of cell fate, influencing proliferation, differentiation and apoptosis (Nosaka et al., 1999). Moreover, TSLP targets the same signal transduction pathway as granulocyte/monocyte-colony stimulating factor (GM-CSF) does (Liva et al., 1999; Yamaoka et al., 1998), a molecule involved in the control of microglia proliferation (Liva et al., 1999), activation (Natarajan et al.,

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**FIGURE 4**: TSLP expression in the myelin-degenerative spinal cord of PLP-transgenic Lewis rats. All tissue samples derived from aged PLP-transgenic Lewis rats with chronic, subclinical myelin degeneration, which is especially pronounced in CNS gray matter (Bauer et al., 2002; Bradl et al., 1999). The spinal cord sections were reacted with anti-TSLP antibodies (a–d) or anti-Iba1 (e) (brown reaction products) and counterstained with hematoxylin to visualize nuclei (blue). Shown here are TSLP<sup>+</sup> astrocytes at the subpial (a) and perivascular (b) glia limitans, in gray matter (c,d), and in white matter close to the gray/white matter junction (c). The rabbit anti-Iba1 antibody used as control does react with microglia, but not with neurons or astrocytes (e). (f) The number of TSLP<sup>+</sup> cells in spinal cord gray matter (thoracic and lumbar segments) differs between wildtype Lewis (gray bars) and PLP-transgenic rats (open bars) according to Mann-Whitney U-tests (P < 0.001). In the superficial glia limitans, no significant difference in cell number was observed. Three thoracic and three lumbar spinal cord sections were analyzed per animal, using six PLP-transgenic and 5 wt Lewis rats.

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**FIGURE 5**: The phenotype of microglia isolated from PLP-transgenic rats. Shown here are log2-fold changes in the expression of TSLP-instructed transcripts in microglia isolated from PLP-transgenic animals, as compared with their wildtype derived counterparts. Note the upregulation of the microbial activation marker CD68, of MHC class II molecules (as an example, RT1-B<sup>+</sup> is shown), of the costimulatory molecule CD86, of CD40 and Ox40 ligand, and the clear downregulation of IL-12b and of CCL17 in PLP-transgenic microglia in comparison to its wildtype counterpart. The microglial cells tested derived from seven wildtype and eight PLP-transgenic rats.
2004) and initiation of microglial differentiation along the DC lineage (Hochmeister et al., 2008; Santambrogio et al., 2001). It is even possible, that TSLP and GM-CSF jointly act in the PLP-transgenic CNS, since microglia of PLP-transgenic rats also approximately two-fold upregulate the expression of Csf2rb1 (the GM-CSF/IL-3/IL-5 receptor common beta subunit; data not shown).

Microglial cells of PLP-transgenic rats do not show all features of TSLP-instructed DCs, since they upregulate transcripts for MHC class II products, CD40, CD86, and OX40L, but not for the chemokines CCL17 or CCL22. The most likely explanation for this finding is the fact that microglial cells in vivo can only partially differentiate along the DC lineage in vivo, and are arrested at an immature stage (Fischer et al., 2000; Fischer and Reichmann, 2001; Reichmann et al., 2002).

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