BRAIN
ORIGINAL ARTICLE

ALCAM on human oligodendrocytes mediates CD4 T cell adhesion

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Multiple sclerosis is a chronic neuroinflammatory disorder characterized by demyelination, oligodendrocyte damage/loss and neuroaxonal injury in the context of immune cell infiltration in the CNS. No neuroprotective therapy is available to promote the survival of oligodendrocytes and protect their myelin processes in immune-mediated demyelinating diseases. Pro-inflammatory CD4 Th17 cells can interact with oligodendrocytes in multiple sclerosis and its animal model, causing injury to myelinating processes and cell death through direct contact. However, the molecular mechanisms underlying the close contact and subsequent detrimental interaction of Th17 cells with oligodendrocytes remain unclear.

In this study we used single cell RNA sequencing, flow cytometry and immunofluorescence studies on CNS tissue from multiple sclerosis subjects, its animal model and controls to characterize the expression of cell adhesion molecules by mature oligodendrocytes. We found that a significant proportion of human and murine mature oligodendrocytes express melanoma cell adhesion molecule (MCAM) and activated leukocyte cell adhesion molecule (ALCAM) in multiple sclerosis, in experimental autoimmune encephalomyelitis and in controls, although their regulation differs between human and mouse. We observed that exposure to pro-inflammatory cytokines or to human activated T cells are associated with a marked downregulation of the expression of MCAM but not of ALCAM at the surface of human primary oligodendrocytes. Furthermore, we used *in vitro* live imaging, immunofluorescence and flow cytometry to determine the contribution of these molecules to Th17-polarized cell adhesion and cytotoxicity towards human oligodendrocytes. Silencing and blocking ALCAM but not MCAM limited prolonged interactions between human primary oligodendrocytes and Th17-polarized cells, resulting in decreased adhesion of Th17-polarized cells to oligodendrocytes and conferring significant protection of oligodendrocytic processes.

In conclusion, we showed that human oligodendrocytes express MCAM and ALCAM, which are differently modulated by inflammation and T cell contact. We found that ALCAM is a ligand for Th17-polarized cells, contributing to their capacity to adhere and induce damage to human oligodendrocytes, and therefore could represent a relevant target for neuroprotection in multiple sclerosis.

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Received March 17, 2023. Revised July 25, 2023. Accepted August 08, 2023. Advance access publication August 28, 2023

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Keywords: oligodendrocytes; multiple sclerosis; Th17 cells; ALCAM; MCAM

Introduction

Multiple sclerosis (MS) is the prototypical neuroinflammatory demyelinating disease, affecting more than two million individuals worldwide.[1] Although the exact pathobiology of MS has yet to be elucidated, the invasion of peripheral immune cells in the CNS is considered to play a major role in the formation and expansion of lesions, which are characterized by myelin and oligodendrocytes damage/loss as well as neuroaxonal injury.[2] Preservation of oligodendrocytes and their myelin processes remains an unmet need to prevent axonal degeneration and abrogate accumulation of neurological disability over time in MS and other inflammatory demyelinating diseases.[3-5] The mechanisms underlying immune-mediated oligodendrocytes injury are only partially understood, a major gap in the effort to develop neuroprotective strategies. Zaguia *et al.*[6] reported that activated CD4 T cells can show toxicity towards human oligodendrocytes. Recently, we showed that oligodendrocytes damage and death can be induced by close contact with polarized T helper 17 (Th17) cells,[7] a pro-inflammatory subset of CD4 T cells considered pathogenic in MS[8-11] and its animal model experimental autoimmune encephalomyelitis (EAE).[10,12] Following direct contact with oligodendrocytes, human pro-inflammatory Th17 cells can indeed secrete glutamate and granzyme B, which will induce damage to oligodendrocytes processes and lead to oligodendrocytes cell death, respectively.[7,13] Identification of molecules implicated in prolonged Th17 cell–oligodendrocyte interaction could therefore open novel therapeutic avenues in MS, to prevent chronic demyelination and subsequent neuroaxonal degeneration.[14-17]

Previous studies suggested that oligodendrocytes can express major histocompatibility complex (MHC) II and potentially interact with CD4 T cell receptor in human and murine tissue.[18-22] These observations are mainly based on single nuclei RNA sequencing; the expression at the protein level is found in a very small proportion (~1%) of murine oligodendrocytes in EAE,[23] and remains to be demonstrated on mature human oligodendrocytes.[18] In line with this, we previously reported that the prolonged contact between Th17 cells and oligodendrocytes was not limited to antigen-specific cells.[7] Cell adhesion molecules (CAMs) are implicated in non-antigen-restricted cell–cell interactions, contributing to multiple immunological processes in neuroinflammatory diseases such as the formation of the immune synapse and the transmigration of leucocytes across the blood–brain barrier.[11,24-27] Herein we aimed to identify potential candidates mediating human Th17 cell–oligodendrocyte direct interactions in neuroinflammatory conditions, and to investigate the contribution of identified CAMs to contact and to subsequent Th17-mediated oligodendrocyte injury.

Materials and methods

Ex vivo human CNS material

Human brain tissue was obtained from the resection path during neurosurgery for non-tumoral epilepsy, or within 4 h post-mortem from subjects with MS according to McDonald's criteria,[28] or with amyotrophic lateral sclerosis diagnosis or hereditary spastic paraplegia (non-MS control subjects). All donors provided prior written informed consent and all experiments received full ethical approval (BH07.001, Nagano 20.332). MS lesions were classified using Luxol fast blue/haematoxylin and eosin staining and Oil Red O staining, as previously published.[2] Human tissue was processed for *ex vivo* flow cytometry analysis, as previously described.[29,30] Briefly, tissue was washed, minced with scalpels and passed through a 70 μm filter (Falcon) before digestion with DNAse I (14 μg/ml, Roche Diagnostic) and collagenase D (2 mg/ml, Roche). Percoll gradient centrifugation was used to isolate immune and CNS cells before removal of red blood cells using a commercial kit (Stemcell). Characteristics of human tissue used in this study are described in Supplementary Table 1.

Human primary oligodendrocytes and MO3.13 cell line culture

Oligodendrocytes were isolated from fresh non-pathological brain tissue from neurosurgery for non-tumoral epilepsy, as previously published[31] and approved by the Biomedical Ethics Unit of McGill University (Protocol ANTJ 1988/3). Briefly, blood vessels were removed from the tissue. Digestion with a deoxyribonuclease (Roche)/tryspin (Gibco) cocktail and manual pressing through a strainer resulted in a cell suspension. After a 33% Percoll ultracentrifugation, cells were plated in flasks. The day after, floating cells (oligodendrocytes) were harvested and plated on poly-L-lysine (10 μg/ml) and extracellular matrix (1:200, Sigma) coated wells. Cells were plated at 200 000 cells/well for flow cytometry and 100 000 cells/well for immunofluorescence and live imaging experiments. Oligodendrocytes were used for experiments at 8–10 days *in vitro* (8–10 DIV). For *in vitro* stimulation experiments, recombinant human cytokines were used: TNFα 200 U/ml (Gibco), IFNγ 1000 U/ml (Thermofisher Scientific), IL-4 200 ng/ml (R&D Systems), IL1β 100 ng/ml (R&D Systems), IL-17 100 ng/ml (R&D Systems), IL-12 20 ng/ml (R&D Systems), IL-10 100 ng/ml (R&D Systems), IL-6 20 ng/ml (R&D Systems), GM-CSF 20 ng/ml (BD Biosciences) and TGFβ 5 ng/ml (R&D Systems).

Human oligodendrocytic cell line MO3.13 cells were cryopreserved in Dulbecco's modified Eagle medium (DMEM) 40% fetal bovine serum (FBS)/20% dimethyl sulphoxide. After gentle thawing, MO3.13 cells were plated with DMEM supplemented with 10% FBS, 1% glutamine and penicillin/streptomycin. When confluent

MO3.13 cells were harvested using warm trypsin in PBS and plated according to the experiment. Cells were used between passage three and six for experiments.

Human CD4 T cells primary culture

Blood collection

Written informed consent was obtained from every donor and samples used with full ethical approval (SL05.022, SL05.023 and BH07.001; Nagano 19.088). Clinical information on MS patients is presented in Supplementary Table 2. Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation (Ficoll), as previously published.[11]

Polarization of memory CD4 T cells

Memory CD4 T cells from healthy donors were magnetically sorted (negative selection) according to the manufacturer's instructions (Miltenyi) and activated with plate-bound αCD3 (2.5 μg/ml, eBioscience) and αCD28 (2 μg/ml, BD Bioscience) in the presence of αIFNγ antibody (5 μg/ml, Bio X Cell) in X-VIVO medium (Lonza). For Th2 polarization, human recombinant IL-4 (200 ng/ml) and αIL-12 antibody (5 μg/ml, Bio X Cell) were added on Day 0 and IL-2 (20 U/ml, R&D Systems) on Day 2. For Th17-polarization, cells were cultured with human recombinant IL-23 (25 ng/ml, R&D Systems) added on Days 0, 2 and 3, and αIL-4 antibody (5 μg/ml, Bio X Cell), as previously published.[7,11] Polarized T cells were used at 6 days in culture (D6) for experiments.

Activation of CD4 T cells

Frozen PBMCs from MS patients and healthy controls were thawed in nuclease S7 (20 μl/ml, Sigma Aldrich) diluted in FBS and washed in Roswell Park Memorial Institute medium (Wisent) complemented with 10% FBS. CD4 T cells were extracted using a positive selection kit (Miltenyi) and activated with plate-bound αCD3 and soluble αCD28 (0.5 μg/ml each), as previously published.[7]

Co-culture experiments

Polarized or activated CD4 T cells were harvested and washed before resuspension in X-VIVO medium and addition to oligodendrocytes at the indicated oligodendrocyte:T cell ratio of 1:10 (MO3.13 cells:T cells) or 1:2.5 (primary oligodendrocytes:T cells) for immunofluorescence and flow cytometry experiments, and 1:1 for live imaging experiments.

Single cell RNA sequencing experiments

Two preparations of human primary oligodendrocytes were cultured with medium only (control condition) or co-cultured with human Th17-polarized cells (1:2 oligodendrocyte:T cell ratio) directly ('contact' condition) or separated by an insert ('no contact' condition) for 12 h (Fig. 1A). Cells were then processed for single cell RNA sequencing as previously published.[13] Data were normalized using the SCTransform workflow. The data from these preps were integrated by condition with a previously published dataset from our laboratory (GEO: GSE196953) using the Seurat integration pipeline for SCT normalized data (total of $n = 3$ oligodendrocyte donors).[32] Each population was identified by conducting a pathway analysis on the markers expressed by each population (identified with the FindAllMarkers function from Seurat). Differentially expressed genes (DEGs) between two studied conditions ('contact' versus 'no contact', 'contact' versus 'control', 'no contact' versus 'control') were found with the FindMarkers function from Seurat across oligodendrocytes. For further functional annotation of transcriptional differences, only DEGs with an adjusted P-value < 0.01 were used. The network of significant GO Biological processes (P-value < 0.01) was obtained with Cytoscape (https://cytoscape.org/) and the ClueGO tool (https://apps.cytoscape.org/apps/cluego).

RT-qPCR

Total RNA was extracted from human primary oligodendrocyte culture using the RNeasy Mini Kit and transcribed into cDNA using QuantiTect Reverse Transcription kit (Qiagen) according to instructions. Activated leukocyte cell adhesion molecule (ALCAM), melanoma cell adhesion molecule (MCAM) and 18S-specific primers were used for the PCR.[11]

Flow cytometry

Flow cytometry staining was performed as previously described.[7,33,34] Briefly, live cells were identified using LIVE/DEAD fixable Aqua Dead Cell stain (Invitrogen) before non-specific sites were blocked with mouse IgG (60 μg/ml, ThermoFisher) and incubation with appropriate fluorochrome-conjugated antibodies (Supplementary Table 3).

Fluorescence activated single cell sorting

Fluorescence activated single cell sorting (FACS) was performed on Th17-polarized T cells stained with the LIVE/DEAD fixable Aqua Dead Cell stain and either ALCAM-BV786 or MCAM-APC. $ALCAM^{low}$ or $MCAM^{low}$ Th17-polarized cells were FACS-sorted at low pressure before co-culture with human primary oligodendrocytes, as indicated.

Immunofluorescence

Human CNS tissue

Frozen sections of brain autopsy specimen from MS patients or controls (Supplementary Table 1) were stained following established protocols.[11,35-37] Sections were incubated overnight at 4°C with primary antibodies: mouse anti-human ALCAM (1/200, R&D Systems) or mouse anti-human MCAM (1/100, Miltenyi) and rabbit anti-human NogoA (1/100, EMD Millipore). Following secondary antibodies (goat anti-mouse AF635 1:250, Invitrogen; donkey anti-rabbit Rhodamine Red™-X 1/400, Jackson ImmunoResearch; donkey anti-mouse AF488 1/400, Invitrogen), nuclei were stained with DAPI.

Human primary oligodendrocytes

After fixation with 4% paraformaldehyde, permeabilization with Triton 1% and blocking with donkey serum, as previously described,[7] cells were incubated overnight at 4°C with rabbit anti-human NogoA (1/100) and mouse anti-human ALCAM (1/200) or mouse anti-human MCAM (1/100). After washing, cells were incubated with appropriate secondary antibodies. Finally, nuclei were stained with DAPI. As T cells were loaded with CellTracker Green and MO3.13 with CellTracker Orange CMRA (Supplementary material, 'Methods' section), no additional staining was required for visualization. Images were acquired on a Leica SP5 MP confocal machine or a Zeiss AxioObserver Z1 Yokogawa CSU-X1 confocal microscope.

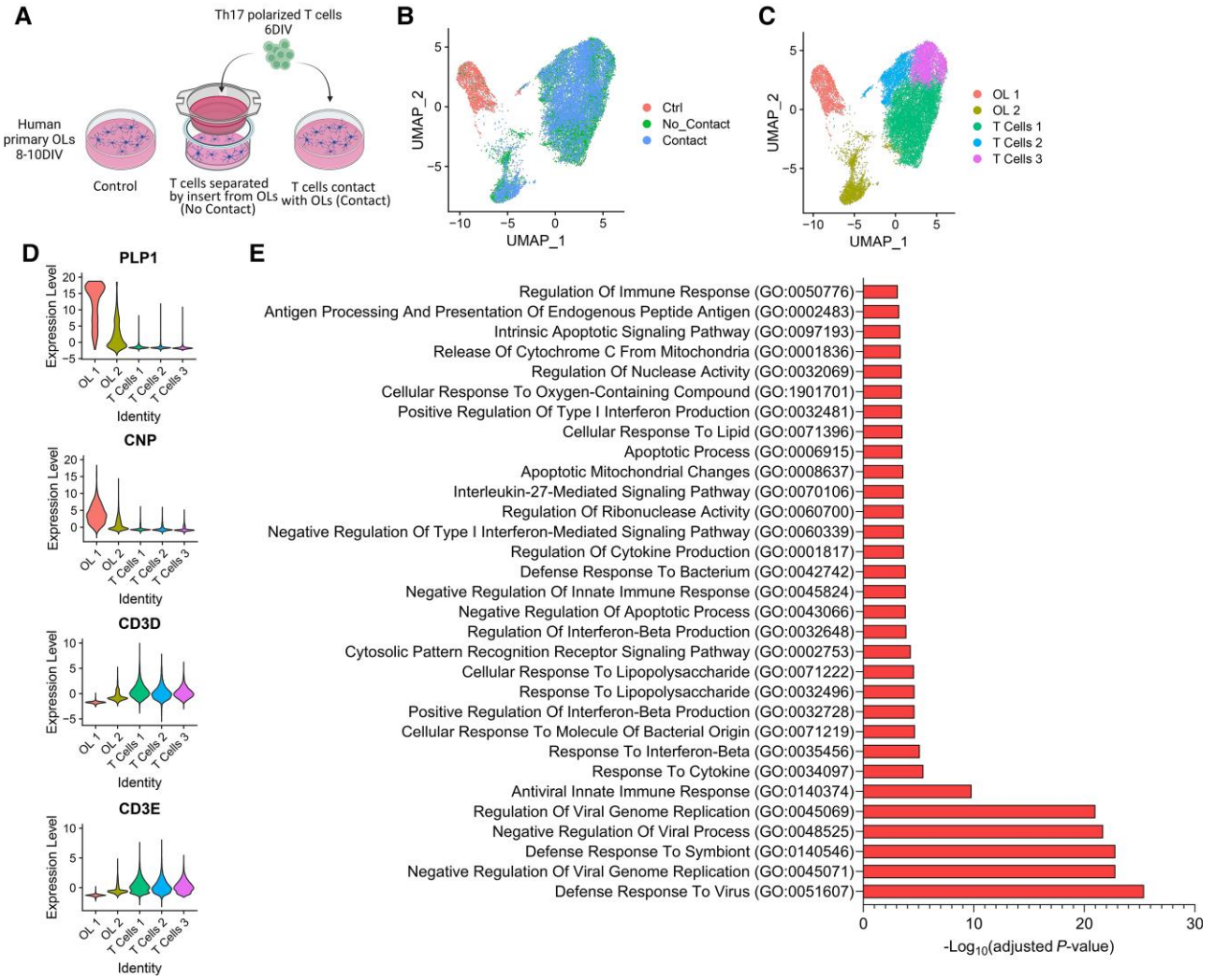

Figure 1 Contact with T cells leads to significant alterations in the transcriptional profile of oligodendrocytes. (**A**) Schematic of single cell sequencing experiments. Created with BioRender.com. (**B** and **C**) Uniform manifold approximation and projection (UMAP) of all preps ($n = 3$) grouped by (**B**) experimental condition or (**C**) cell type. Doublets, cells with high mitochondrial content and residual myeloid cells were removed. (**D**) Violin plots of canonical oligodendrocyte (OL) and T cell marker expression in each cell type cluster. (**E**) Top 30 identified gene ontology (GO) biological processes of differentially expressed genes (DEGs) between oligodendrocytes directly exposed to Th17 cells (contact condition) and oligodendrocytes separated from Th17 cells by an insert (no contact condition).

RNAscope *in situ* hybridization

In situ hybridization was performed using commercially available kits from ACD Bio company (RNAscope Multiplex Fluorescent V2 Assay) following the manufacturer's protocol (https://acdbio.com/technical-support/user-manuals, document 323100-USM). See Supplementary material, 'Methods' section for details and probes.

Analysis

A first step for brightness/contrast adjustment and Z-stack projection was performed with a macro in ImageJ (FIJI, RRID: SCR_002285, http://fiji.sc).[38] To quantify staining overlaps, mean fluorescence intensity and RNAscope dot quantification (number of dots), analysis pipelines were generated using the software CellProfiler (CellProfiler Image Analysis Software, RRID: SCR_007358, http://cellprofiler.org).[39] RNAscope confocal images were analysed following the manufacturer's instructions (available online: https://acdbio.com/technical-support/user-manuals, document SOP 45-006).

MO3.13 cell line transfection

MO3.13 cells were incubated for 5 h at 37°C in Opti-MEM media (Invitrogen) with Lipofectamine 2000 (2 μl/ml, Invitrogen) and 125 pmol siRNA: ALCAM siRNA (Invitrogen, siRNA ID HSS176657), MCAM siRNA (Invitrogen, siRNA ID HSS106379) or siRNA control (Stealth RNAi^{TM} siRNA negative control low GC, Invitrogen). Medium was then replaced and cells were used 3 days later for experiments.

Functional assays with neutralizing antibodies

To pharmacologically block ALCAM or MCAM on the surface of human primary oligodendrocytes, neutralizing antibodies were used, as previously published.[11,35,40,41] Oligodendrocytes were incubated with anti-MCAM[11,35] (40 μg/ml, Chemicon, P1H12), anti-ALCAM (30 μg/ml, R&D, MAB656) or appropriate isotypes (mouse IgG1, 40 μg/ml for MCAM and 30 μg/ml for ALCAM) in oligodendrocyte medium for 30 min at 37°C before addition of Th17-polarized cells.

Live imaging

Staining of oligodendrocytic cells MO3.13 with CellTracker Orange CMRA (Thermo Fisher), of mature oligodendrocytes with Via Fluora 647 live cell microtubule stain (Biotum) and of human T cells with CellTracker Green CMFDA (ThermoFisher) was performed as previously published.[13] Image acquisition and movie analysis were conducted, as previously published[13] and described in the Supplementary material, 'Methods' section.

Experimental autoimmune encephalitis model

All experiments were approved by the CHUM animal protection committee (N21015CLs). Active EAE was induced in 6–8 week-old (16–20 g) female C57BL/6J mice (Jackson) with 200 μg of mouse $MOG_{35–55}$ in a 100 μl emulsion of Complete Freund's Adjuvant (CFA), as previously published.[11,35,37] Pertussis toxin was administered (500 ng, Sigma-Aldrich) 48 h later. CFA immunization (sham EAE) followed the same protocol, omitting MOG.[35-55]

Statistical analysis and tests

Statistics were generated using R software[42] with ggplot2,[43] dplyr[44] and stats packages.[42] All data were tested by Shapiro normality test to assess their distribution. One-way ANOVA with Tukey's *post hoc* test, Kruskal-Wallis, Wilcoxon or paired/unpaired *t*-test were used as indicated. All statistical tests were two-tailed and the alpha-level set at $P < 0.05$ to determine significance. Graphs were generated using R software[42] with ggplot2[43] and dplyr[44] packages.

Results

Contact with T cells leads to significant alterations in the transcriptional profile of oligodendrocytes

To identify molecules expressed by human oligodendrocytes that could mediate contact with Th17 cells and regulate Th17 cell-mediated damage, we performed single cell RNA sequencing of primary human oligodendrocytes in the following conditions: (i) oligodendrocytes alone (control); (ii) oligodendrocytes in co-culture with Th17-polarized cells but separated by a permeable insert (No contact); or (iii) oligodendrocytes in direct contact with Th17-polarized cells (Contact) (Fig. 1A). The sequencing of two new preparations and inclusion of the control condition added an additional 18 000 cells to our previous dataset[13] after removal of residual myeloid cells and dying cells. The datasets ($n = 3$ oligodendrocyte donors) were integrated to minimize batch effect and clustered to identify cell subtypes. Two oligodendrocyte subpopulations (OL1 = 1990 cells, OL2 = 2505 cells) expressing high levels of the canonical markers *PLP1* and *CNP* and three T cell subpopulations (T Cells 1 = 10 691 cells, T Cells 2 = 2267 cells, T Cells 3 = 3855 cells) expressing high levels of *CD3D* and *CD3E* were identified (Fig. 1B–D and Supplementary Fig. 1). While the OL1 cluster was predominantly present in the control condition, the OL2 cluster was primarily present in the 'contact' and 'no contact' conditions, suggesting a large transcriptional shift in the oligodendrocytes when exposed to Th17 cells (Fig. 1B and Supplementary Fig. 1). Indeed, the OL2 cluster across all the datasets was differentiated from OL1 by the enriched expression of stress-associated genes like *GBP1*, *IFIT2* and *IFIT3*, while the OL1 cluster had enriched expression of homeostatic genes such as *PLP1* and *NKX6-2* (Supplementary Fig. 1A). DEG analysis of the oligodendrocytes in contact versus not in contact with Th17 cells identified 141 significant DEGs (Supplementary Table 4). Gene ontology analysis of the biological processes associated with these 141 genes identified inflammation and cell death/apoptosis associated pathways as differentially regulated by direct contact with Th17 cells (Fig. 1E), in line with previously published studies.[13] In particular, changes in expression of genes associated with cell death, inflammation, oxidative stress and inhibition of translation/protein synthesis were exacerbated upon contact with Th17 cells (Supplementary Fig. 1B and Supplementary Table 4), in line with previous observations that direct contact with oligodendrocytes increases Th17-mediated oligodendrocyte damage.[7] Overall, these data demonstrate that oligodendrocytes undergo significant transcriptional changes upon interaction with Th17 cells.

Mature oligodendrocytes do not express significant levels of MHC-II on their surface

To infer major signalling pathways implicated in human oligodendrocyte–Th17 cell interactions in neuroinflammatory conditions, we used CellChat on scRNAseq data from human Th17 cells and oligodendrocytes in direct contact (Fig. 2). While we reported that Th17–oligodendrocyte prolonged interactions are not limited to antigen-specific T cells *in vivo*,[7] MHC-II was one of the membrane-bound molecules identified in our unbiased analysis of cell–cell contact outgoing communication patterns (Fig. 2A). CellChat chord diagram analysis of the MHC-II signalling pathway in our human scRNAseq dataset, however, showed a significant interaction only between T cells, not with oligodendrocytes, implicating MHC-II in T cell–T cell interaction but not T cell–oligodendrocyte interactions (Fig. 2B). We nevertheless set to assess whether MHC-II on oligodendrocytes could contribute to oligodendrocyte–Th17 cell interactions. In line with previous RNA sequencing studies reporting expression of MHC-II-related gene transcripts by oligodendrocytes in EAE and MS,[18-22] RNAscope *in situ* hybridization on spinal cords from EAE mice showed that some oligodendroglial cells, recognized by their expression of *Olig1* transcript, co-expressed the MHC-II-related *H2-Ab1* transcript (15.5%). The majority showed a low level of expression (66.8% of *OLIG1*+ *H2-Ab1* + mRNA + cells with 1–3 dots) (Supplementary Fig. 2A and B). We then investigated MHC-II expression at the protein level on mature oligodendrocytes (NogoA +cells) in EAE tissue (cerebellum and spinal cords) (Supplementary Fig. 2C and D). We did not find any significant expression of MHC-II on mature oligodendrocytes at presymptomatic, onset or peak time point (<1% signal overlap). Quantification specifically in EAE infiltrates yielded a small (2%) NogoA–MHC-II signal overlap that was largely secondary to areas of oligodendrocytes in contact with cells expressing MHC-II, such as $CD4^+$ cells (Supplementary Fig. 2C). In addition, on the contrary to infiltrating CD4 T cells, we could not detect significant levels of MHC-II on the surface of oligodendrocytes ($O4^+$ cells) by flow cytometry in the CNS of EAE mice (Supplementary Fig. 2F). As shown in Supplementary Fig. 2 and as previously published,[7] while HLA-DRA was upregulated by oligodendrocytes in contact condition (Supplementary Table 4), we similarly did not observe expression of MHC-II-related molecule HLA-DR by flow cytometry on resting or inflamed $O4^+$ primary human oligodendrocytes *in vitro*. Based on these data, we concluded that MHC-II was not likely playing a major role in contact between oligodendrocytes and Th17 cells in neuroinflammatory conditions.

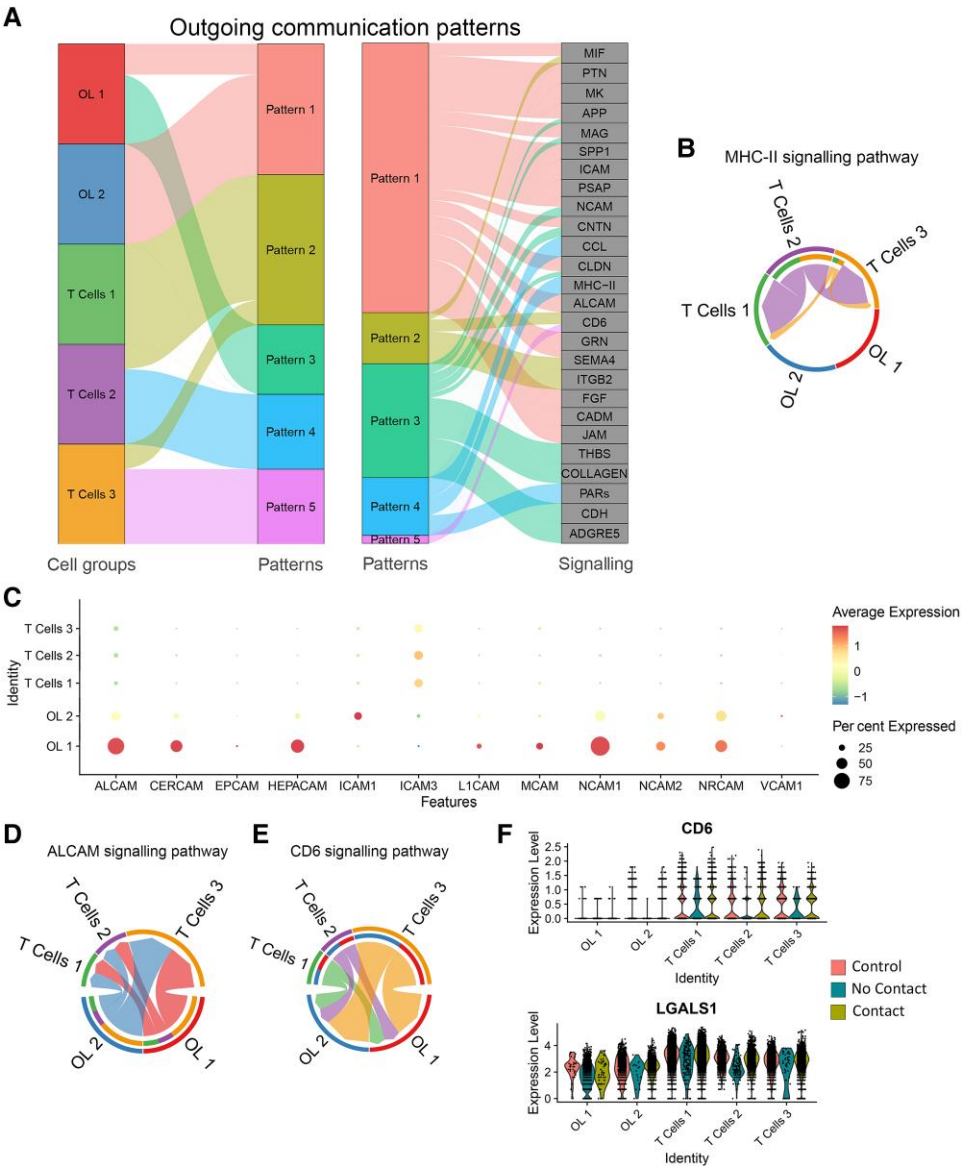

Figure 2 Cell adhesion molecules are implicated in oligodendrocyte–T cell contact. (**A**) Alluvial plot of all preps ($n = 3$) identifying significant outgoing communications between T cells and oligodendrocytes (OLs) in direct contact. The flow thickness shows the contribution of the cell group or signalling pathway to the inferred pattern, and the height of the patterns indicates the number of associated cell groups or signalling pathways. (**B**) Chord diagram of MHC-II signalling pathway network in the contact condition. (**C**) Dot plot of cell adhesion molecule (CAMs) expression in each cell population across conditions. (**D** and **E**) Chord diagram of (**D**) ALCAM and (**E**) CD6 signalling pathway networks in the contact condition. The inner bar colours of the chord diagram represent the targets that receive signal from the corresponding outer bar. The inner bar size is proportional to the signal strength received by the targets. (**F**) Violin plots of expression of CD6 and LGALS1, the corresponding ligands of ALCAM and MCAM, in the different cell subpopulations.

ALCAM and MCAM are expressed on oligodendrocytes in multiple sclerosis

Based on our intercellular communication analysis (Fig. 2A), we next investigated expression of CAMs by oligodendrocytes as potential contributors to T cell-mediated damage. ALCAM and its cognate ligand CD6 were indeed both identified as top candidates for membrane-bound molecules mediating cell–cell communication in the contact condition (Fig. 2A). Importantly, different CAMs strongly detected in the human primary oligodendrocytes from our dataset (Fig. 2C) and in particular ALCAM, were also found in oligodendrocytes from published single nuclei RNA sequencing[19,46] of control human white matter post-mortem tissue (Supplementary Fig. 3A and B). CellChat chord diagram analysis of the ALCAM signalling pathway suggested a significant outward interaction with T cells through ALCAM expressed on oligodendrocytes in our human scRNAseq dataset, while the reverse was observed for its ligand CD6 expressed on T cells (Fig. 2D and E). ALCAM and MCAM both mediate T-cell adhesion on endothelial cells in neuroinflammation[11,26,35] and CNS-infiltrating CD4 T cells expressing their cognate ligand are found in MS tissues.[29] We focused on these two CAMs based on higher gene expression in oligodendrocytes relative to T cells (Fig. 2C) and expression of cognate ligands on T cells in our human scRNAseq dataset (Fig. 2F). Analysis of snRNAseq data from Jakel *et al.*[19] showed strong

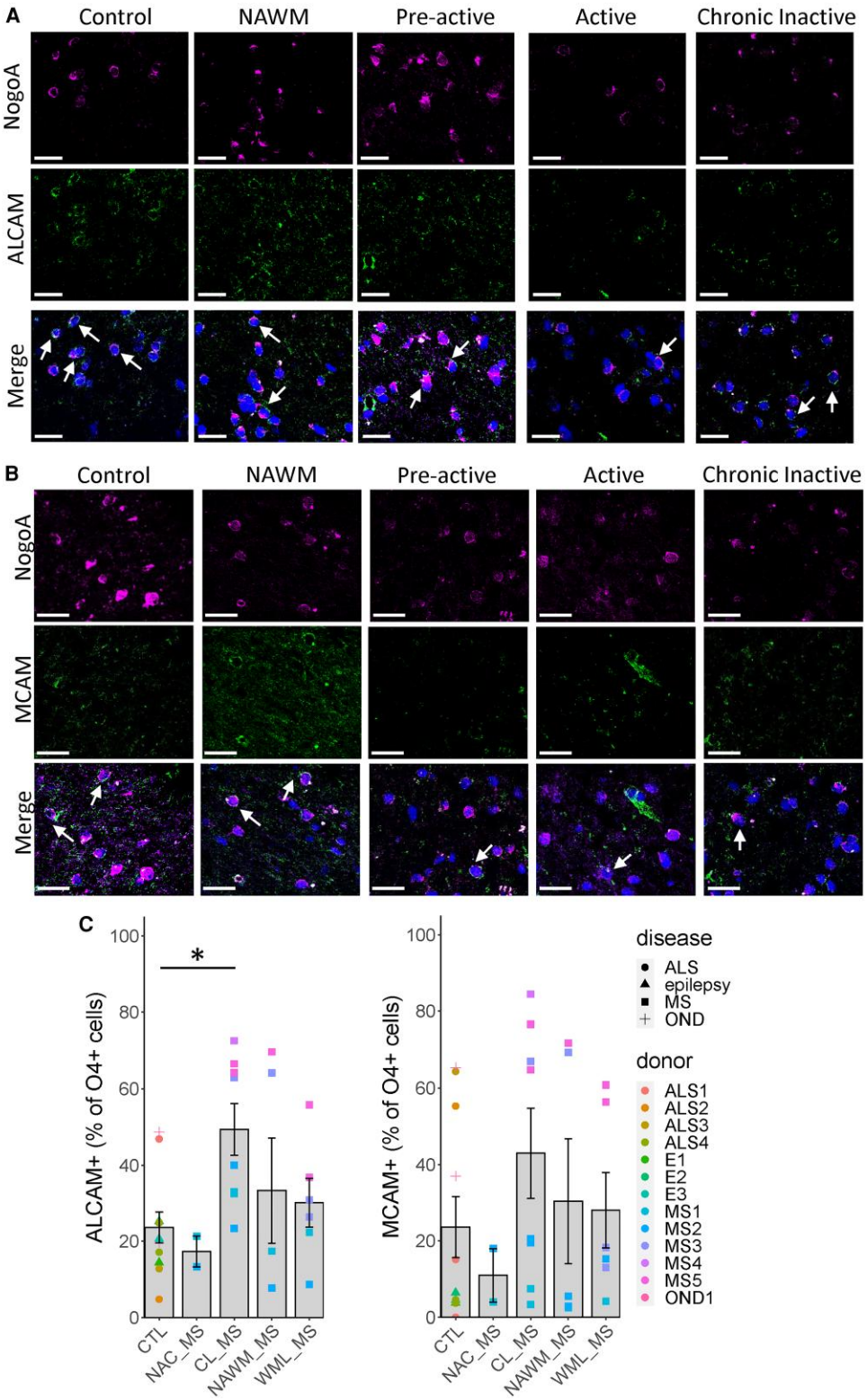

Figure 3 ALCAM and MCAM are expressed by oligodendrocytes in MS. (**A** and **B**) Representative confocal images of (**A**) ALCAM or (**B**) MCAM staining on CNS post-mortem tissue from controls with epilepsy (temporal lobe, white matter) and multiple sclerosis (MS) patient normal-appearing white matter (NAWM), pre-active, active and chronic inactive lesions (frontal lobe, white matter). Oligodendrocytes (OLs) are stained in magenta (NogoA) and nuclei in blue (DAPI). Scale bar = 25 µm, representative of $n = 3$ MS and two control donors. Arrows point to examples of oligodendrocytes positive for ALCAM or MCAM. (**C**) Proportion of $O4^+$ OL expressing ALCAM and MCAM on their surface by flow cytometry analysis of *ex vivo* tissue of MS patients in normal-appearing cortex (NAC), cortical lesion (CL), NAWM or white matter lesion (WML) tissue. Control (CTL) tissue corresponds to non-inflammatory neurological disease: epilepsy, hereditary spastic paraplegia and amyotrophic lateral sclerosis (ALS). Each dot represents one area of tissue, bar plots representing mean ± standard error of the mean (SEM). Kruskal-Wallis test, $n = 5$ MS donors, $n = 4$ ALS donors, $n = 3$ epilepsy donors, $n = 1$ other neurological disease (OND) donor. One-way ANOVA, Tukey's multiple comparison's test, $^*P \leq 0.05$.

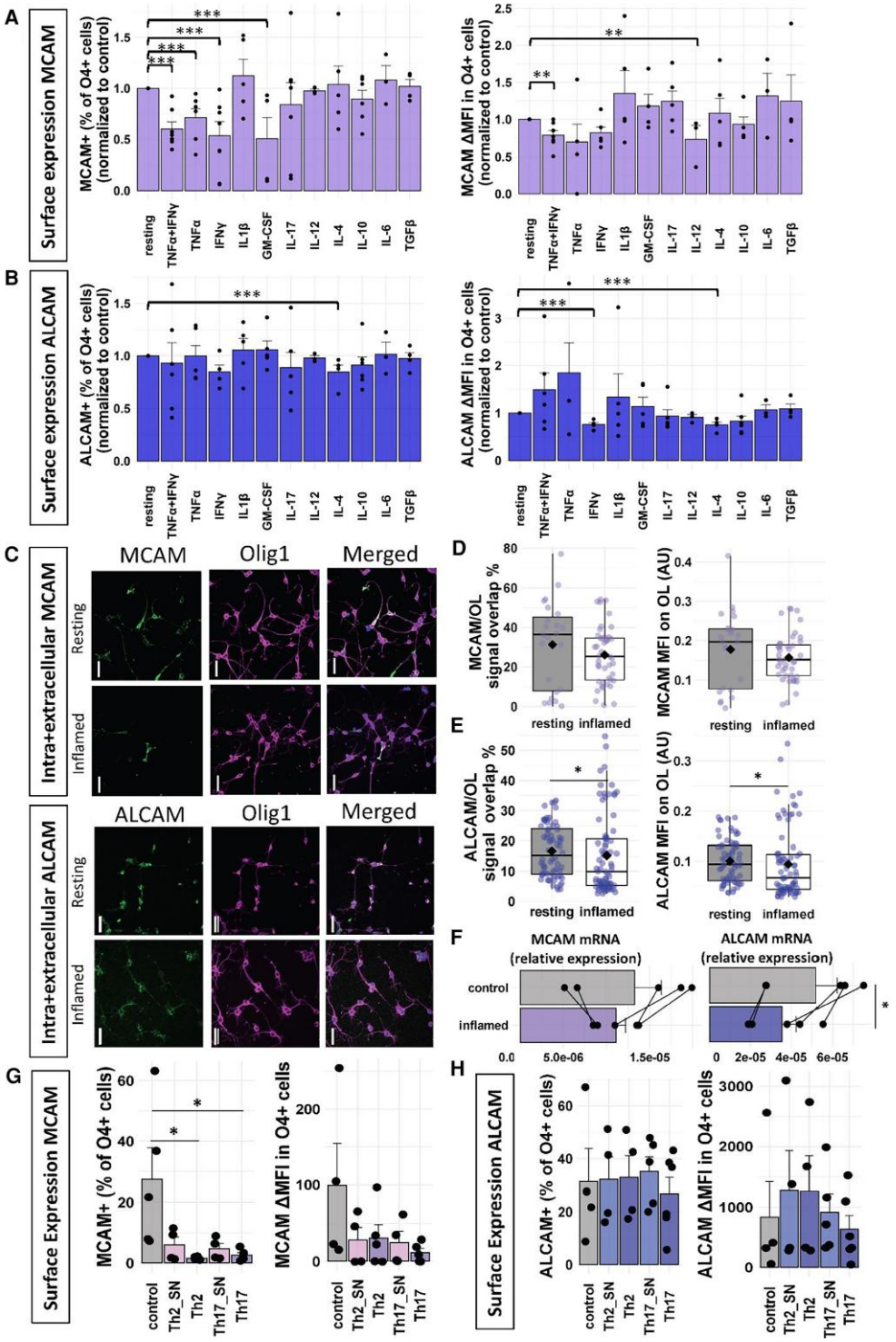

Figure 4 ALCAM and MCAM are expressed by human primary oligodendrocytes in resting and inflamed conditions. (**A** and **B**) Proportion and delta median fluorescence (ΔMFI) for surface expression by oligodendrocytes (OLs) of (**A**) MCAM or (**B**) ALCAM after exposure to indicated cytokines for 16 h (flow cytometry analysis). Each dot represents one prep (one human donor), bar plots representing mean ± SEM, Kruskal-Wallis test. (**C**) Representative confocal images of ALCAM or MCAM (green) expression by human primary OL (Olig1, magenta) in resting condition or after activation with TNFα/IFNγ for 16 h. Staining performed after permeabilization. Nuclei (DAPI) = blue. Scale bar = 50 µm. (**D**) Quantification of ALCAM or MCAM staining overlap with oligodendrocyte staining (Olig1, CNPase or NogoA) (**E**) and mean fluorescence intensity (MFI) of ALCAM or MCAM on oligodendrocyte. Box plots representing data spreading (box for the interquartile interval, horizontal bar for median, diamond for mean), each dot represents one field of view, Wilcoxon test, $n = 3$ preps. (**F**) Relative expression of ALCAM or MCAM mRNA by human primary oligodendrocyte in resting condition or after activation with TNFα. Relative mRNA level was quantified based on 18S RNA expression using RT-qPCR. Each dot represents one experiment, bar plots representing mean ± SEM, paired *t*-test, $n = 3$ preps. (**G** and **H**) Percentage and delta MFI quantification of (**G**) MCAM and (**H**) ALCAM expression by human oligodendrocytes in primary culture as assessed by flow cytometry after coculture with human Th2- or Th17-polarized CD4 T cells for 36 h. Each dot represents one donor, bar plots representing mean ± SEM. One-way ANOVA with Tukey's *post hoc* test (MCAM analyses and ALCAM percentage) or Kruskal-Wallis test (ALCAM MFI), $n = 3$ preps. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$.

expression of ALCAM and some detectable expression of MCAM through the oligodendrocyte lineage in control white matter and across all MS lesion types (Supplementary Fig. 3C). To determine whether ALCAM and/or MCAM could contribute to oligodendrocyte–Th17 cell contact, we first assessed the expression of MCAM and ALCAM on the surface of oligodendrocytes in MS and EAE. At the protein level, we found that some NogoA+ cells (mature oligodendrocytes) express MCAM and numerous express ALCAM in controls and in the normal-appearing white matter (NAWM), pre-active, active and chronic inactive lesion post-mortem tissue from MS subjects (Fig. 3A and B). To confirm and measure the expression at the surface of oligodendrocytes, we used flow cytometry and gated on live $O4^+$ cells isolated from human non-MS control brain tissue and from MS brain tissue samples obtained within 4 h post-mortem. We observed that in some samples, 0–20% of oligodendrocytes express MCAM and 7–40% of oligodendrocytes express ALCAM, while in others >50% of oligodendrocytes express these CAMs (Fig. 3C and Supplementary Fig. 3D). We observed no significant difference between control and MS brains except for a higher proportion of ALCAM+ oligodendrocytes in infiltrated cortical grey matter from MS subjects.

To determine if ALCAM and MCAM are similarly found in murine inflamed CNS tissue, we used the MOG-induced active EAE, a common animal model for MS. In line with the analysis of CAMs in published scRNAseq dataset (Supplementary Fig. 4A),[56] in the active EAE mouse model, we found that MCAM transcripts were expressed by oligodendroglial cells, identified as *OLIG1*+ cells, in 69.4% of cells, but *ALCAM* transcripts were found in only 18.5% of *OLIG1*+ cells (Supplementary Fig. 4C and D). Assessing expression of MCAM and ALCAM at the surface of mature oligodendrocytes in EAE (brain and spinal cord) by flow cytometry, we found that 15–30% of $O4^+$ oligodendrocytes express MCAM and 10–25% express ALCAM in control and EAE conditions. Whereas the frequency of oligodendrocytes expressing MCAM was relatively similar between controls and EAE mice, the mean fluorescence intensity (MFI) for ALCAM and the percentage of overlap with NogoA was lower for ALCAM after immunization, especially at peak of disease in infiltrated CNS areas (Supplementary Fig. 4E–G), with a majority of ALCAM+ cells not identified as NogoA+. In line with this, no significant interactions between T cells and oligodendrocytes was found for the ALCAM pathway in published EAE scRNAseq (Supplementary Fig. 4A and B). Our data therefore show that both human and murine mature oligodendrocytes can express MCAM and ALCAM on their surface, but that their relative expression and regulation in neuroinflammation compared to controls is different in humans with MS, where ALCAM is upregulated or similar in MS tissue, versus in EAE where ALCAM is downregulated.

Expression of ALCAM and MCAM on human primary oligodendrocytes is modulated by inflammation

To gain insight on the regulation of the protein expression of MCAM and ALCAM on the surface of human oligodendrocytes, we exposed oligodendrocytes in primary culture to various cytokines for which oligodendrocytes express the receptors.[47-49] Expression of MCAM specifically on the surface of mature oligodendrocytes (no permeabilization) was lower upon exposure to pro-inflammatory cytokines TNFα, IFNγ, TNFα+IFNγ and GM-CSF (Fig. 4A). However, this reduction was not found when MCAM was measured on inflamed oligodendrocytes by immunofluorescence after a permeabilization step (Fig. 4C and D), suggesting the presence of MCAM intracellular pools, as previously described in human tissue and cells.[50,51] *MCAM* mRNA levels, as measured by RT-qPCR, were not significantly changed in inflamed oligodendrocytes (Fig. 4F), suggesting post-translational regulation of surface expression. On the contrary, surface expression of ALCAM on human primary oligodendrocytes was mildly reduced only upon exposure to IL-4 (Fig. 4A and B) but inflammation of human primary oligodendrocytes led to a reduction of ALCAM expression as measured by immunofluorescence after permeabilization (Fig. 4C–E) and by RT-qPCR (Fig. 4F).

CNS-infiltrating CD4 T cells can secrete high levels of multiple cytokines and we have previously shown that contact of Th17 cells with oligodendrocytes is associated with an upregulation of Th17/1 pro-inflammatory cytokines.[13] To understand how contact with activated CD4 T cells could affect the expression of MCAM and ALCAM by oligodendrocytes in MS, we characterized their expression on mature primary human oligodendrocytes after 36 h of co-culture with Th2- and Th17-polarized cells. We found that, similar to the effects of pro-inflammatory Th17/1 cytokines TNFα, IFNγ and GM-CSF, activated T cells and to a lower extent their supernatants significantly reduced the expression of MCAM but not ALCAM at the surface of oligodendrocytes (Fig. 4G and H). Of note, while Th2-polarized cells expressed lower levels of pro-inflammatory cytokines and showed lower toxicity towards oligodendrocytes (Supplementary Fig. 5),[7] the activation of human memory CD4 T cells in the presence of IL-4 rather than IL-23 led to the expression and secretion of a combination of cytokines such as TNFα and IFNγ.[11] When expression of CAMs was measured by immunofluorescence after permeabilization of human primary oligodendrocytes, as was the case for exposure to cytokines TNFα and IFNγ, no reduction was observed in the expression of MCAM, while a small decrease was observed in the ALCAM levels following co-culture with Th2- or Th17-polarized cells. MCAM and ALCAM levels were slightly increased by exposure to Th17 supernatants (Supplementary Fig. 5D–F), suggesting that a combination of soluble factors secreted by Th17 cells increased the MCAM and ALCAM intracellular pool, as seen in the tumoral environment.[52] Finally, although as we previously reported[7] T cells from MS subjects induced greater oligodendrocyte damage, we observed similar trends when comparing the impact of activated CD4 T cells from MS and age- and sex-matched controls on the surface expression of MCAM and ALCAM by remaining oligodendrocytes (Supplementary Fig. 6A–C). Taken together, these data suggest that MCAM and ALCAM expression by oligodendrocytes is regulated at different post-translational levels, and that oligodendrocytes can significantly downregulate their surface expression of MCAM but not ALCAM in inflammatory conditions.

Silencing or blocking ALCAM limits interactions between oligodendrocytes and Th17 cells and subsequent oligodendrocyte injury

As MCAM and ALCAM are expressed at the surface of oligodendrocytes in control and MS brains and are modulated in inflammatory conditions and upon exposure to activated CD4 T cells, we next explored their potential role in the deleterious crosstalk between Th17 cells and oligodendrocytes.[7] As a proof-of-concept, we first used siRNA to silence MCAM or ALCAM expression in the human oligodendrocytic cell line MO3.13. We confirmed that MCAM siRNA and ALCAM siRNA specifically and sustainably reduce the surface expression of the targeted CAM on MO3.13 cells compared to the control siRNA for the whole duration of the co-culture with human Th17-polarized cells (up to 16 h) (Fig. 5A and B). We then performed live imaging as previously done[13] during the first 2 h of

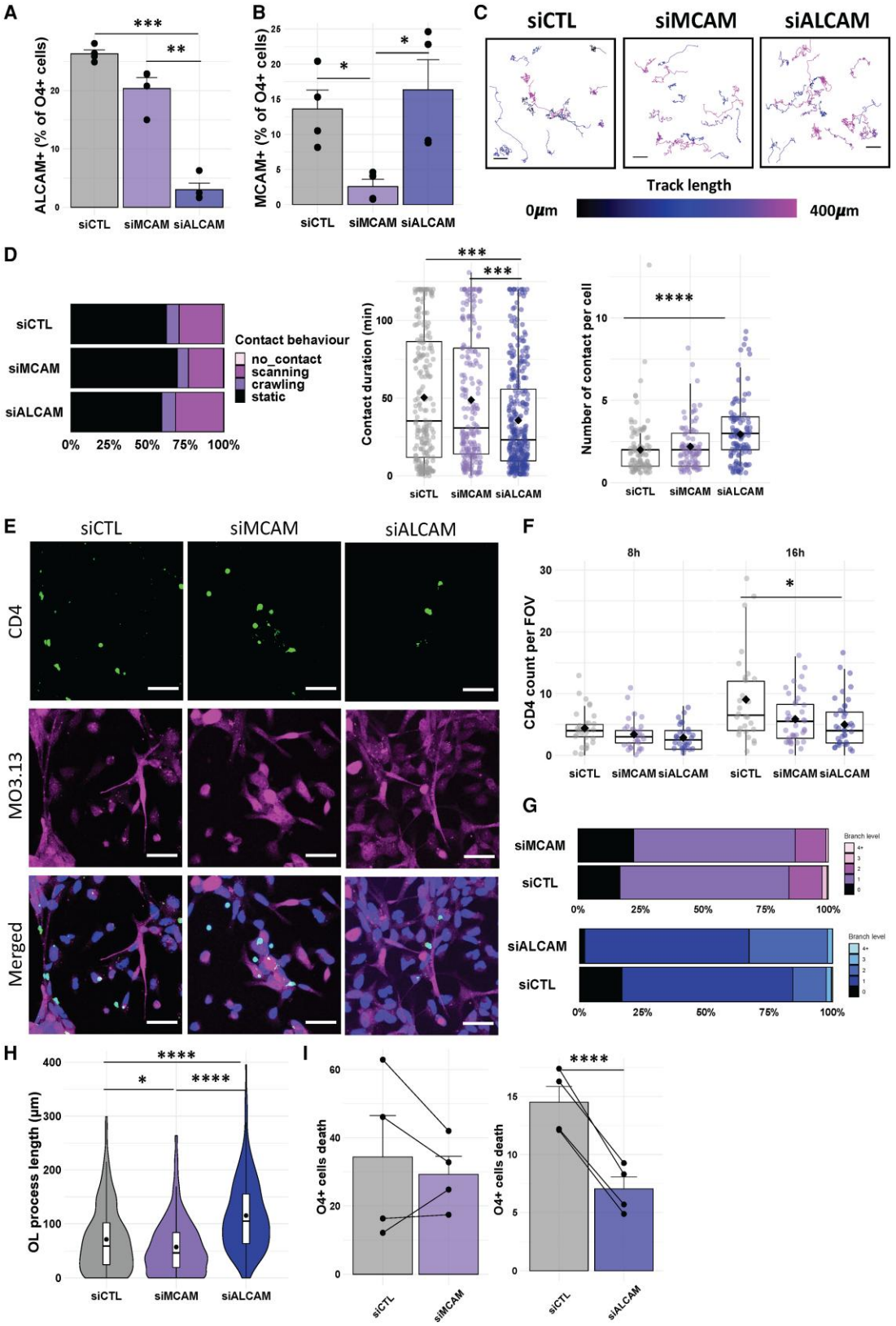

Figure 5 ALCAM silencing in oligodendrocytic cell line MO3.13 reduces damage induced by Th17-polarized cells. (**A**) ALCAM and MCAM percentage and (**B**) Δ median fluorescent intensity (MFI) on $O4^+$ cells from transfected MO3.13 cells with control (siCTL), ALCAM (siALCAM) or MCAM (siMCAM) silencing RNA in co-culture with polarized Th17 cells. Quantification from fluorescence activated single cell sorting (FACS) acquisition, each dot represents one experiment, bar plots representing mean ± SEM, Wilcoxon test for ALCAM and paired *t*-test for MCAM quantifications. (**C**) Representative tracking of Th17-polarized cells in co-culture with transfected MO3.13 cells over 2 h. Track length is colour-coded. Scale bar = 40 µm. (**D**) Live imaging quantification of contact behaviour (proportion of each type of contact), contact duration and number of contact per cell. Box plots representing data

(Continued)

co-culture to evaluate the contribution of oligodendrocytic MCAM and ALCAM expression to the interaction of Th17-polarized cells with MO3.13 cells. (Fig. 5C). Silencing of MCAM or ALCAM on oligodendrocytic cells did not significantly alter the type of contact made with oligodendrocytes by Th17-polarized cells (contact behaviour). In addition, silencing MCAM did not have a significant impact on contact duration. On the contrary, ALCAM silencing in MO3.13 cells reduced interactions between polarized Th17 cells and the oligodendrocytic cells, as reflected by a significantly shorter duration of contacts compared to control. This was paralleled by a higher number of different (shorter) contacts per cell (Fig. 5D). To assess the impact on firm adhesion of Th17-polarized cells to MO3.13 cells, we performed immunofluorescence after 8 and 16 h of co-culture (Fig. 5E). As expected, the number of adhering cells was higher after 16 h of co-culture compared to 8 h for the control siRNA condition. In line with the shorter duration of interactions, silencing ALCAM on MO3.13 was associated with a significant reduction in the number of Th17-polarized cells adhering to MO3.13 cells compared to controls, whereas a non-significant trend was observed for MCAM siRNA ($P = 0.111$) (Fig. 5F). To determine if limiting interactions of Th17-polarized cells with MO3.13 cells through downregulation of CAM surface expression can promote the survival and myelinating function of oligodendrocytes, we quantified MO3.13 branch level and length of processes, as previously reported.[7] We observed that silencing ALCAM, but not MCAM, prevents the degradation of MO3.13 processes by human Th17-polarized cells (Fig. 5G and H). Moreover, reduced expression of ALCAM but not MCAM on the surface of MO3.13 cells increased their survival after 16 h of co-culture with Th17-polarized cells (Fig. 5I).

Finally, to show that targeting CAM expression on human oligodendrocytes can represent a potential therapeutic avenue in human neuroinflammatory demyelinating diseases, we used well characterized blocking antibodies for MCAM and ALCAM to investigate their impact on the deleterious interaction of Th17-polarized cells with human primary oligodendrocytes.[7] Live imaging during the first 2 h of co-culture revealed that neutralizing MCAM did not significantly affect the contact behaviour, number of contacts per polarized Th17 cell or duration of contact (Fig. 6A and B). In contrast, neutralizing ALCAM reduced static contact proportion and resulted in significantly shorter duration of contact between Th17-polarized cells and oligodendrocytes, in line with our siRNA data (Fig. 6A and B). In addition, we found that blockade of ALCAM, but not MCAM, reduces the adherence of human Th17-polarized cells to human primary oligodendrocytes after 8 h (Fig. 6C and D). Of note, the smaller differences observed between anti-ALCAM and isotype antibodies in adhering Th17 cells at 16 h could be explained by the reduction of the available oligodendrocyte area, most pronounced in isotype condition, due to Th17-mediated oligodendrocyte damage. Importantly, blocking ALCAM, but not MCAM, is indeed associated with a significant reduction of oligodendrocyte damage compared to its isotype, as outlined by a preservation of oligodendrocyte processes complexity and length (Fig. 6E and F). Though we observe much lower expression of ALCAM on Th17-polarized cells compared to oligodendrocytes (Supplementary Fig. 7A and B), to confirm that the effects of the neutralizing antibodies on process length are due to blocking ALCAM expressed on oligodendrocytes, we co-cultured human primary oligodendrocytes with FACS-sorted $ALCAM^{low}$ or $MCAM^{low}$ Th17-polarized cells in the presence of the ALCAM or MCAM neutralizing antibody, respectively. Indeed, neutralizing ALCAM resulted in preserved oligodendrocyte process length at 16 h of co-culture, while neutralizing MCAM showed no effect (Supplementary Fig. 7C and D). These results suggest that limiting interactions between oligodendrocytes and Th17 cells through CAM blockade on oligodendrocytes could represent a therapeutic avenue for protection of oligodendrocytes from neuroinflammatory processes such as seen in MS and EAE.

Discussion

Immune-mediated myelin and oligodendrocytes injury are pathological hallmarks in MS and EAE.[1] The loss of myelination and metabolic support from mature oligodendrocytes disrupts axonal transmission and leads to pathological neuronal alterations, translating into neurological disability.[53] Interactions between CAMs expressed by cells of the oligodendrocytic lineage and complementary ligands expressed by neurons regulate myelin formation.[54,55,57-59] Although the physiological role of ALCAM in oligodendrocytes is not known, ALCAM expression by neurons suggests that homophilic interactions between $ALCAM^+$ neurons and $ALCAM^+$ oligodendrocytes could be implicated in myelin-axon interactions during development.[60,61] RNA sequencing studies have reported expression of transcripts for different CAMs in human oligodendrocytes cells, especially on fetal material and in oligodendrocyte progenitor cells (OPCs).[62] OPCs can differentiate into myelinating oligodendrocytes in physiological conditions and can generate new remyelinating oligodendrocytes in pathological demyelinating conditions.[48,63-65] However, the capacity of OPCs to efficiently remyelinate axons in MS is often limited, especially in chronic disease.[66-68] Mature oligodendrocytes are more resistant than OPCs to metabolic and inflammatory stressors[3,48,69,70] and can retract their processes to promote their survival over luxury myelination function in adverse conditions.[3,7,70] Importantly, mature oligodendrocytes are not only crucial to maintain neuronal homeostasis but can partake into axonal remyelination following injury if favourable conditions are restored.[3,71,72] Here we integrated single cell sequencing of an additional 18 000 cells from two individual preparations to our previously published sequencing dataset to show that mature human primary oligodendrocytes

Figure 5 Continued
spreading (box for the interquartile interval, horizontal bar for median, diamond for mean), each dot represents one contact, Kruskal-Wallis test, $n = 3$ donors, $n = 4$ movies per donor, 218 tracks for siCTL, 206 tracks for siMCAM and 335 tracks for siALCAM. (**E**) Representative confocal images from polarized Th17 cells (CellTracker green, green)—transfected MO3.13 cells (CellTracker Orange CMRA, magenta) 16 h co-cultures. Nuclei are stained by DAPI (blue). Scale bar = 50 µm. (**F**) Adherent polarized Th17 cells quantification after 16 h co-culture with transfected MO3.13 cells. Box plots representing data spreading (box for the interquartile interval, horizontal bar for median, diamond for mean), each dot represents one field of view (FOV), $n = 3$ donors for 8 h co-culture and $n = 4$ donors for 16 h co-culture, Kruskal-Wallis test. (**G**) MO3.13 cells branch level proportion and (**H**) process length after 16 h co-culture with polarized Th17 cells. Violin plot incremented with box plots representing data spreading (box for interquartile interval, horizontal bar for median, dot for mean), $n = 4$ donors, Kruskal-Wallis test. (**I**) Transfected MO3.13 cells death per cent after 16 h co-culture with polarized Th17 cells quantified by FACS ($O4^+$ $Amcyan^+$ cells %). Each dot represents one donor, bar plots representing mean ± SEM, paired *t*-test. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

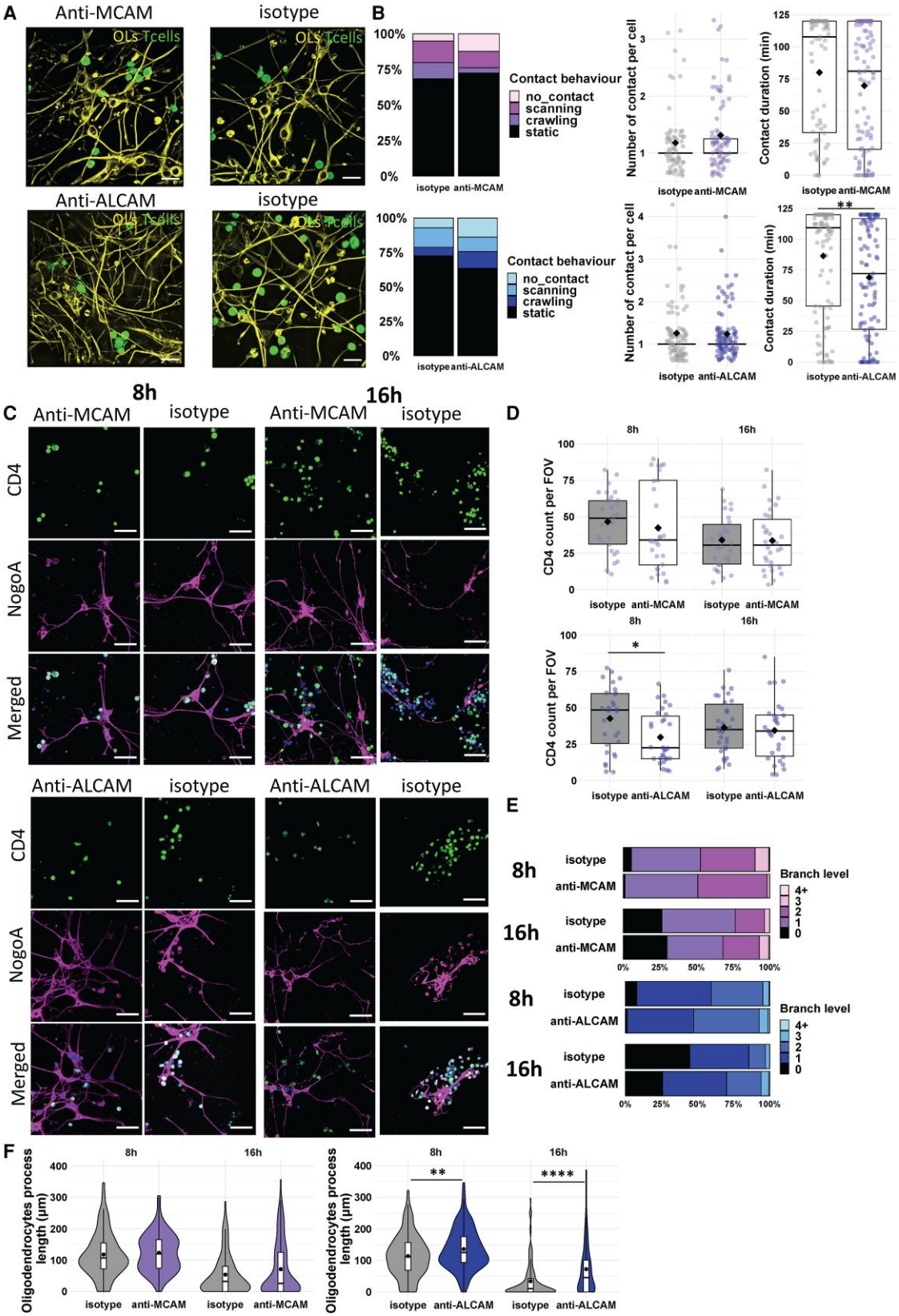

Figure 6 ALCAM blockade protects oligodendrocytes from Th17-mediated injury. (**A**) Representative tracks of Th17-polarized cell–human primary oligodendrocyte (OLs) co-culture ($n = 4$ independent experiments). Scale bar = 20 µm. (**B**) Contact behaviour (proportion of each type of contact), number of contact per cell and contact duration quantification from live imaging experiment on polarized Th17 cell–OL co-culture 2-h videos with ALCAM (30 µg/ml) or MCAM (40 µg/ml) neutralizing antibodies or isotype (IgG1, 30 µg/ml or 40 µg/ml). Box plots representing data spreading (box for the interquartile interval, horizontal bar for median, diamond for mean), each dot represents one contact. Number of tracks: anti-ALCAM = 115;

(Continued)

undergo a transcriptional shift upon direct contact with Th17-polarized cells. The gene ontology analysis identified cell death associated pathways to be differentially regulated in a contact-dependent manner, including upregulation of EIF2AK2, an inhibitor of protein synthesis implicated in myelination processes.[73,74] Importantly, this new dataset allowed identification of potential molecules implicated in intercellular communication between human oligodendrocytes and Th17-polarized cells.

Though MHC-II is implicated in intercellular crosstalk with $CD4^+$ T cell and we found that *HLA-DRA* RNA was upregulated by oligodendrocytes in the contact condition, in line with previous studies reporting MHC-II expression by a very low proportion of oligodendrocytes in mice[75] and no expression in MS lesions,[76] we show that most murine and human oligodendrocytes do not express significant amounts of MHC-II at the protein level, suggesting that other molecules are mediating this OL-Th17 cell interaction. Based on multiple sequencing datasets, we found that different CAMs were expressed by murine and human oligodendrocytes. While the role of the other CAMs expressed by oligodendrocytes remains to be explored, we report that a notable proportion of oligodendrocytes express MCAM and ALCAM on their surface in MS, EAE and control tissue. We found that while both murine and human oligodendrocytes express ALCAM on their surface, regulation in neuroinflammatory disease is different in humans and mice. Indeed, we observed that a higher proportion of human oligodendrocytes express ALCAM compared to MCAM at their surface in some of the samples from MS lesion tissue. Interestingly, we found that ALCAM expression is higher in grey matter cortical tissue with infiltration, while the highest number of reads for ALCAM was found in oligodendrocytes, and especially in oligodendrocytes from grey matter tissue, in a large dataset by Macnair *et al.*[23] On the other hand, expression of MCAM was more frequent than ALCAM on murine oligodendrocytes, and EAE was associated with lower expression of ALCAM on murine oligodendrocytes at both the transcriptional and surface protein level. Well described biological differences exist between EAE and MS, and between rodent and human oligodendrocytes,[31,77] and therefore we focused our work on human mature oligodendrocytes to identify molecular mechanisms that could mediate oligodendrocyte interactions with T cells and subsequent injury in MS. We report a downregulation of the surface expression of MCAM on mature human oligodendrocytes by inflammatory cytokines and after exposure to T cell supernatants, which contain a combination of inflammatory cytokines, as well as upon contact with activated CD4 T cells. The absence of alteration in the total (intracellular and extracellular) pool of MCAM and in mRNA levels suggest that endocytosis contributes to downregulation of surface expression, as was described in melanoma cells upon stimulation.[78] Activated CD4 T cells can express MCAM homotypic ligand[11,79,80] and heterophilic ligands such as galectin-3.[81] MCAM downregulation at the surface of oligodendrocytes could therefore represent an adaptive process to protect oligodendrocytes from immune-mediated injury, as previously described for L1CAM expression on neurons.[82] We speculate that this downregulation in inflammatory conditions could underlie the little impact of MCAM neutralization or silencing on Th17 cell–oligodendrocyte interactions.

In contrast to MCAM, we report that ALCAM remains expressed at the surface of a large proportion of human mature oligodendrocytes in inflammatory conditions. Expression of ALCAM on mature oligodendrocytes could enable interactions with immune cells bearing complementary homophilic ligand ALCAM or heterophilic ligand CD6. CD6 is highly expressed at the surface of T cells[40] especially on Th17 cells[80] and on CD4 T cells from MS subjects with active lesions on MRI,[83] and is associated with the development of MS and EAE.[84,85] Interestingly, in light of our observations that ALCAM is expressed on oligodendrocytes in MS lesions, CD6 polymorphisms have been linked to worse recovery after a relapse in MS.[86] Using live imaging, we observed that ALCAM silencing and blockade reduced adherence of Th17-polarized cells to MO3.13 and oligodendrocytes, as indicated by shorter duration of contacts and confirmed by lower numbers of Th17 cells firmly adhering to oligodendrocytes. Limiting ALCAM-mediated interactions with Th17-polarized cells was biologically significant, preserving human oligodendrocyte processes from Th17 cell attack. We have previously shown that close contact of Th17-polarized cells with oligodendrocytes causes release of glutamate and granzyme B, which are associated with oligodendrocyte injury and cell death.[7,13] Moreover, like MCAM, ALCAM is linked to the actin cytoskeleton,[87-90] can induce intracellular signalling[91] and its binding with CD6 induces optimal T-cell activation.[92-95] Therefore, ALCAM blockade could promote integrity of oligodendrocytes by different mechanisms in oligodendrocytes and on the T cell side, and such mechanisms could be dissected in future studies. A small proportion of CD4 T cells can also express ALCAM,[40] the other main (homophilic) ALCAM ligand, however, we show a similar preservation of process length upon co-culture of oligodendrocytes with FACS sorted $ALCAM^{low}$ Th17 cells in the presence of the ALCAM neutralizing antibody, supporting that blocking ALCAM on oligodendrocytes is protective. Using siRNA, we have also shown that expression of ALCAM on oligodendrocytic cells mediates adhesion of Th17 cells and is required for Th17 cells to exert their full toxicity towards MO3.13 cells. Of note, ALCAM modulation did not entirely abrogate contact with Th17 cells and its deleterious consequences on oligodendrocytes, suggesting that other molecules are implicated in Th17 cell–oligodendrocyte nefarious crosstalk. Finally, from a therapeutic perspective, since other CNS-infiltrating immune cells in MS and EAE can express ligands for MCAM and ALCAM, e.g. pathogenic B cells express ALCAM in MS,[25] future studies should assess whether these subsets also directly contact oligodendrocytes through CAMs-ligands interactions in neuroinflammatory diseases, and whether ALCAM plays a role in such interactions.

In conclusion, we have identified expression of MCAM and ALCAM at the surface of mature oligodendrocytes in the CNS of

Figure 6 Continued
isotype ALCAM = 113; anti-MCAM = 105; isotype MCAM = 79. (**C**) Representative confocal images of human primary OL (NogoA, magenta) in co-culture with human polarized Th17 cells (CellTracker green) for 8 h or 16 h in the presence of ALCAM neutralizing antibody, MCAM neutralizing antibody or isotype. Nuclei are stained in blue. Scale bar = 50 µm. (**D**) Quantification of adherent polarized Th17 cells remaining on human primary oligodendrocyte. Box plots representing data spreading (box for the interquartile interval, horizontal bar for median, diamond for mean), each dot represents one field of view (FOV), $n = 4$ preps and donors for 8 h co-culture and $n = 3$ preps and donors for 16 h co-culture, Wilcoxon test. (**E**) Quantitative analysis of branch level complexity per oligodendrocyte and (**F**) length of $n = 190$ (8 h, ALCAM isotype), $n = 165$ (8 h, anti-ALCAM), $n = 139$ (16 h, ALCAM isotype), $n = 151$ (16 h, anti-ALCAM), $n = 180$ (8 h, MCAM isotype), $n = 175$ (8 h, anti-MCAM), $n = 140$ (16 h, isotype), $n = 154$ (16, anti-MCAM) oligodendrocyte after co-culture with Th17 cells in the presence of ALCAM or MCAM neutralizing antibody or isotype. Violin plot incremented with box plots representing data spreading (box for interquartile interval, horizontal bar for median, dot for mean), Wilcoxon test, $n = 4$ preps and donors for 8 h co-culture and $n = 3$ preps and donors for 16 h co-culture. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$.

control and MS subjects. Importantly, we have uncovered that ALCAM, which is not downregulated in inflammatory conditions, mediates adhesion of human Th17 cells to human oligodendrocytes and contributes to immune-mediated oligodendrocytes damage. Our results support targeting CAM-mediated interactions of mature oligodendrocytes with immune cells as a potential neuroprotective strategy in MS.

Data availability

All data are available upon request. Single nuclei sequencing datasets from Jäkel *et al.*[19] and Absinta *et al.*,[46] and single cell sequencing datasets from Fournier *et al.*[56] were extracted from the NCBI Gene Expression Omnibus (GEO) database (accession GSE118257, GSE180759 and GSE199460 respectively). The single cell RNAsequencing datasets we generated and used for this study have been deposited in GEO (GSE230828; GSE196953; GSE180670).[45]

Acknowledgements

We thank Lyne Bourbonnière, Sandra Larouche, Dr Aurélie Cléret-Buhot, Dr Gaël Dulude, Dominique Gauchat and Philippe St-Onge, the imaging platform, and the flow cytometry platform of the CRCHUM for their assistance.

Funding

H.J. received a scholarship from the Multiple Sclerosis Society of Canada (MSSOC), V.H.M from the Fonds de recherche du Québec-Santé (FRQS) and the MSSOC, H.S. from the FRQS. C.L. holds a Junior 2 clinician-researcher award from the FRQS. This work was supported by the MSSOC (EGID 3322) and Canadian Institutes of Health Research (CIHR 162430).

Competing interests

The authors report no competing interests.

Supplementary material

Supplementary material is available at *Brain* online.

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