Mucosal-associated invariant T (MAIT) cells in the pathogenesis of central nervous system inflammation

Dissertation

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Abbreviations

5-A-RU	5-amino-6-D-ribitylaminouracil
5-OE-RU	5-(2-oxoethylideneamino)-6-D-ribitylaminouracil
5-OP-RU	5-(2-oxopropylideneamino)-6-D-ribitylaminouracil
6-FP	6-formyl pterin
α-galactosylceramide	α-Gal-Cer
AIRE	Autoimmune regulator
APCs	Antigen-presenting cells
Areg	Amphiregulin
BBB	Blood brain barrier
bp	Base pairs
BV	Brilliant violet
CCL	C-C motif chemokine ligand
CCR	C-C motif chemokine receptor
CD	Cluster of differentiation
CDR	Complementarity-determining regions
CFA	Complete Freund's adjuvant
CIS	Clinically isolated syndrome
CNS	Central nervous system
CO_2	Carbon dioxide
CSF	Cerebrospinal fluid
cTEC	Cortical thymic epithelial cells
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCL	C-X-C motif chemokine ligand
CXCR	C-X-C motif chemokine receptor
D	Diversity
DAMP	Damage-associated molecular patterns
DCs	Dendritic cells
DEG	Differentially expressed genes
DN	Double-negative
dNTP	Deoxynucleoside triphosphates
DP	Double-positive
dpi	Days post immunisation
EAE	Experimental autoimmune encephalomyelitis
EBV	Epstein-Barr virus
EDTA	Ethylenediaminetetraacetic acid
ER	Endoplasmic reticulum
FACS	Fluorescence-activated cell sorting
FCS	Fetal calf serum
FDR	False discovery rate
FMO	Fluorescence minus one
FoxP3	Forkhead-box protein P3
FSC	Forward scatter
fwd	Forward
g	Gram
GATA3	GATA binding protein 3
GF	Germ-free
GFP	Green fluorescent protein
GM-CSF	Granulocyte macrophage colony-stimulating factor
GO	Gene Ontology
GSEA	Gene set enrichment analysis
GWAS	Genome wide association studies

h	Hour
HLA	Human leukocyte antigen
i	Invariant
ICAM-1	Intercellular adhesion molecule 1
IFN-γ	Interferon γ
IgG	Immunoglobulin G
IL	Interleukin
iNKT	Invariant natural killer T
Iono	Ionomycin
i.p.	Intraperitoneal
IS	Immunological synapse
J	Joining
JCV	John Cunningham virus
KLRG1	Killer cell lectin-like receptor subfamily G member 1
l	Liter
LFA-1	Lymphocyte function-associated antigen 1
LN	Lymph nodes
m	Milli
M	Molar
MAIT	Mucosal-associated invariant T
MBP	Myelin basic protein
MEC	MAIT EAE CNS
MES	MAIT EAE Spleen
MFI	Mean fluorescent intensity
MHC	Major histocompatibility complex
min	Minute
MNS	MAIT Naïve Spleen
MOG	Myelin oligodendrocyte glycoprotein
MR1	MHC class I – related protein 1
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
MS	Multiple sclerosis
mTEC	Medullary thymic epithelial cells
n	Nano
NES	Normalised enrichment score
NGS	Next-generation sequencing
NIH	National Institutes of Health
NK	Natural killer
NOD	Non-obese diabetic
O_2	Oxygen
PAMP	Pathogen-associated molecular patterns
PBS	Phosphate buffered saline
PCA	Principal component analysis
PCR	Polymerase chain reaction
pDC	Plasmacytoid dendritic cells
PFA	Paraformaldehyde
PLP	Proteolipid protein
PLZF	Promyelocytic leukemia zinc finger
PMA	Phorbol 12-myristate 13-acetate
PML	Progressive multifocal leukoencephalopathy
PPMS	Primary progressive multiple sclerosis
PRR	Pattern recognition receptors
PSGL-1	P-selectin glycoprotein ligand 1

pTα	Pre-TCR α chain
pTreg	Peripheral regulatory T cells
R	Receptor
RA	Rheumatoid arthritis
RAG	Recombination-activating gene
rev	Reverse
RORγt	Retinoic acid receptor-related orphan receptor γt
ROS	Reactive oxygen species
rpm	Revolutions per minute
RRMS	Relapsing-remitting multiple sclerosis
RT	Room temperature
SAPE	Streptavidin-phycoerythrin
SARS-CoV-2	Severe acute respiratory syndrome-coronavirus 2
s.c.	Sub-cutaneous
SEM	Standard error of the mean
SLE	Systemic lupus erythematosus
SLO	Secondary lymphoid organs
SNP	Single nucleotide polymorphism
SP	Single-positive
SPF	Specific-pathogen free
SPL	Spleen
SPMS	Secondary progressive multiple sclerosis
SSC	Side scatter
STAT	Signalling transducer and activator of transcription
T1D	Type 1 diabetes
T-bet	T-box expressed in T cells
Tc	Cytotoxic T cells
TCM	Central-memory T cells
TCRs	T cell receptors
TEM	Effector-memory T cells
Tfh	Follicular helper T cells
TGF-β	Transforming growth factor β
Th17	T helper 17
TiReDB	Tissue repair database
TLR	Toll-like receptor
TMEV	Theiler's murine encephalomyelitis virus
TNFα	Tumour necrosis factor α
Tregs	Regulatory T cells
TRM	Tissue-resident memory T cells
tTreg	Thymic regulatory T cells
µ	Micro
UKE	University Medical Center Hamburg-Eppendorf
UV	Ultraviolet
V	Variable
VCAM-1	Vascular cell adhesion molecule 1
VEGF	Vascular endothelial growth factors
VLA-4	Very late antigen 4
ZMNH	Center for Molecular Neurobiology Hamburg

1 Introduction

Multiple sclerosis (MS) is a chronic neuroinflammatory disease of the central nervous system (CNS) hallmarked by inflammatory lesions resulting in demyelination and neuro-axonal degeneration. Thereby, neuronal signal transduction gets affected and disrupted leading to heterogenous symptoms in MS patients ranging from different motor and sensory impairments to cognitive deficiencies[1]. MS is considered to be an autoimmune disease most likely caused by peripherally activated autoreactive T cells. However, a defined trigger for developing MS or a specific T cell autoantigen remain elusive[1,2].

The differential contribution of immune cells to damage and repair of the CNS in MS pathogenesis is unresolved and a major focus of MS research. A better understanding of the immune system will help to disentangle mechanisms of MS pathogenesis. In 2005, a new effector subpopulation of T cells was discovered and termed T helper 17 (Th17) cells[3]. Whereas these proinflammatory cells play a central role in autoimmunity and the animal model of MS[4,5], their contribution to MS pathogenesis is still controversial[6].

Interestingly, another type of newly discovered immune cells are mucosal-associated invariant T (MAIT) cells, which share many functional similarities with Th17 cells and were also found in MS lesions[7]. MAIT cells are a large population of T cells in humans comprising 1-10% in the blood and up to 45% of all T cells in the liver[8,9]. However, their exact contribution to MS is still under investigation, mainly because functional characterisation of MAIT cells in the animal model of MS are lacking. Due to newly emerging tools it is possible to identify and specifically stain MAIT cells in mice only since 2016, thereby enabling to decipher their contribution to the animal model of MS that is the aim of this work.

1.1 The immune system

The immune system is a complex interaction of specialized cells and defence strategies to ensure protection against pathogens and neoplastic cells. Continuous evolution and adaptation, which occur daily as well as over thousands of years, are necessary to keep up a balance between strong and fast clearance of pathogens, detection of abnormal cell growth due to malignant transformations and maintaining self-tolerance. A disbalance can lead to severe consequences like persisting infections, cancer or autoimmunity. The immune system of vertebrates consists of physical barriers like the skin or mucus layers of the gut and respiratory tract as well as innate and adaptive effector cells[10]. The vast majority of immune cells develop from hematopoietic stem cells and are the backbone of immune responses. Hematopoietic stem cells differentiate in the bone marrow into common myeloid or common lymphoid progenitor cells. The cells of the adaptive immune system, T and B cells, originate from lymphoid progenitor cells, whereas the majority of innate immune cells, e.g. macrophages, mast cells, eosinophils, neutrophils and most of the dendritic cells (DCs) differentiate from myeloid stem cells[11].

An immune response has four main functions: recognition of pathogens and infected or neoplastic cells, attack of those and initiation of responses by other immune and surrounding tissue cells as well as restoring homeostasis by initiating tissue repair and wound healing. Recognition by the innate immune system relies on the detection of broader and evolutionarily conversed patterns. These are molecular structures expressed by a large variety of microbes called pathogen-associated

molecular patterns (PAMP). The detection of PAMP as well as damage-associated molecular patterns (DAMP) or alarmins, which are intracellular molecules released during cell stress or tissue injury, is mediated by pattern recognition receptors (PRR)[12,13]. Most cells of the innate immune system are equipped with PRR enabling rapid responses of the innate immune system. After recognition and activation, innate immune cells directly attack pathogens or infected cells via diverse strategies like phagocytosis or release of cytotoxic molecules or cytokines. Professional antigen-presenting cells (APCs) like DCs and macrophages present antigens, mainly peptides, to T cells via major histocompatibility complex (MHC) molecules and thereby activate the adaptive immune response. Activated DCs migrate to lymph nodes (LN) and active naïve T cells[14], whereas macrophages stay in the tissue and reactivate the infiltrating T cells. Next to cellular responses, also humoral components like antimicrobial peptides and the complement system are part of the innate immune system able to mark pathogens and cells for a better detection of other immune cells and to directly attack these cells[15].

In contrast, the adaptive immune system is characterised by a diverse antigen specificity. The hallmark of this diversity are antigen-specific receptors carried by B and T cells. These receptors are generated via somatic rearrangement and enable millions of different receptor combinations, each with a different antigen specificity. The majority of B and T cells express only one specific antigen receptor, although cells with two different receptors have been identified[16,17]. Therefore, the adaptive immune response is slow due to the necessity of selecting the cell with the respective matching antigen receptor and inducing their proliferation. After the first response to a specific antigen, memory cells persist and are able to respond in a much faster way to a second encounter with the same antigen. Even if the innate and adaptive immune system use different mechanisms, both rely on mutual interaction and synergy to ensure an effective immune response, while the need to activate both systems restrains undesired immune responses and protects against autoimmunity.

1.2 T cells

T cells are a diverse group of cells of the adaptive immune system carrying specific T cell receptors (TCRs) able to recognise specific antigens. Functionally, they play a central role in the immune system by killing target cells using cytotoxic molecules and by interacting with other immune cells via cytokines and direct receptor-mediated contact, thereby orchestrating the whole immune response. T cells can be classified by their TCR, co-receptors, produced cytokines and effector function. Classical T cells recognise peptides presented by MHC molecules using a diverse TCR repertoire and are subdivided into regulatory T cells (Tregs), cluster of differentiation 4^+ ($CD4^+$) T helper cells (Th) and $CD8^+$ cytotoxic T cells (Tc). After differentiation, $CD4^+$ and $CD8^+$ T cells can be further classified into Th1/Tc1, Th2/Tc2 and Th17/Tc17 cells due to their respective effector function and cytokine production[3,18]. In addition to classical T cells, the unconventional T cell subsets γδ T cells, natural killer T (NKT) cells and MAIT cells exist, which have a limited TCR repertoire and recognise non-peptide antigens[19].

1.2.1 TCR rearrangement

The specificity of T cells relies on a diverse TCR repertoire originated from a complex TCR rearrangement. Four different TCR chain loci (α, β, γ, δ) exist, which each have a variable and a

constant region. The variable region of the TCR β and δ gene loci consists of various variable (V), diversity (D) and joining (J) gene segments, whereas only V and J gene segments are present in TCR α and γ loci. TCR chains are rearranged by somatic recombination of the V, (D), and J gene segments. In this process, recombination-activating gene (RAG) 1 and RAG2 enzymes cut the DNA at recombination signal sequences adjacent to every V, D and J segment[20]. These gene segments are rejoined by DNA repair enzymes resulting in a TCR chain having only one combination of V, (D) and J segments connected to the constant region. Two TCR chains form a heterodimeric TCR and only two combinations are possible generating either an αβ or γδ TCR. To ensure a high diversity of TCRs recognising as many distinct antigens as possible, different V, (D) and J gene segments are required. The human TCR β locus consists of 42 V, 2 D and 12 J gene segments and the TCR α locus has 43 V and 58 gene segments[21,22]. In addition to these combinatorial possibilities, deletion or addition of two to three nucleotides at the end of the selected V, (D) and J gene segments results in further diversity[23]. These mechanisms and a high diversity are especially important in hypervariability regions of the V gene segment, which are involved in antigen recognition and named complementarity-determining regions (CDR). The TCR α and β chain each have three CDR and the CDR3 regions directly contact the antigen[24,25]. In summary, somatic recombination ensures that millions of different TCR combinations are possible and a diverse TCR repertoire is generated.

1.2.2 Development of T cells

Hematopoietic stem cells committed to T cell differentiation migrate from the bone marrow to the thymus for their maturation. These T cells enter the thymus at the cortico-medullar junction as $CD4^-$ $CD8^-$ double-negative (DN) cells and start to rearrange their TCR β, γ and δ gene segments while migrating to the cortex[26]. At this point, the decision if T cells become αβ or γδ T cells is made. A requirement for further development is that a functional TCR is formed, otherwise the cells initiate apoptosis. γδ T cells stay $CD4^-$ $CD8^-$ DN and require only a single TCR selection step before they leave the thymus already with effector functions[27]. During the development process more αβ T cells are generated and only 0.5-5% of the circulating T cells are γδ T cells[28]. The rearranged β chain of αβ T cells is paired and functional tested together with a pre-TCR α chain (pTα). The assembly of a functional pTα/β TCR leads to the expression of CD4 and CD8 co-receptors and rearrangement of the TCR α gene locus. The further development involves positive and negative selection processes mediated by different APC-like cortical and medullary thymic epithelial cells (cTEC and mTEC), DCs, B cells and CD4 and CD8 double-positive (DP) thymocytes[29]. Positive selection ensures that a TCR is able to interact with the antigen-presenting MHC or MHC-like molecules, whereas negative selection should prevent the survival of T cells recognising self-antigens. Therefore, the thymus is divided into the cortex, where positive selection takes place and the medulla, where negative selection and further maturation occurs[30]. Without getting a signal during positive selection as well as receiving a strong signal during negative selection result in apoptosis.

Classical αβ T cells recognise peptides presented by MHC molecules. During positive selection their TCR is tested for a weak interaction with peptide-MHC complexes presented on the surface of cTEC. During this selection process the further fate of classical αβ T cells is determined by becoming either $CD4^+$ or $CD8^+$ single-positive (SP) T cells. The exact mechanism is still incompletely

understood and different models are discussed. The instruction model suggest that differential intracellular signals are generated if the TCR and CD8 coreceptor interact with a peptide-MHC I complex or if the TCR and CD4 engage a peptide-MHC II complex. These signals lead to a downregulation of the respective other co-receptor[31]. The selection model proposes that DP T cells are already stochastically committed to a $CD8^+$ or $CD4^+$ lineage[32]. After positive selection, SP T cells migrate to the medulla and are negatively selected by self-peptide presenting mTEC as well as other APC. For this purpose, thymic B cells and mTEC express the transcription factor autoimmune regulator (AIRE), which promotes the expression and presentation of normally tissue-restricted self-antigens[33,34]. After approximately four days of negative selection, naïve T cells egress the thymus and migrate into the circulation[35].

1.2.3 T cell activation

In the steady state, in the blood and lymphoid system T cells show minimal peripheral proliferation. The lifespan of naïve human T cells is 5-10 years, whereas mouse T cells only persist for 6-10 weeks[36,37]. Activation of naïve T cells takes place in secondary lymphoid organs (SLO) like spleen, LN, Peyer's patches and mucosa-associated lymphoid tissue. Activated APCs, mainly DCs, migrate to SLO and present processed antigens to T cells in a compact environment to increase the likelihood that an APC encounters the corresponding T cell. This results in proliferation and further differentiation of T cells into long-living effector and memory cells. For this purpose, antigen binding via the TCR and co-stimulation are required for activation and surrounding cytokines mediate the differentiation into specific effector cells.

The activation process of T cells starts with the formation of a so called immunological synapse (IS) between APC and T cell stabilizing their contact area. Cell-cell junctions and reorganization and polarization of proteins and cytoskeleton takes place to form distinct supramolecular activation clusters[38,39]. Antigen recognition of T cells is mediated by binding of the TCR to a specific peptide presented via MHC molecules. Classical MHC molecules are highly polymorphic and subdivided into MHC I and MHC II. MHC I molecules are expressed by all nucleated cells and consist of a heavy α subunit and an invariant β2-microglobulin. In the endoplasmic reticulum (ER) degraded endogenous peptides with a length of 8 to 10 amino acids are loaded on MHC I molecules. Upon transport to the cell surface, theses complexes are recognised by $CD8^+$ T cells. MHC II is composed of an α and β chain and presents 8 to 20 amino acids long peptides degraded from exogenous proteins ingested by the cell. Professional APCs express MHC II and present peptides recognised by $CD4^+$ T cells[40,41]. Many other surface receptors of T cells, e.g. CD3, CD4 or CD8, cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte function-associated antigen 1 (LFA-1) and CD28, and of APCs like CD80, CD86, and intercellular adhesion molecule 1 (ICAM-1) are involved and can contribute to either inhibition or activation of T cells[42,43,44]. The TCR is noncovalently associated with CD3εγ, CD3εδ, and CD3ζζ dimers, which are together with the co-receptors CD4 and CD8 responsible for the intracellular signalling of the TCR resulting in further downstream activation and signal amplification[45,46].

TCR activation alone is not sufficient to induce a fully activated T cell and without a proper co-stimulation this can lead to a hyporesponsive and anergic state[47]. CD28 is the best characterised

costimulatory molecule, which binds to CD80 or CD86 on APC. A sustained T cell activation leads to the removal of CD28 and induction of CTLA-4, which recruits inhibitory phosphatases to the IS and downregulates co-stimulatory molecules on APC via transendocytosis[48,49].

1.2.4 Differentiation of T cells

Upon activation, T cells undergo differentiation into distinct effector T cells mainly induced by the cytokine milieu in SLO before they participate in immune responses and acquire a memory phenotype.

$CD4^+$ T helper cells:

The major three subclasses of $CD4^+$ Th cells are Th1, Th2 and Th17. Nevertheless, also other Th effector cells like Th9, Th22, Treg and follicular helper T cells (Tfh) exist[50]. In 1986, T cells were classified for the first time by their secretion of signature cytokines into Th1 and Th2 cells[18]. The differentiation into Th1 cells is initiated by stimulation with interleukin-12 (IL-12) and leads to the activation of signalling transducer and activator of transcription 4 (STAT4)[51,52]. Thereby, the master transcription factor of Th1 differentiation T-box expressed in T cells (T-bet) is induced resulting in expression of Th1-associated genes and repression of other Th determining fate genes[53]. Th1 cells are marked by expressing interferon γ (IFN-γ), IL-2 and tumour necrosis factor α (TNFα) and are important for antiviral and antibacterial immunity[18,50]. In contrast, Th2 fate is mainly induced through IL-4 resulting in activation of STAT6 and STAT5 and initiation of the master transcription factor GATA binding protein 3 (GATA3)[54,55]. GATA3 and T-bet have opposing roles and can directly repress the other to ensure that only one differentiation program is initiated[56,57]. Th2 cells are defined by secretion of signature cytokines IL-4, IL-5, IL-9 and IL-13 and take part in clearing extracellular pathogens and stimulate repair of damaged tissue[58]. The third main subtype of Th cells was discovered in 2005 and termed Th17 cells[3]. Differentiation into Th17 cells from naïve T cells is promoted by IL-1β, IL-6, IL-21 and transforming growth factor β (TGF-β) resulting in an intracellular downstream signalling mediated by STAT3[4,59,60,61] inducing the master transcription factor retinoic acid receptor-related orphan receptor γt (RORγt). The signature cytokines of Th17 cells are IL-17A, IL-17F and IL-22 and they are critical for overcoming fungal and bacterial infections[62]. Furthermore, Th17 cells are enriched in the gut and control and regulate gut microbiota[63], but are also involved in the pathogenesis of different autoimmune disease[64]. The presence of IL-6 during differentiation of Th17 cells results in expression of the IL-23 receptor (IL-23R)[65], which is not expressed on naïve T cells[66]. IL-23 is a proinflammatory cytokine composed of the subunits IL-23p19 and IL-12p40 and a key driver of pathology in different autoimmune diseases[66,67,68,69]. Induction with IL-23 modulates the effector profile of Th17 cells leading to pathogenic Th17 cells and upregulation of T-bet. $ROR\gamma t^+$ $T\text{-}bet^+$ Th17 cells are able to produce IL-17A, IFN-γ and granulocyte macrophage colony-stimulating factor (GM-CSF) in mice[64,70]. These cells mainly originate from Th17 and not from Th1 cells shown by experiments using IL-17A fate mapping mice[71]. In contrast to mice, human $ROR\gamma t^+$ $T\text{-}bet^+$ Th17 cells primarily produce IL-17A and IFN-γ[72], while human Th1 cells instead of Th17 cells are the main producers of GM-CSF[73]. Pathogenic Th17 cells contribute to many autoimmune and inflammatory diseases[5,74,75].

Regulatory T cells (Tregs):

The majority of Tregs is positive for $CD4^+$ and expresses the transcription factor forkhead-box protein P3 (FoxP3) and CD25, whereas also $CD8^+$ Tregs exist[76,77,78]. Their main function is to suppress immune responses and to maintain homeostasis and self-tolerance[79]. Tregs are separated due to their ontogeny into peripheral (pTreg) and thymic Tregs (tTreg). tTregs are generated during the negative selection process in the thymus by an intermediate TCR-peptide interaction[80,81]. In contrast, pTreg develop outside the thymus and differentiate from classical naïve T cells after TCR-dependent activation in combination with TGF-β[82]. Furthermore, also a transdifferentiation from Th17 cells into Tregs is possible[83].

$CD8^+$ cytotoxic T cells:

$CD8^+$ T cells can be divided in Tc1, Tc2, Tc9, Tc17 and Treg cells. The best characterised $CD8^+$ T cell subpopulation are Tc1 cells, which require IL-2 and IL-12 for differentiation, resulting in expression of T-bet and enabling secretion of IFN-γ and TNFα[84,85]. $CD8^+$ T cell activation can be supported by $CD4^+$ T cells increasing $CD8^+$ memory T cell formation and responses[86,87]. $CD4^+$ T cells help by secreting cytokines like IL-2 or interacting directly with $CD8^+$ T cells via CD40/CD40L or indirectly via APC, which then activate $CD8^+$ T cells[87]. After activation and differentiation, $CD8^+$ T cells circulate and migrate into inflamed tissues. Activated $CD8^+$ T cells are important for an immune defence against intracellular pathogens, including bacteria and viruses, and for tumour surveillance. These functions are mainly fulfilled by direct killing of target cells via perforin and granzyme B[88].

Natural killer T cells:

NKT cells belong to the class of unconventional T cells that recognise lipid antigens. During development, NKT cells leave the thymus in waves and home to different barrier tissues like skin, intestine and lung, where they become long-lived tissue-resident cells[89]. Nevertheless, they are also present in the blood and other tissues. NKT cells are innate-like and CD1d-restrited αβ T cells[90]. The major and best known subgroup are invariant NKT cells (iNK T cells), which use all the same TCR α chain, Vα24Jα18 in humans and Vα14Jα18 in mice, combined with a limited TCR β chain repertoire[91]. iNKT cells get activated after recognising different lipids. The most and best studied glycolipid that activates iNKT cells is α-galactosylceramide (α-Gal-Cer), which is also used to identify iNKT cells by using CD1d tetramers loaded with α-Gal-Cer[92,93]. A TCR-dependent activation of iNKT cells leads to a rapid immune response reflected by a cytokine burst within two hours after *in vivo* stimulation[92]. A classification in iNKT1, iNKT2 and iNKT17 cells is made based on their effector function. Thus, iNKT1 cells express the transcription factor T-bet and produce IFN-γ, whereas iNKT2 cells have the highest levels of the transcription factor promyelocytic leukemia zinc finger (PLZF) and IL-4. Lastly, NKT17 cells express RORγt and secrete IL-17A[94]. iNKT cells contribute to protective immune responses in autoimmune and inflammatory conditions as well as against pathogens[95]. Furthermore, iNKT play a role in natural immunity against tumours[96].

1.2.5 Resolution of T cell activation and repair of inflammation

TCR-dependent activation induces massive proliferation of T cells and expression of proteins inducing an effector and/or memory phenotype. Activated T cells leave the SLO, migrate to the site of

inflammation and fulfil their respective effector function after receiving a local TCR-dependent reactivation. After clearance of an infection, the majority of these T cells undergo apoptosis and only a small pool of long-living memory T cells persists to ensure a rapid immune response after reinfection involving the same antigens. The mechanisms how and which T cells persist as memory T cells are still under debate and reflected by different models[97,98]. In mice, memory T cells are mainly classified as central-memory T (TCM) cells expressing low levels of killer cell lectin-like receptor subfamily G member 1 (KLRG1^{low}), CD44^{high}, CD127^{high} and CD62L^{high} and effector-memory T (TEM) cells specified as KLRG1^{high}, CD44^{high}, CD127^{low} and CD62L^{low}. The main homing site of TCM cells are SLO, whereas TEM cells are mostly present in non-lymphoid tissues. In addition, tissue-resident memory T (TRM) cells are defined by CD69^{high}, CD44^{high}, CD62L^{low}, CD103^{high} as well as other markers and permanently reside in a respective tissue[99,100].

Furthermore, different T cell subsets participate in resolution of inflammation and tissue repair, whereby Tregs are the most studied subset[101]. Tregs can dampen immune responses, secrete pro-repair mediators like the epidermal growth factor receptor ligand Amphiregulin (AREG)[102,103] and modulate stromal and stem cells to enhance tissue repair[101].

In general, tissue repair and wound healing are a highly orchestrated overlapping events mediated by immune cells, fibroblasts, endothelial cells and stem cells able to prevent infection, to repair damage and to restore tissue function[104,105]. After the inflammatory phase characterised by activation and infiltration of immune cells, the proliferative phase of wound healing is responsible for the closure of lesions and damaged tissue by inducing processes like angiogenesis, fibroplasia and reepithelialisation. Afterwards, the remodelling phase aims to recover normal tissue structure by breaking down and modulating the extracellular matrix and forming scar tissue[106,107].

1.3 Mucosal-associated invariant T cells

The term mucosal-associated invariant T cells was used for the first time in 2003 to describe a population of unconventional T cells in the gut lamina propria of humans and mice[108]. MAIT cells are characterised by expression of a semi-invariant TCR recognising microbial metabolites presented by the MHC class I – related protein 1 (MR1). In humans, the MAIT TCR consists of a Vα7.2 α chain joined to Jα33, Jα20 or Jα12 and paired with a limited array of β chains, mainly Vβ2 or Vβ13, whereas the TCR in mice comprises Vα19Jα33 primarily bound to Vβ6 or Vβ8[109,110,111,112].

1.3.1 Antigen recognition of MAIT cells

MR1 is evolutionarily highly conserved in most mammals and exhibits 90% homology between mice and humans[113]. The MR1 gene and thereby also MAIT cells were independently lost three times during evolution and are absent in rabbits, carnivores and armadillos[114]. Like MHC I, MR1 is stably associated with β2-microglobulin[115,116] and MAIT cells are absent in β2-microglobulin knockout mice[108]. MR1 messenger ribonucleic acid (mRNA) is widely expressed in different tissues and cell lines[117]. However, expression analysis on protein level in specific cell types is challenging due to low surface expression in the absence of MR1 ligands[118]. MR1 protein expression could only be shown for MR1 overexpressing cell lines[119,120] and some APCs like macrophages, DCs and DP thymocytes[121]. In the absence of MR1 ligands the majority of MR1 molecules is empty and located in the ER, while ligand binding results in conformational change and transport via the Golgi to the cell surface[117,119].

A landmark event of MAIT cell research was the discovery that MR1 presents microbial metabolites to MAIT cells, thereby revealing a new class of T cell antigens[120]. MR1 binds and presents different microbial metabolites of the vitamin B pathway present in many bacteria and yeast, but not in mammals. However, not all MR1-metabolite complexes are recognised by the MAIT TCR. The folic acid (vitamin B9) metabolite 6-formyl pterin (6-FP) or its synthetic analogue acetyl-6-FP (Ac-6-FP) are bound by MR1 resulting in increased formation of MR1-6FP on the cell surface without activating MAIT cells[120]. MAIT cell specific antigens are 5-(2-oxoethylideneamino)-6-D-ribitylaminouracil (5-OE-RU) and 5-(2-oxopropylideneamino)-6-D-ribitylaminouracil (5-OP-RU)[122]. These metabolites are formed by a non-enzymatic reaction of the riboflavin (vitamin B2) metabolite 5-amino-6-D-ribitylaminouracil (5-A-RU) with glyoxal to form 5-OE-RU or with methylglyoxal to form 5-OP-RU. Binding to MR1 captures and stabilizes the generally unstable intermediates[122]. Therefore, only bacteria and yeast possessing riboflavin production activate MAIT cells. This was proven by an abolished MAIT cell activation when cultured with bacteria genetically deleted for key enzymes of the riboflavin pathway[122,123].

Until now the specific identity of other MAIT cell-activating ligands remains enigmatic, while some studies propose their existence without identifying specific molecules[124,125]. MAIT cells are nearly completely absent in germ-free (GF) mice, while the few existing MAIT cells in the thymus, which develop without microbial ligands, may be selected by endogenous ligands or empty MR1[126,127]. Furthermore, studies revealed that MR1 is able to present a broader range of ligands like drugs and drug-like molecules[128], bacterial ligands independent of the riboflavin biosynthesis[124] and different synthetic analogues of 5-OP-RU[129]. However, if these molecules occur naturally and can activate MAIT cells *in vivo* has to be proven.

1.3.2 MAIT cell development

In the thymus, $CD4^+$ $CD8^+$ DP T cells with a TCR able to interact with MR1 commit into MAIT cell lineage. The selection of these cells occurs mainly by MR1-expressing DP cortical thymocytes[130] and initiates a three-stage development pathway for MAIT cells in mice and humans[126]. In mice, these stages are defined by expression or absence of CD24, CD44 and PLZF. Stage one ($CD24^+$, $CD44^-$ and $PLZF^-$) MAIT cells are the least mature and become stage two ($CD24^-$, $CD44^-$ and $PLZF^-$) MAIT cells by downregulation of CD24. PLZF knockout (*Zbtb16*$^{-/-}$) mice only exhibit stage one and two MAIT cells, thereby showing that the expression of PLZF is mandatory for the transition to stage three MAIT cells ($CD24^-$, $CD44^+$ and $PLZF^+$). MAIT cell interaction with MR1 is required for the transition through all developmental stages shown by experiments using an anti-MR1 blocking antibody[126]. Furthermore, MR1 presented microbial metabolites are needed for MAIT cell development, since mature MAIT cells are absent in GF mice[126,127]. Microbial colonization of GF mice increased the MAIT cell frequencies in the thymus and other peripheral organs without reaching the levels of mice colonized at birth[127] indicating that a specific time window during the first three weeks of development influences MAIT cell expansion[131]. MAIT cell development shares many similarities with iNKT cells, which were positively selected by other cortical DP thymocytes presenting lipids via CD1d and express PLZF[132]. Furthermore, MAIT and iNKT cells leave the thymus with effector functions and a memory phenotype ($CD44^+$, $CD62L^-$)[133].

The majority of MAIT cells egressing the thymus have downregulated CD4 and CD8 expression, while also $CD4^+$ and $CD8^+$ subsets are present and their respective frequencies vary in a tissue-specific manner[134]. MAIT cells can be further classified based on their transcription factors and effector function into MAIT1 (T-bet^+, $ROR\gamma t^-$) and MAIT17 (T-bet^-, $ROR\gamma t^+$) cells in mice[126,135]. Furthermore, a small population of naïve $CD44^-$ MAIT cells exists outside the thymus, which are mainly $CD4^+$ and reside in the spleen or LN. These cells show the same specificity as effector MAIT cells, but either left the thymus already as stage two MAIT cells[136] or underwent positive selection on mTEC and cTEC[137], which are also able to present 5-OP-RU via MR1[127].

The development of human MAIT cells is similar to mice and human stage one ($CD27^-$, $CD161^-$, $CD218^-$ and $PLZF^-$) MAIT cells develop via stage two ($CD27^+$, $CD161^-$, $CD218^-$ and $PLZF^-$) to mature stage three ($CD27^+$, $CD161^+$, $CD218^+$ and $PLZF^+$) MAIT cells, which egress the thymus with effector/memory functions. However, some stage two MAIT cells could be detected in the cord blood or blood from young donors suggesting that stage two MAIT cells can complete their maturation in the periphery[126]. In contrast to mice, human MAIT cells predominantly express CD8 and only small subpopulations of DN or $CD4^+$ MAIT cells exist. Furthermore, human MAIT cells co-express both transcription facts T-bet and $ROR\gamma t$[126,136].

1.3.3 Activation and effector function of MAIT cells

After leaving the thymus, human MAIT cells expand and their number increases with age until a peak at young adulthood (~ 25-30 years of age) followed by a continuous decline over the following decades[138,139]. Human MAIT cells are highly abundant and represent 1-10% of blood T cells and up to 45% of liver T cells[8,9], while common laboratory mouse strains like C57BL/6J mice show low frequencies of MAIT cells in blood and spleen (below 0.1%) as well as in the liver (0.7%)[134]. MAIT cells distribute in the whole body and the majority resides in tissues as shown by parabiosis mouse experiments[135]. Furthermore, MAIT cells can be directly activated in the tissue without the need to home to SLOs. In addition to a TCR-dependent activation, MAIT cells can be activated via the proinflammatory cytokines IL-12 and IL-18[140] (**Fig. 1.1**). Other cytokines like IL-15[141], IFN-α and IFN-β[142] as well as ligands for toll-like receptors (TLR)[140] can further promote the TCR-independent activation. Therefore, MAIT cell activation can occur as a bystander activation and enables to respond to viral infections and other immune responses without presence of a specific antigen[142,143]. The TCR-dependent activation of MAIT cells is also enhanced and shaped by many different cytokines like IL-7[144], IL-12[144], IL-18[141] and IL-23[145].

Recent RNA sequencing studies implicate that activation of MAIT cells via their TCR, cytokines or a combination of both induces respectively different effector functions. The upregulation of genes linked to a tissue repair signature occurred only after TCR-dependent activation shown by *in vitro* stimulation of human MAIT cells[146]. Furthermore, this tissue repair signature was also upregulated in activated MAIT cells in a *Legionella* infection mouse model[147] and a functional contribution of cutaneous MAIT cells to wound healing has been described[131].

By contrast, activated MAIT cells secrete a variety of cytokines and cytotoxic molecules associated with proinflammatory function. Unchallenged human MAIT cells express granzyme A and K, but low levels of perforin and granzyme B. However, upon activation MAIT cells strongly upregulate

perforin and granzyme B expression enabling to lyse and induce destruction in bacterial infected target cells[148,149]. Furthermore, the repertoire of proinflammatory cytokines produced by activated MAIT cells comprises TNF-α, IFN-γ, Il-2, IL-17A, IL-22 and GM-CSF[134,150,144,8], whereas *in vivo* production depends on the tissue and the context of activation. The majority of human blood MAIT cells secretes TNF-α and IFN-γ and only to a lesser extend IL-17A[8,151,152], whereas in mice the production of these cytokines is, in general, mutually exclusive. T-bet^{+} MAIT1 cells express IFN-γ and RORγt^{+} MAIT17 cells IL-17A[134]. However, during *Salmonella* infection RORγt and T-bet DP MAIT cells produce IFN-γ and IL-17A[153] and thereby closer resemble human MAIT cells. In summary, MAIT cells can fulfil diverse effector functions probably induced by different activation pathways suggesting that their specific contribution to immune responses is determined in the respective *in vivo* situation (**Fig. 1.1**).

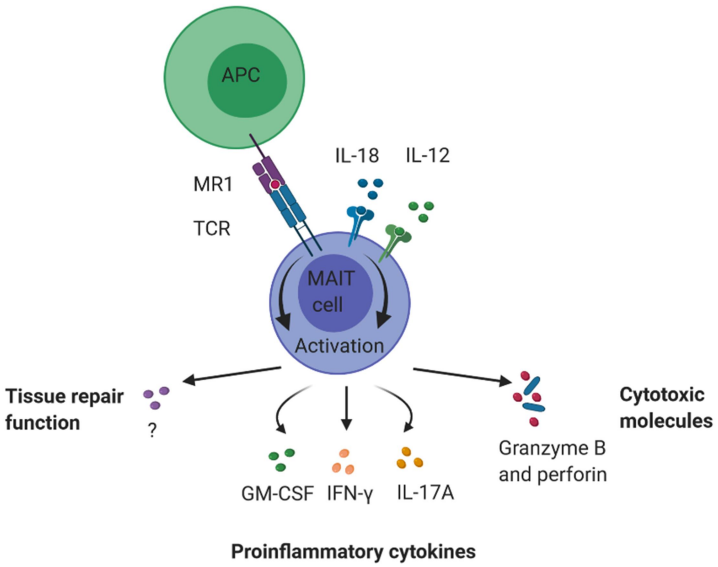

Figure 1.1: Activation and effector function of MAIT cells. MAIT cell activation occurs via their TCR recognising metabolites presented by MR1, in a cytokine-dependent activation by IL-12 and IL-18 or via both stimuli. After activation, MAIT cells can secrete proinflammatory cytokines like GM-CSF, IFN-γ and IL-17A and the cytotoxic molecules granzyme B and perforin. MAIT cells also have a tissue repair function, whereas no specific molecules have been identified yet.

1.3.4 Tools for studying MAIT cells

MAIT cell research was recently advanced through the development of different tools to study MAIT cells in mice. In humans, MAIT cells could already be identified by using antibodies against CD161 and Vα7.2, whereas a specific antibody against the mouse Vα19 chain is missing. Therefore, the generation of MR1-5-OP-RU tetramers in 2013, which became publicly available by the National Institutes of Health (NIH) in 2016, enabled for the first time the specific detection and characterisation of MAIT cells in mice[111].

In addition, different mouse strains were analysed and it has been shown that the wild-derived inbred mouse strain CAST/EiJ harbours more MAIT cells than C57BL/6J mice. Therefore, the congenic C57BL/6J^{CAST} strain was generated and the increased MAIT cell frequency is attributed to a single locus in the TCR α-chain. This locus can be identified by two single nucleotide polymorphisms (SNPs) and lead to an increased generation of Vα19Jα33 rearrangements resulting in higher MAIT cell frequencies shown by a frequency of 0.1-1% of T cells in the spleen and 1-10% in the liver[135,154]. In

contrast, C57BL/6J$^{Mr1-/-}$ (*Mr1*$^{-/-}$) mice lack MAIT cells due to the missing positive selection in the thymus[108]. However, these mice exhibit an increased intestinal permeability resulting in more bacterial infiltration possibly able to influence immune responses[155].

1.3.5 MAIT cells in infection and immunological diseases

MAIT cells have been investigated in human patients and animal models in cancer research[156] as well as in infectious[157,158], metabolic[159] and autoimmune diseases[160,152]. Most studies of infectious diseases propose a protective role of MAIT cells, since MAIT cells recognise microbial antigens, are able to kill infected cells[149] and are activated in bacterial[161] and viral infections[142,162]. Moreover, low MAIT cell frequency of patients at the intensive-care unit correlated with the severity of acquired infections further indicating a protective effect of MAIT cells[163]. MAIT cell frequencies in the blood of patients are often decreased and an infiltration in the inflamed tissue is suggested[152,164]. A recent study analysing MAIT cells in severe acute respiratory syndrome-coronavirus 2-(SARS-CoV-2) infected patients described increased MAIT cell frequencies in the airways. The activation of MAIT cells indicated by CD69 expression appeared predictive of the clinical course suggesting a beneficial role of activated MAIT cells during severe SARS-CoV-2 infection[165]. Furthermore, MAIT cell kinetics in *Salmonella enterica* serovar Typhimurium-infected patients revealed next to a decrease in blood frequency also strong activation, upregulation of homing markers and proliferation[166]. Investigations using mouse models confirmed the infiltration and accumulation of activated MAIT cells in the inflamed tissue of either *Salmonella*- or *Francisella tularensis*-infected mice[153,167]. Moreover, adoptive transfer of MAIT cells into immune-deficient *Rag2*$^{-/-}$*γC*$^{-/-}$ mice rescues them from otherwise lethal infections with *L. longbeachae* showing direct functional relevance of MAIT cells when unmasking their potential by removing other immune cell populations[168].

Investigations of autoimmune diseases are difficult, since it must be deciphered if certain immune responses ameliorate or worsen the disease, especially for MAIT cells able to mediate proinflammatory and tissue repair function. Systemic lupus erythematosus (SLE) is an autoimmune disease characterised by autoantibodies targeting nuclear antigens and leading to inflammation in different organs[169]. MAIT cells are reduced in the blood of SLE patients compared to healthy controls and the activation status of MAIT cells correlates with disease severity[170]. Furthermore, MAIT cells enhance the autoantibody production of B cells *in vitro*. An ameliorated disease was observed in a spontaneous SLE mouse model when MAIT cells are either missing using *Mr1*$^{-/-}$ mice or their activation is blocked by administration of isobutyryl-6-FP indicating a proinflammatory function of MAIT cells in SLE[171].

In rheumatoid arthritis (RA) patients compared to healthy controls, either a diminished or unchanged MAIT cell frequency has been reported[172,173]. The activation status of MAIT cells quantified by CD69 expression correlates with disease activity of RA patients[173] and activated MAIT cells produced more IL-17A compared to healthy controls indicating a disease augmenting effect[172]. This is further strengthened by reduced severity of *Mr1*$^{-/-}$ mice in animal models of RA[160].

In the context of type 1 diabetes (T1D), MAIT cells were able to kill pancreatic β-cells *in vitro*. Moreover, in an *in vivo* model using non-obese diabetic (NOD) mice MAIT cells are decreased in lymphoid tissues, whereas they are increased and activated in pancreatic islets and produce IFN-γ

and granzyme B[155]. In contrast, NOD *Mr1*$^{-/-}$ mice have an exacerbated disease implicating a protective function of MAIT cells. However, this effect is most likely due to their role of maintaining intestinal homeostasis and barrier integrity lost in *Mr1*$^{-/-}$ mice[155].

Therefore, MAIT cells seem to have distinct and diseased-dependent functions in different autoimmune diseases. New insights about MAIT cells and their effector functions, like the newly discovered tissue repair function of MAIT cells in 2019[131,147], require to constantly revisit and adapt the functional contribution of MAIT cells to diseases.

1.4 Multiple Sclerosis

The term multiple sclerosis arose from the first systematic description of patients with sclerotic lesions in brain and spinal cord in 1868 by Jean-Martin Charcot[174]. Nowadays, about 2.5 million people worldwide are affected by MS. It is a heterogenous disease and due to distinct disease patterns classified into relapsing-remitting MS (RRMS) and primary progressive MS (PPMS). About 85% of MS patients are diagnosed with RRMS having a disease course marked by intermittent attacks followed by remission phases. However, approximately 80% of these RRMS patients exhibit a progressive disease course called secondary progressive MS (SPMS) after one to two decades. RRMS patients have a mean disease onset of 29 years of age and a gender bias towards females with a women to men ration of 3:1[175]. In contrast, the mean disease onset of PPMS patients is 39 years[176] without showing a gender bias[177]. MS is characterised by different clinical manifestations and an unpredictable disease course. It begins with a first episode of neurological disturbance named clinically isolated syndrome (CIS), which passes over into either RRMS or PPMS. Further disease progress and disabilities are driven by neuroinflammatory and neurodegenerative processes. However, it is still under debate if one process triggers the other and how they interact over the disease course[1,178,179]. Good biomarkers for MS, especially for the different disease courses, are lacking and the diagnosis mainly relies on magnetic resonance imaging (MRI). MS patients commonly have oligoclonal immunoglobulin G (IgG) in the cerebrospinal fluid (CSF) and transient gadolinium-enhancing lesions on MRI scans indicating blood brain barrier (BBB) break-down and inflammation. CNS lesions are the hallmark of MS and occur in the grey and white matter further resulting in demyelination, oligodendrocyte loss, reactive gliosis, neuro-axonal degeneration and loss of neuronal signalling[1,176]. Depending on the localization of the lesions, MS patients can suffer from diverse symptoms including sensory and motor impairments, fatigue, pain, depression and cognitive dysfunctions[180].

1.4.1 Aetiology of MS

The initial cause of developing MS still remains elusive and it is assumed that MS develops in genetically predisposed individuals triggered by different environmental factors. Genome wide association studies (GWAS) linked the appearance of 233 SNPs to the risk of developing MS[181]. Many alleles of immune cell-associated genes were identified as risk factors for MS like MHC, IL-2R and IL-7R. One of the overall strongest association has been shown for the MHC II allele HLA-DRB1*1501, whereby also protective alleles exist (HLA DRB1*14)[182,183,184]. Furthermore, protective (HLA-A*0201) and risk (HLA-A*0301) alleles for the MHC I locus have been identified[185,186]. These findings strongly suggest a central role of the immune system in MS pathogenesis and substantiate the view of MS as an autoimmune disease.

Next to genetics, many different environmental factors contribute to the risk of developing MS. Geoepidemiology studies demonstrate a latitudinal gradient with a higher MS risks with increasing distance from the equator[187]. Furthermore, migration studies showed that the environment before adolescence is especially important and moving before adolescence from a high-risk to a low-risk area reduces the disease rate and vice versa[188,189]. Ultraviolet (UV) radiation and vitamin D are two environmental factors strongly influenced by the latitude and possibly contributing to these findings. Higher UV radiation and vitamin D levels are associated with a lower risk of MS potentially by influencing the immune system to a more regulatory instead of an inflammatory response[190,191]. Additional factors correlating with a higher prevalence of MS are smoking and an infection with the Epstein-Barr virus (EBV)[192,193]. Furthermore, the microbiome and its contribution to diseases got into the focus of research during the last years. The human microbiome inhabits several trillion microbial cells mainly located in the gastrointestinal tract. Changes or imbalances in its functional composition are associated with inflammatory and metabolic diseases, cancer and autoimmunity especially by altering and shaping immune responses[194]. Analyses of MS patients compared to healthy controls revealed microbiome alterations possibly either promoting or preventing MS development[195]. However, understanding the contribution of the microbiome to MS is at the very beginning and most of the data relies on findings in the animal model of MS, experimental autoimmune encephalomyelitis (EAE). Many studies using the EAE model have already shown that the microbiome heavily influences the disease leading to a protection or an exacerbation of EAE[196,197,198,199]. Transplantations of the gut microbiome from MS patients to GF mice worsened the disease in comparisons to the microbiome of unaffected twins[200]. The commensal gut flora is important in inducing EAE in a spontaneous EAE model using myelin oligodendrocyte glycoprotein (MOG)-specific TCR-transgenic mice. Nearly all mice raised in a specific-pathogen free (SPF) environment spontaneously develop EAE, while mice kept in GF conditions are protected. After exposure of GF mice to SPF-derived faeces, these mice develop EAE[199]. Furthermore, a recent study shows that two gut microorganisms co-ordinately foster activation of MOG-specific autoreactive T cells in the small intestine. A bacterial strain of the family Erysipelotrichaceae enhances the activation of Th17 cells similar to an adjuvant, while a strain of *Lactobacillus reuteri* carries peptides potentially mimicking MOG. Mice co-colonised with both strains developed a more severe EAE than GF or mono-colonised mice, further suggesting a critical role for the microbiome in EAE development and in expansion of autoreactive T cells[201].

1.4.2 MS treatment

So far, there is no preventative or curative treatment for MS. The majority of drugs and treatment strategies target components of the immune system, thereby helping RRMS patients mainly by reducing and preventing relapses. However, neurodegenerative processes are not directly stopped resulting in further axonal damage and disease progression. In general, these immunomodulatory drugs restrain immune cells in the periphery, decrease Th1, Th17 and B cells, induce Treg cells or block cytokines. Corticosteroids have potent anti-inflammatory and immunosuppressive properties and are often used to reduce active relapses and speed up the recovery[202]. Natalizumab is a humanized antibody targeting the α4 subunit of very late antigen 4 (VLA-4) expressed by immune cells and blocking the interaction with vascular cell adhesion molecule-1 (VCAM-1) expressed on endothelial

cells. This inhibits T and B cell migration into the CNS[203]. The sphingosine-1-phosphate receptor modulator Fingolimod also affects migration and sequesters lymphocytes in LN by inhibiting their egress[204]. Alemtuzumab, an anti-CD52 antibody, downregulates the immune system by depleting B and T cells resulting in a reduced relapse rate of RRMS patients[205]. B cell depletion is also mediated by ocrelizumab, a humanised monoclonal antibody targeting the CD20 antigen on B cells, which is approved for RRMS and PPMS patients[206,207]. Since Th1 and Th17 are mainly implicated in MS pathogenesis, skewing of the T cell differentiation towards non-pathogenic Th2 cells is ameliorating the disease and achieved by treating MS patients with glatiramer acetate, IFN-β or dimethyl fumarate, whereas also other mechanisms and effects are discussed[208,209,210,211]. However, directly interfering with the T cell differentiation into Th1 and Th17 by using the antibody ustekinumab failed to reduce lesions in RRMS patients[212]. Ustekinumab targets the p40 subunit of IL-12 and IL-23. Possible explanations suggest that the antibody was not able to cross the BBB or that ustekinumab is more important in the early phase of the disease and the treated patients already had a too advanced disease state[213]. Administration of secukinumab, an IL-17A blocking antibody, reduced MRI lesion activity[205].

Since all these drugs target general mechanisms of the immune system instead of inhibiting specific pathways, unspecific and treatment-dependent side effects can occur. One of the most concerning side effects is the development of progressive multifocal leukoencephalopathy (PML) caused by the John Cunningham virus (JCV), which is often seen under natalizumab treatment[214,215]. Next to side effects, some treatments exacerbated MS like the application of anti-TNF-α or IFN-γ[216,217]. Therefore, treatment decisions are mainly determined on an individual basis and associated with a certain risk.

1.4.3 Immunopathology of MS

MS is considered to be an autoimmune disease. This hypothesis is supported by the positive effects of immunomodulatory drugs, GWAS studies, data from the EAE model and the presence of activated lymphocytes in CNS lesions. The lesion pattern is very heterogenous between MS patients and four different lesion types can be defined based on myelin protein loss, geography and extension of plaques, oligodendrocyte destruction and the presence of T cells, macrophages, IgGs and complement proteins. Importantly, lesions within individual MS patients have the same pattern further suggesting that distinct treatment strategies are required depending on the underlying pathogenic pathway[218]. Lymphocyte infiltration of the CNS results in break-down of the BBB and inflammatory lesions. T cells, B cells, NK cells, monocytes and macrophages are infiltrating the CNS and are contributing, whereby specific effects of each cell type are not fully understood and difficult to disentangle.

Next to CNS-infiltrating immune cells, CNS-resident microglia play a role in EAE/MS pathogenesis. Distinct microglia subsets exist[219,220,221], which have either proinflammatory or regulatory function and can switch between these profiles upon activation[222]. Microglia can induce tissue damage in the CNS by secreting proinflammatory cytokines, chemokines, reactive oxygen species (ROS) and glutamate[223]. On the other hand, they contribute to immunoregulation and tissue repair by producing anti-inflammatory cytokines and neurotrophic factors[224,225]. Moreover, activated

microglia upregulate MHC and costimulatory molecules and phagocytose myelin debris. Based on these observations, it has been assumed that microglia function as APCs and mediate reactivation of CNS-infiltrating T cells in EAE/MS[226,227]. However, a recent study revealed that MHC II-mediated T cell priming by microglia is not required for EAE induction or progression[228], suggesting that microglia are redundant for antigen presentation to $CD4^+$ T cells in MOG_{35-55}-mediated neuroinflammation.

The initial activation of autoreactive T cells is still under debate and addressed by two hypotheses[229]. The “inside-out” hypothesis postulates that CNS-intrinsic neurodegenerative processes activate the immune system. The activation and CNS infiltration of autoreactive T cells occurs as a secondary phenomenon according to this model. This view is supported by data showing that oligodendrocyte loss in the brain of MS patients can occur in the absence of inflammation[230]. Furthermore, data using a mouse model demonstrate that killing oligodendrocytes by genetic activation of diphtheria toxin fragment A results in $CD4^+$ T cell infiltration and a demyelinating disease[231]. In contrast, the “outside-in” hypothesis argues that dysregulation of the immune system generates and activates autoreactive T cells against myelin components[229]. These cells infiltrate the CNS and get locally reactivated resulting in a break-down of the BBB and in further inflammation and demyelination. Possible mechanisms for the development of autoreactive T cells could be co-expression of an additional TCR, escape of negative selection or molecular mimicry, with the latter mediating cross-activation of T cells due to sequence similarities between foreign and self-peptides.

Most of the research of the last decades focussed on $CD4^+$ effector T cells due to the strong MS association of the MHC II allele HLA-DRB1*1501 in GWAS and their necessity for nearly all EAE models. $CD4^+$ effector T cells are present in CNS lesions and able to recruit and activate other T cells, macrophages and microglia[227,232]. Th1 and Th17 cells, particularly pathogenic Th17 cells producing IFN-γ and IL-17A, are present in MS lesions[72,233,234]. The specific role of these proinflammatory cytokines and their contribution to MS pathogenesis is still incompletely understood. IL-17A was detected in MS lesions and may play a role in MS pathogenesis by inducing ROS within endothelial cells contributing to the break-down of the BBB[235]. In addition, IL-17A can also be secreted by $CD8^+$ T cells, oligodendrocytes and astrocytes[236].

However, depletion of $CD4^+$ T cells in MS patients did not diminish inflammatory activity in lesions nor did it reduce the relapse rates[237]. A partial explanation for this observation is that also regulatory $CD4^+$ T cells have been depleted, but it also points out that $CD8^+$ T cells seem to play an important role. Furthermore, $CD8^+$ T cells outnumber $CD4^+$ T cells in CNS lesions and the number of $CD8^+$ T cells correlates with the degree of axonal damage[238,239,240]. $CD8^+$ T cells probably actively contribute to axonal damage since they were found in close proximity to damaged axons and *in vitro* experiments showed that $CD8^+$ T cells impair neuronal signalling and promote neuronal cell death [241]. These processes are mainly mediated by the cytotoxic molecules granzyme B and perforin, which were upregulated in the CSF of MS patients at relapse compared with healthy controls and patients in remission[242]. Moreover, $CD8^+$ T cells might also contribute to MS pathogenesis by secreting IFN-γ and IL-17A[236,243]. Interestingly, it was shown that the majority of the IL-17A-producing $CD8^+$ T cells express CD161 and belong to the MAIT cells[7,244].

1.5 Animal models of MS

The use of animal models in MS research has been hugely informative about the pathogenesis of MS. Animal models enable to collect CNS samples during different and especially early timepoints of CNS inflammation. Investigation of genetically identical mice reduces the complexity of disease-contributing possibilities. Furthermore, genetic modifications allow to stain specific cell populations by using reporter mice or analyse the impact of certain genes using knockout animals.

MS presents as a complex disease with variable clinical and pathological manifestations and different pathogenic processes, which cannot be depicted in one animal model. Therefore, various models exist partially reflecting different aspects of the disease. In the cuprizone model, animals, mainly rodents, are fed with the neurotoxic copper chelator cuprizone leading to oligodendrocyte damage and demyelination in the CNS. This results in only little inflammation and is therefore suited to investigate neurodegenerative aspects of MS[47,245]. In contrast, the Theiler's murine encephalomyelitis virus (TMEV) model can be used to investigate the association of MS with viral infections like EBV and the contribution of $CD8^+$ T cells[245]. Certainly, the most commonly used animal model for MS is EAE initiated by autoreactive T cells leading to CNS inflammation and demyelination.

1.5.1 Experimental autoimmune encephalomyelitis

The first unintentional cases of EAE occurred in 1885 as a side effect of Louis Pasteur's rabies vaccination with dried spinal cords of rabbits. Sporadic paralysis occurred in some of these patients due to contamination with CNS antigens. This observation lead to first active immunisation experiments with repeated injections of CNS tissue in rabbits and monkeys[246,247]. Since then, different EAE induction protocols were established and optimized in rodents and non-human primates.

Nowadays, mainly rat and mouse strains are used and EAE is induced by a single subcutaneous (s.c.) injection of an emulsion of complete Freund's adjuvant (CFA) and defined peptides of myelin sheath proteins. Furthermore, the immunisation is often enhanced by an injection of pertussis toxin[248,249]. CFA consist of non-metabolizable oils and heat-killed *Mycobacterium tuberculosis* and can enhance and maintain a strong immune response against CNS antigens to trigger an autoimmune reaction. Thereby, repeated injections of CNS tissue, up to 80 per animal[250], could be replaced by a single injection[248]. Defined peptides of myelin basic protein (MBP), proteolipid protein (PLP) and MOG haven been established. EAE course and susceptibility are highly dependent on the combination of genetic background of the animal and the respective peptide. MBP injection in Lewis rats leads to a monophasic EAE course, while $PLP_{139-151}$ in SJL mice resembles a relapsing-remitting phenotype[251,252]. The most common form of EAE is induced in C57BL/6J mice immunised with the MOG_{35-55} peptide.

Next to active EAE immunisation protocols, also passive and spontaneous EAE models exist. Spontaneous models use genetic modifications to overcome the intrinsic regulatory mechanisms preventing autoimmunity by using mice expressing myelin-specific TCR and BCR[253,254,255]. However, the incidence of the spontaneous models is very low and highly depends on the genetic background of the mice and the microbiome in the respective animal facility[256]. Passive EAE induction is defined as transfer of activated myelin-specific T cells into recipient mice. Autoreactive T cells for transfer are generated by *in vitro* activation of either naïve T cells from TCR transgenic mice or T cells isolated

from LN of actively immunised mice[257]. Advantages of such models are that T cells can be labelled either genetically or with a fluorescent protein and that T cells can be differentiated into specific subtypes like Th1 or Th17 cells allowing the investigation of the function of specific T cell populations in EAE pathogenesis[258].

In the most commonly used active EAE model, myelin-reactive T cells get activated after immunisation in the LN and migrate into the CNS leading to break-down of the BBB and inflammation in brain and spinal cord. In general, the first symptoms occur 7-11 days post immunisation (dpi) followed by increased severity until a peak of the acute phase arises 13-15 dpi. Afterwards, the animals recover and enter either a relapse or chronic phase 25-30 dpi. Two different EAE courses, described as classical and atypical EAE, can be distinguished based on contrasting symptoms. Mice developing classical EAE symptoms show an ascending paralysis from the tail to the hind- and sometimes even to forelimbs correlating with spinal cord inflammation[252,259]. Signs of atypical EAE correspond to inflammation in the brain, and these mice show symptoms such as head tilt, spinning or axial rotation[260,261]. These EAE types are not mutually exclusive and animals can develop both types of symptoms indicating CNS infiltration in brain and spinal cord. This shows that like in MS patients the localization of inflammatory lesions results in diverse symptoms.

1.5.2 T cells in EAE

Transfer EAE experiments showed that Th1 and Th17 are capable to induce EAE in recipient mice[258,262]. Nevertheless, pathogenic Th17 able to produce IFN-γ, GM-CSF and IL-17A are proposed to be the main contributors to EAE pathogenesis[72,263,264]. This is supported by studies showing that IL-12, which is required for Th1 differentiation, is not essential for EAE since *Il-12*$^{-/-}$ mice are fully susceptible[265,266]. In contrast, *Il-23p19*$^{-/-}$ mice are resistant to EAE induction[67] as IL-23 mediates the development of Th17 to pathogenic Th17 cells[72,263]. Th17 cells differentiated in the absence of IL-23 still produce high levels of IL-17A but are not able to induce EAE. These cells express higher amounts of IL-10 and seem to have a more regulatory function[267].

The differential contribution of Th1 and Th17 cells to EAE pathogenesis is reflected by distinct disease outcomes and EAE courses. In general, Th1 cells mainly infiltrate the spinal cord and produce IFN-γ leading to classical EAE, whereas Th17 cells induce atypical EAE symptoms by mainly infiltrating the brain and producing IL-17A, GM-CSF and partially IFN-γ[264]. A combination of the genetic background, mainly because of the MHC haplotype, and the peptide used for immunisation strongly influences the activation and differentiation of T cells and thereby the Th1 to Th17 ratio important for EAE pathogenesis[261,264]. Infiltration of myelin-specific T cells into the meninges of the CNS occurs independently of the Th1 to Th17 ratio, but inflammation in the brain parenchyma develops only if Th17 cells outnumber Th1 cells[261].

Th1 and Th17 cells use distinct entry routes into the CNS depending on the expression of different integrins and chemokine receptors. Spinal cord infiltration of Th1 cells requires expression of VLA-4[268], which is a heterodimer consisting of an α4 (CD49d) and β1 (CD29) integrin subunit. VLA-4 interacts with VCAM-1 expressed on endothelial cells and is required for firm adhesion and diapedesis of encephalitogenic T cells[269]. Adoptive Transfer EAE using only Th1 cells is completely abrogated when the mice are treated with an anti-α4 integrin antibody. Th1 cells express a significantly higher

amount of VLA-4 compared to Th17 cells[268]. Mice having a conditional knockout of α4 integrin in $CD4^+$ T cells exhibit atypical EAE symptoms, indicating that Th17 cells infiltrate the CNS independent of VLA-4. Treating conditional α4-integrin knockout mice with an anti-LFA-1 antibody made the mice resistant to EAE showing that Th17 cells enter the CNS via LFA-1[268]. The heterodimer LFA-1 is composed of the subunits αL- (CD11a) and β2-integrin (CD18) and interacts with ICAM-1 and ICAM-2 thereby mediating transendothelial migration[270]. The C-C motif chemokine receptor 6 (CCR6) enables crossing of the blood–CSF barrier and thereby infiltration of autoreactive T cells to the CNS via the choroid plexus[271]. CRR6 is mainly but not exclusively expressed by Th17 cells and was also found on Treg cells and MAIT cells[154,272]. Epithelial cells of the choroid plexus constitutively express the CCR6 ligand C-C motif chemokine ligand 20 (CCL20). During EAE, CCR6-mediated CNS infiltration seems to be important in the early disease phase, when the first wave of T cells enters the uninflamed CNS[271].

Next to their entry routes also dissimilar effector functions of Th1 and Th17 cells, mainly mediated by their cytokine secretion profile, differentially affect EAE pathogenesis. Th1 cells are characterised by expression of IFN-γ, which has contrasting effects on EAE pathogenesis depending when and where it is secreted. Treating mice with IFN-γ during EAE induction increased severity, while administration at EAE onset ameliorated the disease[273]. Protective effects of IFN-γ are supported by studies using knockout mice for IFN-γ or IFN-γR showing an exacerbating effect[274,275]. Moreover, opposing effects of IFN-γ in brain and spinal cord have been reported[276]. IFN-γ supresses brain inflammation but induces inflammation in the spinal cord. This effect is possibly mediated by higher IFN-γR expression in the brain compared to the spinal cord[261]. Therefore, IFN-γ or IFN-γR knockout mice develop an atypical EAE characterised by brain inflammation[277]. Brain and spinal cord are distinct microenvironments where IFN-γ has different effects on the recruitment of other immune cells. IFN-γ in the spinal cord promotes monocyte, macrophage and neutrophil infiltration by upregulation of CCL2 and C-X-C motif chemokine ligand 2 (CXCL2)[278], whereas IFN-γ inhibits CXCL2-dependent neutrophil infiltration in the brain[279].

In contrast, IL-17A increases the CXCL2-dependent neutrophil infiltration in the brain indicating the contrary functions of IFN-γ and IL-17A[279]. Another function of IL-17A in EAE is to induce tertiary lymphoid tissue-like structures within the meninges[280,281]. These structures resemble SLOs, support the priming of immune responses at the site of inflammation and were also detected in MS patients[282,283]. Moreover, IL-17A is participating in BBB break-down by increasing ROS production of brain endothelial cells resulting in loss and disorganization of tight junction proteins[235]. IL-17A can also contribute to neuronal damage by exacerbating TNF-α-induced loss of oligodendrocytes[284]. However, IL-17A is dispensable for EAE, since *Il-17a*$^{-/-}$ mice or mice treated with an anti-IL-17A antibody have an unaltered or only slightly ameliorated disease[285,286,287].

In multiple mouse strains including C57BL/6J mice, GM-CSF, encoded by the *Csf2* gene, is an essential cytokine for EAE induction. *Csf2*$^{-/-}$ mice or mice treated with an anti-GM-CSF antibody are resistant to actively induced EAE[288,289]. Many different cell types are able to produce GM-CSF. However, GM-CSF production by encephalitogenic T cells is primarily necessary for EAE induction as indicated by experiments with GM-CSF reporter mice[290] and transfer EAE using *Csf2*$^{-/-}$ T cells[291]. Nevertheless, the absence of GM-CSF in *Csf2*$^{-/-}$ mice is also affecting effector T cell priming showing

that other GM-CSF driven mechanisms next to GM-CSF production of CNS-infiltrating encephalitogenic T cells also contribute to EAE pathogenesis[292,293]. Furthermore, it was postulated that a certain level of either GM-CSF or IL-17A is needed for EAE induction. Absence of both cytokines strongly reduces classical EAE and completely protects C3HeB/FeJ mice from developing atypical EAE symptoms[293]. GM-CSF, independent of IL-17A, recruits neutrophils to the brain and thereby promotes induction of atypical EAE[293]. Furthermore, GM-CSF sustains neuroinflammation by affecting CNS-infiltrating myeloid cells[70]. Activating the pathogenic function of monocytes by GM-CSF contributes to inflammation and tissue damage[294]. In summary, GM-CSF is an important cytokine strongly influencing EAE pathogenesis, while the exact contribution seems to be model- and strain-dependent.

1.6 MAIT cells in MS and EAE

Investigations of MAIT cells in patients with MS revealed opposing results concerning MAIT cell frequencies. Whereas most of the studies showed decreased MAIT cell abundance in the periphery[7,295,296], also unchanged[297] or elevated[298,299] MAIT cell frequencies in the peripheral blood compared to healthy controls have been reported. Reasons for these discrepancies may be differences in the study cohorts varying in age, MS subtype, smoking behaviour, current treatment or treatment history. Furthermore, in the CSF of MS patients either increased[295] or reduced[7] MAIT cell frequencies were reported. However, MAIT cells could be detected in inflamed brain lesions of MS patients[7,300,301] indicating a possible role for these cells in MS pathogenesis. Characterisation of MAIT cells from the peripheral blood of MS patients showed that they have an increased expression of VLA-4[7], CCR5, CCR6, C-X-C motif chemokine receptor 6 (CXCR6), LFA-1, P-selectin glycoprotein ligand 1 (PSGL-1) and LFA-3[295], which are chemokine receptors and adhesion molecules involved in migration into the CNS. MAIT cells highly express the IL-18 receptor[8] and stimulating MAIT cells with IL-18 alone or in combination with a TCR-dependent activation results in activation of MAIT cells and an upregulation of VLA-4. IL-18 is increased in the serum of MS patients compared to healthy controls[302] and the levels of IL-18 in the serum of MS patients negatively correlated with their MAIT cell frequency. It was therefore hypothesised that IL-18 is involved in activation and migration of MAIT cell from the blood to the CNS in MS[7]. Moreover, cytokine production of MAIT cells from MS patients indicates a proinflammatory phenotype. MAIT cells from the peripheral blood of MS patients produced significantly more IL-17A compared to healthy controls[151,295], whereas no difference in IL-17A production was observed in non-MAIT T cells[151]. Furthermore, the IL-17A production of MAIT cells positively correlated with the IL-7 receptor expression, which is elevated on MAIT cells of MS patients in one out of two cohorts[151].

In contrast, a protective or regulatory phenotype of MAIT cells was proposed by a study showing that MAIT cells from MS patients as well as from healthy controls inhibit IFN-γ production of Th1 cells[296]. Furthermore, a protective effect of MAIT cells was supported by data from the EAE model. The role of MAIT cells in EAE has only been investigated in one study published in 2006, before the MR1-5-OP-RU tetramer became available[303]. Therefore MAIT cells were studied in iVα19 transgenic mice having an increased frequency of T cells expressing the MAIT TCR[304]. The authors showed that *Mr1*$^{-/-}$ mice had an exacerbated EAE course and that transferring liver T cells from iVα19

transgenic mice into wildtype recipient mice leads to an ameliorated EAE course. This protective effect was further investigated by *in vitro* experiments showing that iVα19 transgenic T cells reduce proinflammatory cytokines in LN and induce IL-10 production of B cells in an MR1-independent manner[303]. However, nowadays it is known that the majority of these iVα19 transgenic MAIT cells show huge discrepancies with non-transgenic MAIT cells like a naïve phenotype and the lack of PLZF[134,152] and results obtained from experiments using iVα19 transgenic mice have to be considered with caution[305].

Taken together, the role of MAIT cells in MS is still incomplete understood and requires more functional characterisation to estimate beneficial or detrimental effects of MAIT cells to MS pathogenesis. Therefore, it is necessary to revisit the role of MAIT cells in EAE and use the newly developed MAIT cell tools to investigate the functional contribution of MAIT cells to EAE.

1.7 Aim of this work

MAIT cells are a newly discovered and in humans highly abundant T cell population able to fulfil diverse functions ranging from proinflammatory to tissue repair effects. Alterations of MAIT cells have been reported in the MS patients, while functional investigations in humans are difficult and limited. Hence, deciphering the functional role of MAIT cells in CNS inflammation requires investigations in the MS mouse model EAE. Due to the just recently availability of MR1-5-OP-RU tetramers to specifically identify MAIT cells in mice, such studies are still sparse. The overall aim of this work is to analyse MAIT cells in EAE and thereby to decipher the contribution of MAIT cells to MS pathogenesis.

To achieve this, the following aims were addressed:

1. Combining newly available tools to study MAIT cells in mice with the EAE model to establish a model system to analyse MAIT cells in EAE.

2. Characterisation of CNS-infiltrating MAIT cells in EAE by flow cytometry and transcriptome analysis to investigate proinflammatory as well as protective effector functions.

3. Decipher the role of MAIT cells in EAE using genetic *Mr1* deletion and MR1 blocking antibodies.

2 Material and methods

2.1 Material

2.1.1 Laboratory animals

Animal experiments were approved by the local ethics committee (Behörde für Justiz und Verbraucherschutz in Hamburg; G16/79).

Table 2.1: Used mouse strains.

Mouse strain	Official symbol	Origin
C57BL/6J	C57BL/6J	The Jackson Laboratory, stock number 000664
C57BL/6J^{CAST} × RORγtGFP	B6-MAIT^{CAST}	O. Lantz (Institut Curie, Paris)
C57BL/6J$^{CAST\ Mr1-/-}$ × RORγtGFP	B6-MAIT$^{CAST\ Mr1-/-}$	O. Lantz (Institut Curie, Paris)
Mr1$^{-/-}$	C57BL/6J$^{Mr1-/-}$	Crossing of C57BL/6J and C57BL/6J$^{CAST\ Mr1-/-}$ mice
Nur77GFP	B6N.B6-Tg(Nr4a1-EGFP/cre)820Khog/J	The Jackson Laboratory, stock number 018974

2.1.2 Reagents

Table 2.2: Reagents for genotyping.

Reagent	Company
Agarose Ultra Pure	Life Technologies (Merck)
ddH_2O	Generated in house
dNTP Mix (10 mM)	Thermo Fisher Scientific
DreamTaq GREEN Hot Start Buffer 10x	Thermo Fisher Scientific
DreamTaq Hot Start Green DNA Polymerase	Thermo Fisher Scientific
GeneRuler 1 kb DNA Ladder	Thermo Fisher Scientific
Genotyping master mix (2x)	Thermo Fisher Scientific
QuickExtract DNA Extraction Solution	Lucigen
RotiSafe	Carl Roth
TaqMan SNP genotyping assays (40x)	Thermo Fisher Scientific

Table 2.3: Primers for genotyping.

Name	Sequence	Mouse line	Company
Nur77GFP 12445 rev	CAGTTTCAGTCCCCATCCTC	Nur77GFP	biomers
Nur77GFP 12444 fwd	CGGGTCAGAAAGAATGGTGT	Nur77GFP	biomers
Nur77GFP 8745 rev	GTCAGTCGAGTGCACAGTTT	Nur77GFP	biomers
Nur77GFP 8744 fwd	CAAATGTTGCTTGTCTGGTG	Nur77GFP	biomers
MR1 fwd 8763-8783	AGCTGAAGTCTTTCCAGATCG	*Mr1*$^{-/-}$ and C57BL/6J^{CAST}	biomers
MR1 rev 10451-10431	GATTCTGTGAACCCTTGCTTC	*Mr1*$^{-/-}$ and C57BL/6J^{CAST}	biomers
MR1 rev 9188-9168	ACAGTCACACCTGAGTGGTTG	*Mr1*$^{-/-}$ and C57BL/6J^{CAST}	biomers
RORgt3 fwd	CCCCCTGCCCAGAAACACT	C57BL/6J^{CAST}	biomers
RORgt4B rev	GGATGCCCCATTCACTTACTTCT	C57BL/6J^{CAST}	biomers

Table 2.4: SNP genotyping assays.

Assay ID	Fwd primer sequence	Rev primer sequence	Reporter 1 sequence	Reporter 1 dye	Reporter 2 sequence	Reporter 2 dye
AH6RVCU	TCCTGGGTGAGTCAAATATCCTGATA	TGTGATGCAAGGCTTCATGATGA	CACTGTTTCTCTTACTTAAT	VIC	CTGTTTCTCTCACTTAAT	FAM
AH6R0R1	CCACCTTTTCTAGGAGCCTACTACT	GCATGGTGTGTGCAGAATCG	CCAGATCGAAGTGCC	VIC	CCAGATCGCAGTGCC	FAM

Table 2.5: Reagents for animal experiments.

Reagent	Company
Anti-MR1 antibody (clone 26.5)	BioLegend
CO_2/O_2 gas mixture (80% CO_2, 20% O_2)	SOL Deutschland
CO_2 gas (100%)	SOL Deutschland
DietGel Recovery	Clear H_2O
Incomplete Freund‘s adjuvant,	BD Biosciences
Mouse IgG2a, κ isotype Ctrl (clone MOPC-173)	BioLegend
Mycobacterium tuberculosis	BD Biosciences
MOG_{35-55} peptide	Peptides & elephants
PBS (1x)	Pan-Biotech
Pertussis toxin (*Bordetella pertussis*)	Calbiochem (Merck)

Table 2.6: Reagents for cell culture.

Reagent	Company
Anti-CD28 (Clone 37.51)	BioLegend
Anti-CD3 (Clone 145-2C11)	BioLegend
Ionomycin	Sigma-Aldrich
Monensin solution (2 mM)	BioLegend
PBS (1x and 10x)	Pan-Biotech
Percoll (1.13 g/ml)	GE Healthcare
Phorbol 12-myristate 13-acetate (PMA)	Sigma-Aldrich
Recombinant mouse IL-12	PeproTech
Recombinant mouse IL-18	MBL
Trypanblue solution	Sigma-Aldrich

Table 2.7: Reagents for magnetic-associated cell sorting.

Reagent	Company
LD columns	Miltenyi Biotec
MS columns	Miltenyi Biotec
Pan T cell isolation kit II, (mouse)	Miltenyi Biotec

Table 2.8: Reagents for RNA isolation.

Reagent	Company
β-mercaptoethanol	Sigma-Aldrich
Ethanol (100%)	Carl Roth
QIAshredder homogenizer	Qiagen
RNeasy Micro Kit	Qiagen

Table 2.9: Reagents for flow cytometry and fluorescence-activated cell sorting.

Reagent	Company
BD CompBeads (Anti-rant and anti-hamster Ig κ/ negative control compensation particles set)	BD Biosciences
BD Cytofix (fixation buffer)	BD Biosciences
BD FACS Clean Solution	BD Biosciences
BD FACSDIVA CS&T Research beads	BD Biosciences
BD FACS Flow (20 l)	BD Biosciences
BD FACS Rinse Solution	BD Biosciences
BD Fiaxable Viability Stain 700	BD Biosciences
BD Trucount tubes	BD Biosciences
Brilliant Stain Buffer	BD Biosciences
ddH_2O	Generated in house
Ethanol absolute	Th. Geyer
Fetal calf serum (FCS)	Biochrome (Merck)
Fixable Aqua Dead Cell Stain Kit	Invitrogen (Thermo Fisher Scientific)
Fixation Buffer	BioLegend
Intracellular Staining Perm Wash Buffer (10x)	BioLegend
MR1 monomers biotinylated	NIH
Streptavidin, R-phycoerythrin-conjugate (SAPE)	Invitrogen (Thermo Fisher Scientific)
True-Nuclear Transcription Factor Buffer Set	BioLegend
TruStain FcX (anti-mouse CD16/32) antibody	BioLegend
UltraComp eBeads (compensation beads)	Invitrogen (Thermo Fisher Scientific)

2.1.3 Antibodies

Table 2.10: Antibodies for flow cytometry.

Antigen	Clone	Fluorochrome	Dilution	Company
CD3ε	145-2C11	FITC	1:100	BioLegend
CD4	GK1.5	BV711	1:100	BioLegend
CD4	GK1.5	BV786	1:100	BioLegend
CD8	53-6.7	BV786	1:100	BioLegend
CD11a	M17/4	PE-Cy7	1:66	BioLegend
CD11b	M1/70	PerCP-Cy5.5	1:100	BioLegend
CD11b	M1/70	BV650	1:100	BioLegend
CD11c	N418	PE-Cy7	1:100	BioLegend
CD19	6D5	BV650	1:100	BioLegend
CD44	IM7	PerCP-Cy5.5	1:100	BioLegend
CD44	IM7	PE-Cy7	1:100	BioLegend
CD44	IM7	APC	1:100	BioLegend
CD45	30F11	Alexa Fluor 700	1:100	BioLegend
CD49d	R1-2	APC	1:100	BioLegend
CD69	H1.2F3	PerCP-Cy5.5	1:100	BioLegend
CD69	H1.2F3	PE-Cy7	1:100	BioLegend
CD317	927	APC	1:100	BioLegend
B220	RA3-6B2	BV510	1:100	BioLegend
B220	RA3-6B2	BV650	1:100	BioLegend
B220	RA3-6B2	BV650	1:100	BD Biosciences
CCR6	29-2L17	BV605	1:66	BioLegend
F4/80	BM8	BV421	1:100	BioLegend
GM-CSF	MP1-22E9	PerCP-Cy5.5	1:20	BioLegend
IFN-γ	XMG1.2	BV605	1:20	BioLegend
IL-17A	TC11-18H10.1	APC	1:20	BioLegend
Ly6G	1A8	APC-Cy7	1:100	BioLegend
MHC II	M5/114.15.2	BV711	1:100	BioLegend
NK1.1	PK136	PE	1:100	eBioScience
T-bet	4B10	PE-Cy7	1:50	eBioScience
TCR-β	H57-597	BV421	1:100	BioLegend
TCR-Vβ6	RR4-7	APC	1:100	BioLegend
TCR-Vβ8	F23.1	BV605	1:100	BD Biosciences

2.1.4 Solutions, buffers and media

Table 2.11: Solutions, buffers and media.

Name	Reagent	Concentration/volume	Company
CNS digestion solution	Collagenase A	1 mg/ml	Roche
	DNaseI	0.1 mg/ml	Merck
	RPMI 1640 medium	50 ml	Pan-Biotech
Complete RPMI medium	β-mercaptoethanol	0.1%	Sigma-Aldrich
	Fetal calf serum (FCS) (BC BW9645)	10%	Sigma-Aldrich
	Penicillin and streptomycin	1%	Thermo Fisher Scientific
	RPMI 1640 medium	500 ml	Pan-Biotech
Erylysis buffer	Potassium bicarbonate ($KHCO_3$)	10 mM	Sigma-Aldrich
	Amoniumchloride (NH_4Cl)	0.15 M	Sigma-Aldrich
	Na_2EDTA	0.1 mM	Thermo Fisher Scientific
	ddH_2O	500ml	Generated in house
	pH 7.3 – 7.4		
FACS buffer	BSA	2.5 g	Merck
	Sodium azide NaN_3	0.1 g	Carl Roth
	PBS 1x	500 ml	Pan-Biotech
Liver digestion solution	Collagenase D	1 mg/ml	Roche
	DNaseI	0.1 mg/ml	Merck
	RPMI 1640 medium	50 ml	Pan-Biotech
MACS buffer	Bovine serum albumin (BSA)	0.5 %	Carl Roth
	Ethylendiaminetetraacetic acid (EDTA)	2 mM	Thermo Fisher Scientific
	PBS 1x	500 ml	Pan-Biotech
Tris-acetate-EDTA (TAE) 50x buffer	Tris	2 M	Applichem
	EDTA	0.5 M	Sigma-Aldrich
	Acetic Acid	5.7%	Merck
	ddH_2O		Generated in house

2.1.5 Devices

Table 2.12: Devices

Device	Company
BD FACS Aria III cell sorter	BD Biosciences
BD FACS LSR II analyser	BD Biosciences
Centrifuge	Heraeus
Computer	HP
FlexCycler2 (PCR cycler)	Analytik Jena
Freezer (-20 °C)	Liebherr
Freezer (-80 °C)	Sanyo
Fridge (4 °C)	Liebherr
Fume hood	Belec Vario Lab
Gel documentary device	INTAS Science Imaging
HT 7900 real-time PCR instrument	Thermo Fisher Scientific
INC153 incubator	Memmert
MACS cell separators (magnets)	Miltenyi Biotec
Nanodrop Nd-1000	Peqlab
SevenCompact pH-meter	Mettler-Toledo
Sterile hood	Thermo Fisher Scientific
Tabletop centrifuge	Thermo Fisher Scientific
Thermomix	Eppendorf
Water bath with shaker	GFL

Flow cytometer configuration:

Table 2.13: BD FACS LSR II analyser configuration.

Laser	Detector	Dichroic Mirror	Bandpass Filter	Bandpass Filter	Primary Fluorochrome	Other Fluorochromes
	E	505 LP	530/30 513/17	530/30 513/17	FITC Alternative: GFP	Alexa Fluor 488, CFSE, YFP
488 nm	D	550 LP	575/26	575/26	PE	Cy3 (!cave: 13% excitation only)
(blue)	C	600 LP	610/20	610/20	PE-TxRed	PE-Dazzle594
Laser 1	B	685 LP 635 LP	695/40 670/14	695/40 670/14	PerCP-Cy5.5 Alternative 1: PerCP	PerCP-eFluor710
	A	735 LP	780/60	780/60	PE-Cy7	PE-Vio770
	F		450/50	450/50	Pacific Blue	Alexa Fluor 405, Brilliant Violet 421, BD Horizon V450
405 nm	E	505 LP	525/50	525/50	AmCyan	BD Horizon V500, Brilliant Violet 510
(violet)	D	600 LP	610/20	610/20	BV605	
Laser 2	C	630 LP	660/20	660/20	BV650	
	B	635 LP	710/50	710/50	BV711	
	A	735 LP	780/60	780/60	BV786	
633 nm	C		660/20	660/20	APC	Alexa Fluor 647, eFuor 660
(red)	B	710 LP	730/45	730/45	Alexa700	
Laser 3	A	755 LP	780/60	780/60	APC-Cy7	APC-eFluor780

Table 2.14: BD FACS Aria III cell sorter configuration.

Laser	Detector	Dichroic Mirror	Bandpass Filter	Bandpass Filter	Primary Fluorochrome	Other Fluorochromes
	E	505 LP	530/30 513/17	530/30 513/17	FITC Alternative: GFP	Alexa Fluor 488, CFSE, YFP
488 nm	D	550 LP	575/26	575/26	PE	Cy3 (!cave: 13% excitation only)
(blue)	C	600 LP	610/20	610/20	PE-TxRed	PE-Dazzle594
Laser 1	B	685 LP 635 LP	695/40 670/14	695/40 670/14	PerCP-Cy5.5 Alternative 1: PerCP	PerCP-eFluor710
	A	735 LP	780/60	780/60	PE-Cy7	PE-Vio770
	F		450/50	450/50	Pacific Blue	Alexa Fluor 405, Brilliant Violet 421, BD Horizon V450
405 nm	E	505 LP	525/50	525/50	AmCyan	BD Horizon V500, Brilliant Violet 510
(violet)	D	600 LP	610/20	610/20	BV605	
Laser 2	C	630 LP	660/20	660/20	BV650	
	B	690 LP	710/50	710/50	BV711	
	A	750 LP	780/60	780/60	BV786	
633 nm	C		660/20	660/20	APC	Alexa Fluor 647, eFuor 660
(red)	B	710 LP	730/45	730/45	Alexa700	
Laser 3	A	755 LP	780/60	780/60	APC-Cy7	APC-eFluor780

2.1.6 General consumables

Table 2.15: Consumables

Consumable	Company
CELLSTAR EASYstrainer (40 µm and 100 µm)	Greiner
Disposable hemocytometer	NanoEntek
Eppendorf tubes (0.2, 0.5, 1.5, 2.5 ml)	Sarstedt
FACS tubes (5 ml)	Sarstedt
Falcon tubes (15 and 50 ml)	Greiner
Liquid reservoir for multichannel pipettes	Integra
Multiwell plates (96-well, 24-well, 6-well)	Greiner
Parafilm N	Carl Roth
PCR plate sealing tape	Sarstedt
Pipette tips	Sarstedt
Pre-Separation Filters (30 µm)	Miltenyi Biotec
Serological pipettes (2ml, 5ml, 10ml and 25ml)	Greiner, Sarstedt
Syringes and needles	Braun, BD Biosciences

2.1.7 Software

Table 2.16: Software

Software	Company
Adobe Illustrator CC (version 24.2.3)	Adobe
Excel	Microsoft
FACSDiva	BD Biosciences
FlowJo (version10)	BD Biosciences
Graph Pad Prism (version 5 and 8)	Graph Pad
RQ Manager 2.1.10	Thermo Fisher Scientific
SDS 2.4	Thermo Fisher Scientific
Windows	Microsoft
Word	Microsoft

2.2 Methods

2.2.1 Mouse breeding

All mice were housed under SPF conditions at the University Medical Center Hamburg-Eppendorf (UKE) either in the central animal facility or in the Center for Molecular Neurobiology Hamburg (ZMNH). Mice were provided with food and water *ad libitum*. Two weeks before an experiment was started, mice were transferred into the institute colony.

Nur77GFP mice were imported from the Jackson Laboratory (Jax). The strain has a C57BL/6N background and all matings were performed between heterozygous (+/T) Nur77GFP and C57BL/6J mice to generate Nur77GFP mice on a C57BL/6J background.

C57BL/6J$^{CAST\ Mr1-/-}$ × RORγtGFP mice were crossed to C57BL/6J mice to generate *Mr1*$^{-/-}$ mice, which lack the RORγtGFP transgene and the CAST locus comprising a 600 base pairs (bp) long region on chromosome 13 encompassing the 3′ end of the Vα locus, TCR-δ, Jα, and Cα segments[154].

2.2.2 Genotyping

Mouse tail biopsies were taken by caretakers of the animal facilities at the UKE. Tails were lysed in 50 µl QuickExtract DNA Extraction Solution at 65 °C for 6 min at 500 rpm followed by 2 min in a Thermomix at 98 °C and 350 rpm. The genotype of mice was determined by polymerase chain reaction (PCR) mainly performed by technicians and students. 2 µl DNA was mixed with dNTPs, DreamTaq Hot Start Green DNA Polymerase, primers, DreamTaq GREEN Hot Start Buffer (10x) and ddH_2O and replicated in a PCR cycler. Agarose gels were prepared in TAE (1x) buffer and heated for about 2 min until the agarose dissolved. RotiSafe (1:20000) was added to the agarose solution and filled into gel chambers for polymerisation. 20 µl PCR sample as well as 6 µl DNA GeneRuler 1kb DNA Ladder were pipetted into gel pockets. Gels ran at 150-180 mV for 15-30 min and PCR product sizes were recorded by a gel documentation system using UV light.

Nur77GFP transgene PCR:

The PCR for the Nur77GFP transgene was performed by adding 2 µl template DNA to a master mix containing 18.03 µl ddH_2O, 0.5 µl dNTPs (10 mM), 2.5 µl DreamTaq Green Hot Start Buffer (10x), 0.17 µl DreamTaq Hot Start Green DNA Polymerase and 0.7 µl of the primers Nur77GFP 12445 rev, Nur77GFP 1244 fwd, Nur77GFP 8745 rev and Nur77GFP 8744 fwd respectively. Amplification in the PCR cycler was performed according to the following protocol: 94 °C for 1.5 min, 10 cycles of 94 °C, 66 °C (-0.5 °C/cycle) and 72 °C for 30 seconds each, 25 cycles of 94 °C, 60 °C and 72 °C for 30 seconds each and 72 °C for 5 min. The PCR generated DNA fragments of a size of 200 base pairs (bp) (+/+) and 300 bp (+/T), which were displayed using a 1.5% agarose gel.

RORγtGFP transgene PCR:

The PCR for the RORγtGFP transgene was performed by adding 2 µl template DNA to a master mix containing 17.75 µl ddH_2O, 0.5 µl dNTPs (10 mM), 2.5 µl DreamTaq Green Hot Start Buffer (10x), 0.25 µl DreamTaq Hot Start Green DNA Polymerase and 1 µl of both primers RORγt3 fwd and RORγt4B rev. Amplification in the PCR cycler was performed according to the following protocol: 94 °C for 5 min, 35 cycles of 94 °C for 30 seconds, 63 °C for 1 min and 72 °C for 1 min, followed by 72°C for 10

min. The PCR generated DNA fragments of a size of 188 base pairs (bp) (+/+) and 887 bp (+/T), which were displayed using a 1.5% agarose gel.

MR1 PCR:
The PCR for the deletion of exon 2 and 3 of the *Mr1* gene resulting in *Mr1*$^{-/-}$ mice was performed by adding 2 µl template DNA to a master mix containing 16.63 µl ddH_2O, 0.5 µl dNTPs (10 mM), 2.5 µl DreamTaq Green Hot Start Buffer (10x), 0.17 µl DreamTaq Hot Start Green DNA Polymerase and 0.8 µl of the primers MR1 rev 10451-10431 and MR1 rev 9188-9168 as well as 1.6 µl of the primer MR1 fwd 8763-8783. Amplification in the PCR cycler was performed according to the following protocol: 94 °C for 5 min, 35 cycles of 94 °C for 30 seconds, 63 °C for 1 min and 72 °C for 1 min, followed by 72°C for 10 min. The PCR generated DNA fragments of a size of 480 base pairs (bp) (+/+) and 400 bp (d/d), which were displayed using a 3% agarose gel.

SNP genotyping for the CAST locus of C57BL/6J^{CAST} mice:
Two SNPs of the CAST locus were identified by using custom made TaqMan SNP genotyping assays (Thermo Fischer) (see Table 2.4). The 40x SNP genotyping assay was diluted with TE buffer (1x) to a 20x working concentration and aliquots were stored at -20 °C. SNP genotyping was performed in 96 well plates. 6.25 µl of 2x genotyping master mix (Thermo Fischer) and 0.62 µl of 20x SNP genotyping assays were added per well and mixed with 5.62 µl of DNA in ddH_2O (3.6 ng/µl). The plate was sealed with a cover, vortexed and briefly centrifuged to remove air bubbles. VIC and FAM signals were measured in a HT 7900 real-time PCR instrument using absolute quantification before the PCR started. The following PCR program was used: 95 °C for 10 min, 40 cycles 92 °C for 15 seconds and 60 °C for 1 min. Afterwards, VIC and FAM signals were measured again and analysed using SDS software (version 2.4).

2.2.3 Experimental autoimmune encephalomyelitis induction

Mice were anesthetised with O_2/CO_2 mixed gas and received two s.c. injections of 100 µl of an emulsion containing a 1:1 mixture of $MOG_{35–55}$ peptide in PBS (200 µg/ml) and complete Freund's adjuvant with 2 mg/ml *Mycobacterium tuberculosis* into flanks of the hind limbs. Every mouse received 200 µg $MOG_{35–55}$ peptide accordingly. In addition, 200 ng pertussis toxin was injected intraperitoneally (i.p.) on the day of immunisation and 48 hours (h) later. EAE-induced mice were handled according to TVA G16/79. Animals were weighed on the day of immunisation and 3 days post immunisation (dpi). After 6 dpi mice were weighed and scored daily by the following system: 0, no clinical deficits; 1, tail weakness; 2, hind limb paresis; 3, partial hind limb paralysis; 3.5, full hind limb paralysis; 4, full hind limb paralysis and forelimb paresis; 5, premorbid or dead. Animals reaching a clinical score of ≥ 4 or having more than 25% body weight loss (from starting weight) had to be sacrificed according to regulations of the Animal Welfare Act.

For blockade of MR1 during EAE, C57BL/6J mice received i.p. injections on day 5, 10 and 15 post immunisation containing either an anti-MR1 blocking antibody (250 µg) in PBS or a respective IgG isotype control (250 µg) in PBS.

2.2.4 Cell isolation

Immune cell isolation from spleen:

Mice were anesthetised with O_2/CO_2 gas mixture and killed with CO_2. The spleen was harvested, transferred to cold PBS and homogenised through a 40 µm cell strainer into a 50 ml tube to obtain a single cell suspension. PBS was added to 50 ml and cells were pelleted by centrifugation (300 g, 10 min, 4 °C). Supernatant was discarded and cells were resuspended in 5 ml of ice-cold erylysis buffer for lysis of red blood cells and incubated for 3min. Lysis was stopped by adding PBS to 50 ml and cells were centrifuged (300 g, 10 min, 4 °C). After discarding the supernatant, cells were resuspended in FACS buffer or complete RPMI medium depending on the follow-up application.

Immune cell isolation from lymph nodes:

Mice were anesthetised with O_2/CO_2 gas mixture and killed with CO_2. Inguinal, brachial and axillary LN were harvested, transferred to cold PBS and homogenised through a 40 µm cell strainer into a 50 ml tube to obtain a single cell suspension. PBS was added to 50 ml and cells were pelleted by centrifugation (300 g, 10 min, 4 °C). After discarding the supernatant, cells were resuspended in FACS buffer or complete RPMI medium depending on the follow-up application.

Immune cell isolation from liver:

Mice were anesthetised with O_2/CO_2 gas mixture and killed with CO_2. Immediately after killing, mice were intracardially perfused with 10 ml ice-cold PBS to remove leukocytes from blood vessels. The liver was harvested and cut with a razor blade to obtain smaller tissue parts. Liver pieces were transferred to a 15 ml tube containing 10 ml liver digestion solution and incubated for 30 min at 37 °C in a shaking water bath. Afterwards, liver pieces were homogenised through a 40 µm cell strainer into a 50 ml tube to obtain a single cell suspension. PBS was added to 50 ml and cells were pelleted by centrifugation (300 g, 10 min, 4 °C). The supernatant was discarded, cells were resuspended in 5 ml of ice-cold erylysis buffer for lysis of red blood cells and incubated for 3 min. Lysis was stopped by adding PBS to 50 ml and cells were centrifuged (300 g, 10 min, 4 °C). After discarding the supernatant, cells were resuspended in 10 ml of a 36% Percoll solution in RPMI medium and transferred to a 15 ml tube. Samples were centrifuged (800 g, 20 min, 4 °C, brake 5). The supernatant was discarded and cells were washed again with 15 ml before they were resuspended in 3 ml FACS buffer or complete RPMI medium depending on the follow-up application.

Immune cell isolation from CNS:

Mice were anesthetised with O_2/CO_2 gas mixture and killed with CO_2. Immediately after killing, mice were intracardially perfused with 10 ml ice-cold PBS to remove leukocytes from blood vessels. Brain and spinal cord were harvested and cut with a razor blade to obtain smaller tissue parts. CNS pieces were transferred to a 15 ml tube containing 10 ml CNS digestion solution and incubated for 45 min at 37 °C in a shaking water bath. Afterwards, CNS pieces were homogenised through a 40 µm cell strainer into a 50 ml tube to obtain a single cell suspension. Cells were washed twice with 50 ml PBS and centrifuged (300 g, 10 min, 4 °C). Cells were resuspended in 4 ml 30% Percoll solution in RPMI medium and 2 ml 78% Percoll solution in PBS was layered underneath. The gradient was centrifuged

(2500 rpm, 30 min, 4 °C, without brake) and immune cells were taken from the gradient interphase. Cells were transferred to a new 15 ml tube, washed with PBS and centrifuged (1800 rpm, 10 min, 4 °C). Cells were resuspended in PBS and centrifuged (1200 rpm, 7 min, 4 °C). After discarding the supernatant, immune cells were resuspended in 1 ml FACS buffer or complete RPMI medium depending on the follow-up application.

2.2.5 Cell culture experiments

Cell counting:
10 µl cell suspension was added to 90 µl of a 10% Trypanblue solution. After mixing, 10 µl were transferred to a disposable hemocytometer (NanoEntek) and either two or four large squares were counted. Blue stained dead cells were excluded.

Magnetic-activated cell sorting (MACS) of T cells:
The pan T cell isolation kit II was used according to the manufacturer's protocol to isolate T cells using a negative selection strategy. Briefly, single cell suspensions of isolated immune cells (see 2.2.4) were centrifuged (300 g, 10 min, 4 °C) and resuspended in MACS buffer (40 µl / 10^7 total cells). Biotinylated antibody cocktail, which labels non-T cells, was added (10 µl / 10^7 total cells). After incubation (5 min, 4 °C), MACS buffer (30 µl / 10^7 total cells) and anti-biotin microbeads were added (20 µl / 10^7 total cells). After vortexing and incubating (10 min, 4 °C), the suspension was transferred to a LD column placed in a strong magnet to hold back all microbead-labelled cells inside the column. All unlabelled T cells in the flow through were collected in a 15 ml tube.

PMA and ionomycin activation for cytokine measurements:
Isolated immune cells or sorted MAIT and non-MAIT T cells from the CNS of C57BL6/J^{CAST} mice in acute EAE were counted and cultured in complete RPMI medium at a cell density of 1 million cells per well in a 48 well round bottom plate. PMA (10 ng/ml) and ionomycin (1 µg/ml) was added directly and the cells incubated at 37 °C and 5% humidity. After 1 h, Monensin solution (2 mM) was added to a final concentration of 2 µM. After 4 hours of incubation, the cells were pooled in a 50 ml tube and washed with PBS (300g, 10, min RT). Subsequently, cells were stained and analysed by flow cytometry (see 2.2.7).

Cytokine- and TCR-dependent activation assays for T cells:
Isolated lymphocytes from spleen and LN of two naïve Nur77GFP mice were counted and cultured in complete RPMI medium at a cell density of 250000 cells per well in a 96 well round bottom plate. Cells were either left unstimulated or were activated by adding the cytokines IL-12 (10 ng/ml) and IL-18 (12.5 ng/ml) or anti-CD3 (clone: 145-2C11, 0.125 µg/ml) and anti-CD28 antibodies (clone: 37.51, 0.125 µg/ml). After 48 h at 37 °C and 5% humidity, cells were washed with PBS (300g, 10, min RT), stained and analysed by flow cytometry (see 2.2.7).

2.2.6 MR1 Tetramer preparation

Biotinylated MR1-5-OP-RU and MR1-6-FP monomers (2mg/ml) were obtained from the National Institutes of Health (NIH), aliquoted and stored at -80 °C. For tetramerisation, 10 µl aliquots were

thawed on ice, gently mixed with 2.11 µl streptavidin-phycoerythrin (SAPE) (1 mg/ml) and incubated for 10 min in the dark at room temperature (RT). This step was repeated 10 times until 21.1 µl streptavidin-PE were added to 10 µl monomers. Tetramers were stored at 4 °C in the fridge. The working concentration varied between 1:300 and 1:800 in different tetramerisation approaches. In order to minimise staining differences, newly prepared tetramers were tested against the older dilutions by analysing LN, spleen or liver cells from naïve C57BL6/J^{CAST} mice by flow cytometry.

2.2.7 Flow cytometry

Identification of dead cells:

Single cell suspensions were transferred to 5 ml FACS tubes and centrifuged (350g, 5 min, RT). After discarding the supernatant, cells were resuspended in 150 µl PBS mixed with fixable dead cell stain solution (1:1000) and incubated for 20 min at 4 °C in the dark. Subsequently, cells were washed with PBS and centrifuged (350g, 5 min, RT). After discarding the supernatant, a surface staining was conducted.

Surface staining:

Up to $2x10^6$ cells were centrifuged (350g, 5 min, RT) and resuspended in 90 µl FACS buffer. MAIT cell staining required a tetramer pre-staining. Therefore, either MR1-5-OP-RU or MR1-6-FP tetramers were added and cells were incubated for 30 min at RT in the dark. Afterwards, 10 µl of a 10 times concentrated master mix solution consisting of Fc receptor block (anti-mouse CD16/32 antibody) and labelled antibodies (see 2.1.3) was added. Cells were stained for 30 min at 4 °C in the dark and the staining was stopped by adding 2 ml FACS buffer followed by centrifugation (350g, 5 min, RT). Cells were either resuspended in 300 µl FACS buffer for direct acquisition, fixed for later measurements or further processed by intracellular or intranuclear stainings. For fixation, cells were resuspended in 150 µl fixation buffer (BD Cytofix) and incubated for 30 min at RT in the dark. Fixation was stopped by adding 2 ml FACS buffer and centrifugation (350g, 5 min, RT). Fixed cells were resuspended in 300 µl FACS buffer and could be stored in the fridge for up to 48 h.

Intracellular staining:

Intracellular stainings were performed to analyse cytokine production of T cells. After surface staining, cells were centrifuged (350g, 5 min, RT) and resuspended in 100 µl fixation buffer (BioLegend). Cells were incubated for 30 min at RT in the dark and the fixation was stopped by adding 2 ml FACS buffer and centrifugation (350g, 5 min, RT). Cells were washed twice (350 g, 5 min, RT) with 1 ml intracellular staining perm wash buffer (BioLegend), which was prediluted 1:10 with ddH_2O. After the second centrifugation step, cells were resuspended in 90 µl intracellular staining perm wash buffer and 10 µl of a 10 times concentrated antibody cocktail including Fc receptor block (anti-mouse CD16/32 antibody) and labelled antibodies (see 2.1.3) were added. After an incubation period of 30 min at 4°C in the dark, cells were washed twice with intracellular staining perm wash buffer (350 g, 5 min, RT) and resuspended in 300 µl FACS buffer for acquisition.

Intranuclear staining:

Intranuclear staining was performed after the surface staining. If cells endogenously expressed GFP like C57BL/6J^{CAST} × RORγtGFP and Nur77GFP reporter mice having a GFP-labelled transgene, cells were fixed with a 3% PFA solution to protect the GFP signal. After centrifugation (350g, 5 min, RT), cells were resuspended in 1 ml 3% PFA solution and incubated for 1h at RT in the dark. Fixation was stopped by adding 3 ml FACS buffer and centrifugation (350g, 5 min, RT). The true-nuclear transcription factor buffer set (BioLegend) was used according to the manufacturer's protocol. Briefly, 1 ml true-nuclear 1x fix concentrate was used to resuspend the cells. After an incubation period of 45-60 min at RT in the dark, 2 ml true-nuclear 1x perm buffer was added to each tube and the tubes were centrifuged (350g, 5 min, RT). Cells were resuspended in 2 ml true-nuclear 1x perm buffer and centrifuged (350g, 5 min, RT). The cell pellet was resuspended in 90 µl true-nuclear 1x perm buffer and 10 µl of a 10 times concentrated master mix solution consisting of Fc receptor block (anti-mouse CD16/32 antibody) and labelled antibodies (see 2.1.3) was added. Cells incubated for 30-60 min at RT in the dark. Afterwards, the reaction was stopped by adding 2 ml true-nuclear 1x perm buffer and cells were washed twice with true-nuclear 1x perm buffer and centrifugation (350g, 5 min, RT). Cell pellets were resuspended in 300 µl FACS buffer for acquisition.

TruCount tubes for cell quantification:

Isolated cells from CNS were resuspended in 1 ml FACS buffer (see 2.2.4) and 50 µl of this cell suspension were transferred to a TruCount tube (BD Biosciences). TruCount tubes contain a defined number of fluorescent beads and can be used to calculate the absolute number of isolated lymphocytes. 50 µl FACS buffer, Fc receptor block (anti-mouse CD16/32 antibody) and anti-CD45-Alexa Fluor 700 antibody (1:100) were added to every tube. After an incubation period of 20 min at 4 °C in the dark, samples were diluted with 300 µl FACS buffer and analysed by flow cytometry. The total number of CD45$^+$ immune cells in the CNS was calculated: CD45$^+$ cells = (total beads per tube / acquired beads) x (acquired CD45$^+$ cells x dilution factor of 20). By initially gating on CD45$^+$ cells in all analyses, this number was used to calculate absolute numbers of all consecutively analysed immune cell subsets.

Sample preparation and staining for cell sorting:

All steps of the cell isolation (see 2.2.4) and staining procedure were performed under sterile conditions and instead of PBS and FACS buffer, PBS containing 10 µM EDTA was used. After the staining procedure, cells were resuspended in 300 µl PBS containing 10 µM EDTA and homogenised with a 40 µm cell strainer to prevent cell aggregations. After rinsing the cell strainer 3 times with 100 µl PBS containing 10 µM EDTA, samples were used for cell sorting. Collection tubes were coated with FCS for 5 min at RT and contained either 2 ml of complete RPMI medium for following cell culture experiments or lysis buffer for RNA isolation.

Gating strategies:

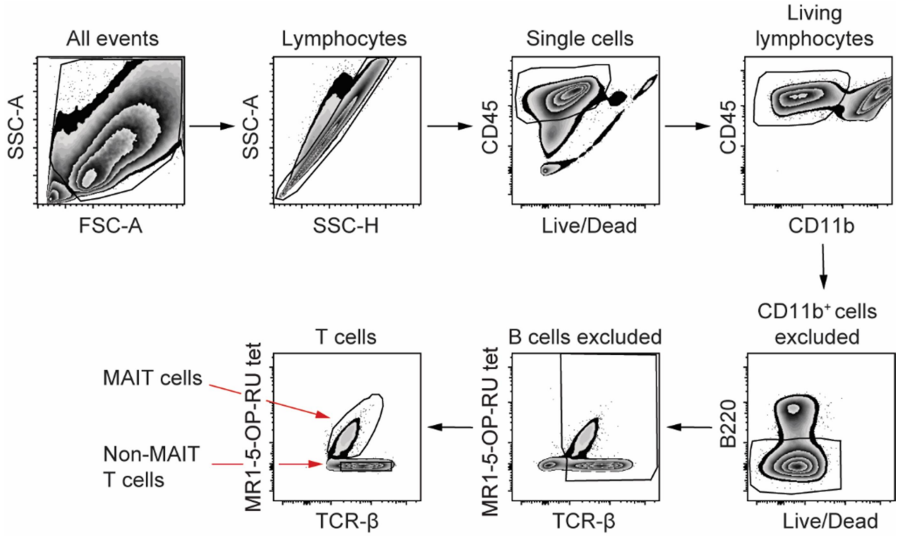

Figure 2.1: Gating strategy for MAIT and non-MAIT T cells. Lymphocytes were identified according to their size and granularity (SSC and FSC). Doublets were excluded by gating height against area of the side scatter. Dead cells were excluded and living CD45^{+} lymphocytes were further analysed by excluding CD11b^{+} and B220^{+} cells. TCR-β^{+} T cells were separated into MR1-5-OP-RU tetramer^{+} MAIT and MR1-5-OP-RU tetramer^{-} non-MAIT T cells.

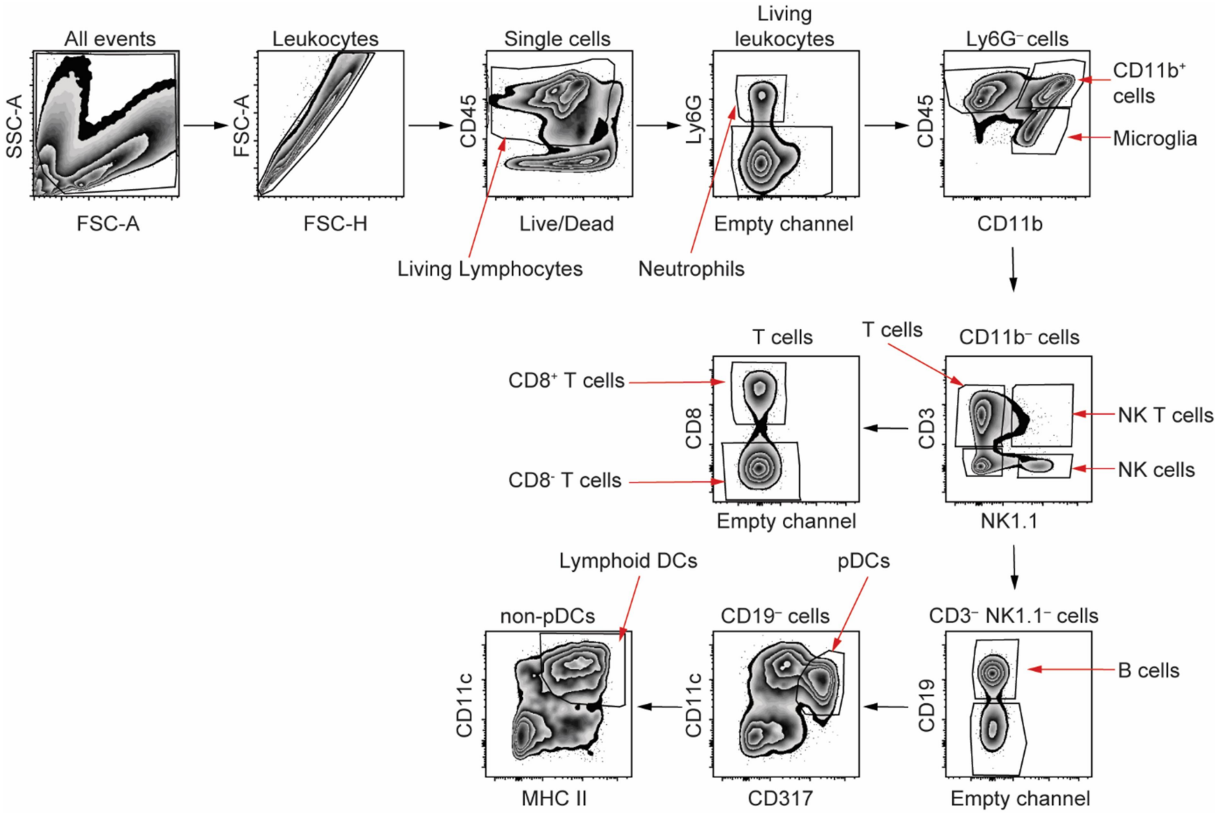

Figure 2.2: Gating strategy for immune cell subsets. Lymphocytes were identified according to their size and granularity (SSC and FSC). Doublets were excluded by gating height against area of the forward scatter. Dead cells were excluded and the living lymphocytes were further analysed by identifying neutrophils (Ly6G^{+}) and microglia (CD45^{low} CD11b^{+}). CD11b^{+} cells comprised mainly macrophages and myeloid DCs, which could not be specifically identified. CD11b^{-} cells were gated by using NK1.1 and CD3 and these cells were classified as T cells (NK1.1^{-} CD3^{+}), NKT cells (NK1.1^{+} CD3^{+}) and NK cells (NK1.1^{+} CD3^{-}). NK1.1^{-} CD3^{-} cells were further separated in B cells (CD19^{+}), plasmacytoid DCs (pDCs) (CD317^{+} CD11c^{+}) and lymphoid DCs (CD11c^{+} MHC II^{+}).

2.2.8 Sample generation for transcriptome analysis of MAIT cells

EAE was induced in C57BL6/J mice by active immunisation (see 2.2.3) and mice were sacrificed in acute EAE having an EAE score above 3. Lymphocytes from CNS and spleen were isolated (see 2.2.4) and cells from five animals were pooled per sample. In addition, the spleen was also taken from naïve C57BL/6J mice and cells from five animals were pooled per sample. T cells from the spleen were enriched by MACS (see 2.2.5). After surface staining of the isolated cells, MAIT cells were sorted in a collection tube containing lysis buffer using the BD FACS Aria III cell sorter. MAIT cells from the spleen of naïve mice (MNS) and from the spleen and CNS of mice in acute EAE (MES and MEC, respectively) were analysed.

Table 2.17: Absolute cell numbers of MAIT cells isolated by cell sorting.

Sample	MAIT Naïve Spleen (MNS)	MAIT EAE Spleen (MES)	MAIT EAE CNS (MEC)
1	6147 cells	3755 cells	8116 cells
2	3612 cells	2248 cells	4614 cells
3	4202 cells	2401 cells	4844 cells
4	2775 cells	3502 cells	5005 cells

The RNA of these cells was isolated by Dr. Jana Sonner. The Qiagen RNeasy MicroKit was used according to the manufacturer's protocol. The RNA was sent to the next-generation sequencing (NGS) integrative genomics core unit (NIG) in Göttingen. RNA-sequencing libraries were prepared using the NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs) with minor modifications in ligation and amplification. Libraries were pooled and sequenced on a HiSeq 4000 sequencer (Illumina) generating 50 base pair single-end reads.

2.2.9 Transcriptome data analysis

The transcriptome data analysis was performed by Dr. Dr. Jan Broder Engler and most of the analyses were performed in the R environment (v.3.6.1) using publicly available packages. The raw read sequences (FASTQ files) were aligned to the Ensembl mouse reference genome (mm10) using the aligner STAR (version 2.5) with default settings[306]. The overlap with annotated gene loci was counted with featureCounts[307]. Contamination was checked by using the FastQ Screen tool to detect reads derived from other genomes. Differential expression analysis was performed with DESeq2 (v.1.26.0) calling genes with a minimal twofold change and false discovery rate (FDR)-adjusted $p<0.05$ differentially expressed[308]. Sample similarity was assessed by using a principal component analysis (PCA) generated from normalized expression values after variance stabilizing transformation in DESeq2 using the top 500 most variable genes.

The Gene Ontology (GO) term analysis was performed using gene set enrichment analysis[309] within the R package clusterProfiler. Input signatures were generated by ranking all expressed genes by the DESeq2-derived *t* statistics of individual comparisons. The main comparisons were MAIT cells from the spleen of mice in acute EAE versus naïve mice (MES vs MNS) as well as MAIT cells in acute EAE from CNS versus spleen (MEC vs MES).

Further gene set enrichment analyses (GSEA) were performed to integrate the results of different datasets. Therefore, gene expression signatures derived from this study as well as published studies were tested for enrichment against gene sets of other published datasets. Gene expression signatures (**Table 2.16**), gene sets (**Table 2.17**) and applied GSEA (**Table 2.18**) are depicted in the following tables.

Table 2.18: Gene expression signatures.

Signature	Description	Reference
MES vs MNS	MAIT cells from the spleen of mice in acute EAE versus naïve mice	Own dataset
MEC vs MES	MAIT cells in acute EAE from CNS versus spleen	Own dataset
CNS-infiltrating $CD4^+$ memory T cells	$CD4^+$ memory T cells in acute EAE from spinal cord versus lymph node	(GSE80978)[310]
CNS-infiltrating Th17 cells	Th17 cells in acute EAE from cerebellum versus lymph node	(GSE125144)[311]

Table 2.19: Gene sets.

Gene set	Description	Reference
TCR-activated MAIT cells	First 200 differentially expressed genes (DEG) according to *t* statistics of MAIT cells activated via their TCR compared to unstimulated MAIT cells	[146]
Cytokine-activated MAIT cells	First 200 DEG according to *t* statistics of MAIT cells activated by cytokines compared to unstimulated MAIT cells	[146]
TCR- and cytokine-activated MAIT cells	First 200 DEG according to *t* statistics of MAIT cells activated via their TCR and by cytokines compared to unstimulated MAIT cells	[146]
Tissue repair Linehan *et al.*, 2018	105 tissue repair associated genes	[312]
Tissue repair database (TiReDB)	First 200 alphabetically ordered genes from the TiReDB	[313]
Pathogenic Th17 cells	First 200 DEG according to *t* statistics of pathogenic Th17 cells compared to non-pathogenic Th17 cells	(GSE39820, samples T16_60hr, rep 1-4 and T36_60hr, rep 1-4)[233]

Table 2.20: Gene set enrichment analyses.

Signature name	Gene set name	Figure reference
MES vs MNS	TCR-activated MAIT cells Cytokine-activated MAIT cells TCR- plus cytokine-activated MAIT cells	Figure 3.6 A
MEC vs MES	TCR-activated MAIT cells Cytokine-activated MAIT cells TCR- plus cytokine-activated MAIT cells	Figure 3.6 B
MES vs MNS	Tissue repair Linehan *et al.*, 2018 Tissue repair database (TiReDB)	Figure 3.13 B
MEC vs MES	Tissue repair Linehan *et al.*, 2018 Tissue repair database (TiReDB)	Figure 3.13 C
CNS-infiltrating $CD4^+$ memory T cells	Tissue repair Linehan *et al.*, 2018 Tissue repair database (TiReDB)	Figure 3.13 D
CNS-infiltrating Th17 cells	Tissue repair Linehan *et al.*, 2018 Tissue repair database (TiReDB)	Figure 3.13 E
MES vs MNS	Pathogenic Th17 cells	Figure 3.14 A
MEC vs MES	Pathogenic Th17 cells	Figure 3.14 B

2.2.10 Statistical analysis

Statistical analyses were performed with Graph Pad Prism 5 and 8. Differences between two groups were investigated by Mann-Whitney-U-test. Comparisons between more than two groups or two groups under multiple conditions were performed by one-way and two-way ANOVA analyses with a Bonferroni *post hoc* test. Significant results were marked by asterisks: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

EAE experiments were analysed in the following way. If mice died during the experiment or had to be sacrificed before the end of the experiment, the average score of the group was carried forward for statistical analysis. The cumulative clinical score per animal was calculated and a ROUT outlier analysis (Q = 5%) was performed. The difference between the groups was tested by Mann-Whitney-U-test.

3 Results

3.1 Establishing a model to study MAIT cells in EAE

The specific identification of MAIT cells in mice became only recently feasible after MR1 tetramers were developed, which enabled us to specifically identify and functionally characterise MAIT cells in EAE. To clarify whether MAIT cells infiltrate the inflamed CNS, EAE was induced in C57BL/6J and *Mr1*$^{-/-}$ mice by active immunisation. Lymphocytes isolated from LN and CNS of naïve mice and mice at different timepoints of EAE were analysed by flow cytometry. MAIT cells were defined as MR1-5-OP-RU tetramer positive cells among T cells (CD45^{+}CD11b^{-}CD45R^{-}TCR-β^{+}). The specificity of the MAIT cell staining was verified by using the MR1-6-FP tetramer and *Mr1*$^{-/-}$ mice as negative controls (**Fig. 3.1 A**). MAIT cells infiltrated the inflamed CNS in acute EAE and were significantly enriched in the CNS compared to LN from naïve mice (p=0.0002) or mice in acute EAE (p<0.0001) (**Fig. 3.1 B**). Moreover, MAIT cells already infiltrated the CNS during preclinical EAE (7 days post immunisation (dpi), score 0). The highest MAIT cell infiltration of the CNS was observed during acute EAE (mean 3.42), when their frequency was significantly increased compared to the uninflamed CNS of naïve mice (p=0.0278) (**Fig. 3.1 C**). Therefore, the EAE model in C57BL/6J mice is well suited to investigate mechanisms and effects of CNS infiltration by MAIT cells in an autoimmune setting.

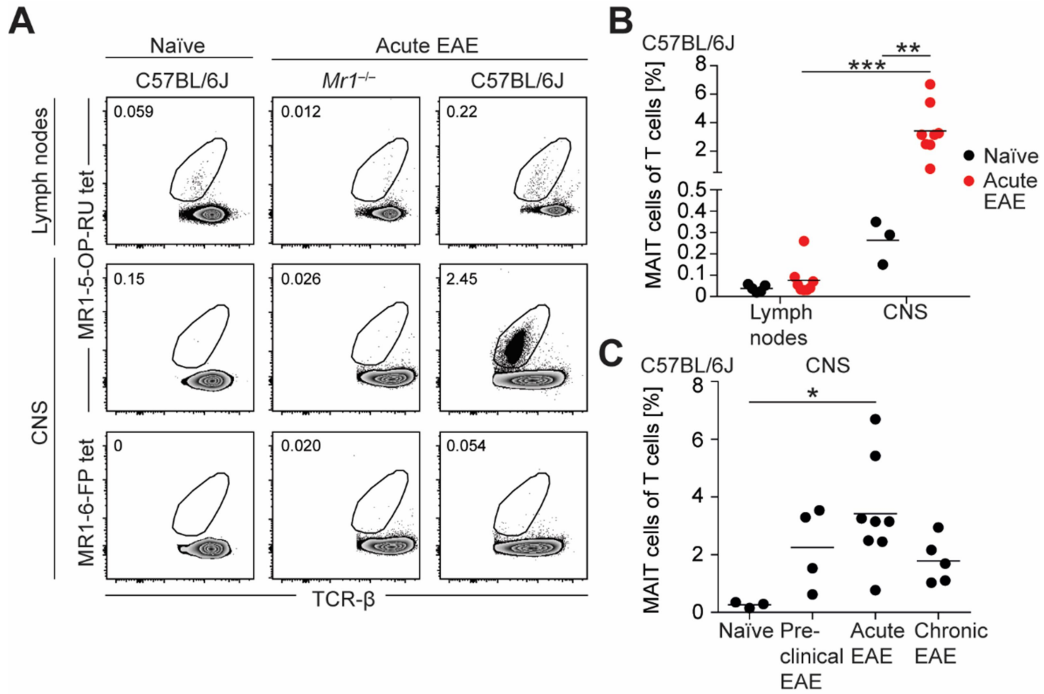

Figure 3.1: CNS infiltration of MAIT cells in EAE. EAE was induced in C57BL/6J and Mr1$^{-/-}$ mice by active immunisation. MAIT cells were analysed by flow cytometry gating on living CD45^{+}CD11b^{-}CD45R^{-}TCR-β^{+}MR1-5-OP-RU tetramer^{+} cells. (A) MAIT cells from lymph nodes (LN) and central nervous system (CNS) of naïve mice and mice in acute EAE were quantified. To verify the specificity of the staining, MR1-6-FP-tetramer was used as a negative control. (B) Frequency of MAIT cells in CNS (n=3) and LN (n=5) from naïve mice and mice with acute EAE (n=8). (C) MAIT cell frequency in the CNS of naïve C57BL/6J mice (n=3) and C57BL/6J mice in preclinical EAE (n=4, 7 days post immunisation (dpi), clinical disease score 0), in acute EAE (n=8, 13-15 dpi, clinical disease score ≥ 3) and in chronic EAE (n=5, 30 dpi, clinical disease score 1.25-2.5). For CNS analyses of naïve and preclinical EAE two independent experiments were performed and five animals were pooled per data point. Statistics: Mann-Whitney-U-test, one-way-ANOVA; *p < 0.05, ***p < 0.001.

As the frequency of MAIT cells in C57BL/6J mice is low in comparison to humans, we made use of C57BL6/J^{CAST} mice, which harbour an increased MAIT cell frequency[154]. To verify a higher MAIT cell frequency in C57BL6/J^{CAST} mice also in our animal facility, lymphocytes from spleen, LN, liver and CNS from naïve C57BL6/J (**Fig. 3.2 A**) and C57BL6/J^{CAST} mice (**Fig. 3.2 B**) were analysed by flow cytometry for their MAIT cell frequency. The staining specificity was again controlled by the MR1-6-FP tetramer as a negative control. Both laboratory mouse strains, C57BL6/J and C57BL6/J^{CAST}, had low MAIT cell frequencies being below 0.5% of all T cells in spleen, LN and CNS. C57BL6/J^{CAST} mice had a significantly increased MAIT cell frequency compared to C57BL6/J mice in the liver (p<0.0001), the analysed tissue with the highest MAIT cell frequency in naïve mice (**Fig. 3.2 C**).

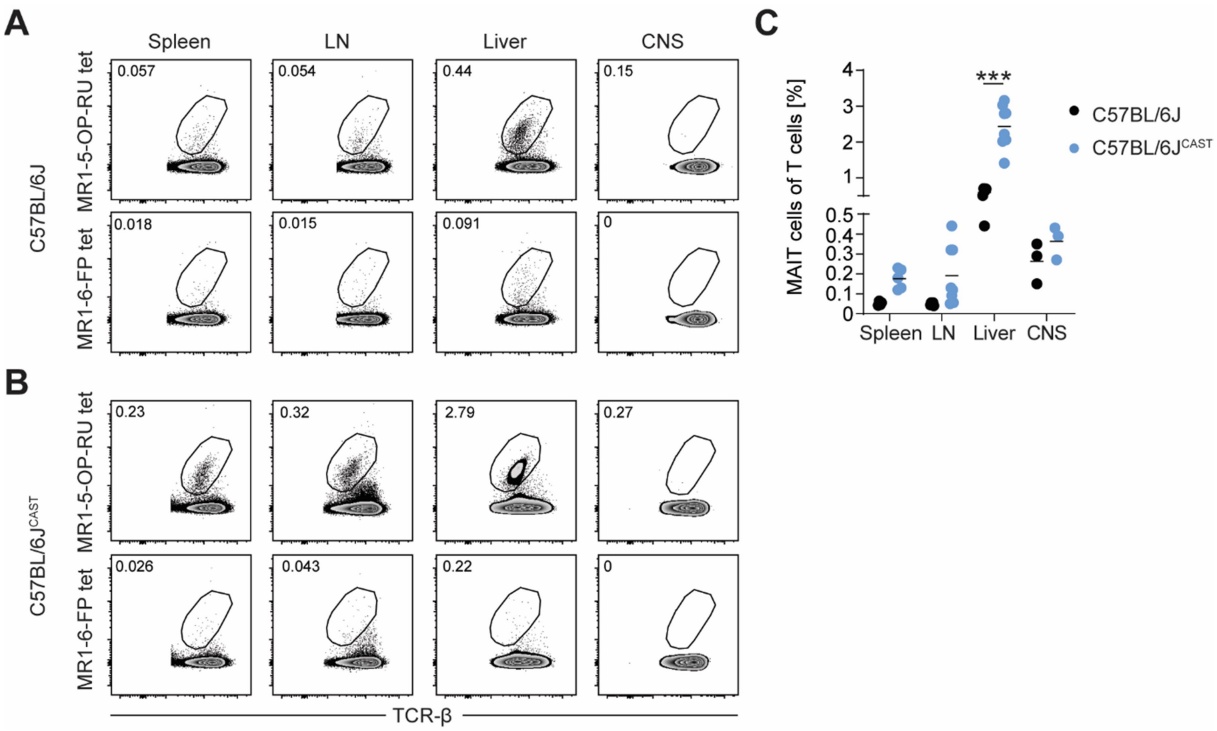

Figure 3.2: Higher MAIT cell frequency in C57BL/6J^{CAST} mice compared to C57BL/6J mice. MAIT cell frequencies of naïve C57BL/6J (A) and C57BL/6J^{CAST} (B) mice were quantified in spleen (n=4, n=5), lymph nodes (LN) (n=5, n=8), liver (n=5, n=8) and central nervous sytems (CNS) (n=3, n=3) by flow cytometry (C). Data from three independent experiments were pooled. MAIT cells were identified by gating on living CD45^{+}CD11b^{-}CD45R^{-} TCR-β^{+}MR1-5-OP-RU-tetramer^{+} cells. To verify the specificity of the staining, MR1-6-FP tetramer was used as a negative control. Statistics: two-way-ANOVA; ***p < 0.001.

To investigate if an increased MAIT cell frequency in naïve mice also results in a higher CNS infiltration of MAIT cells in EAE, C57BL6/J and C57BL6/J^{CAST} mice were immunised and the frequency of CNS-infiltrating MAIT cells was analysed by flow cytometry in acute EAE. Data were obtained from 2016 to 2019 (**Fig. 3.3 A, B**). C57BL6/J^{CAST} mice in 2016 had the highest MAIT cell frequency in the CNS (mean 7.24%), which was significantly increased (p=0.0006) compared to C57BL/6J mice in 2016 (mean 2.76%). However, this difference was lost in 2017, 2018 and 2019 due to a decreased frequency of CNS-infiltrating MAIT cells in C57BL6/J^{CAST} mice, which was significantly reduced in 2018 (p<0.0001) and 2019 (p<0.0001) compared to 2016 (**Fig. 3.3 B**). To exclude that the decreased MAIT cell frequency in the CNS of mice in acute EAE resulted from a generally diminished MAIT cell frequency in C57BL6/J^{CAST} mice, the MAIT cell frequency from the liver of naïve C57BL/6J and

C57BL6/J^{CAST} mice was compared again in 2019 showing still a significant difference (p=0.0079) (**Fig. 3.3 C**).

In summary, MAIT cells infiltrated the inflamed CNS in EAE and were specifically identified with the MR1-5-OP-RU tetramer. Since C57BL/6J mice in contrast to C57BL6/J^{CAST} mice had a stable frequency of CNS-infiltrating MAIT cells in acute EAE over time, these mice were used for the majority of the following experiments to characterise MAIT cells in EAE.

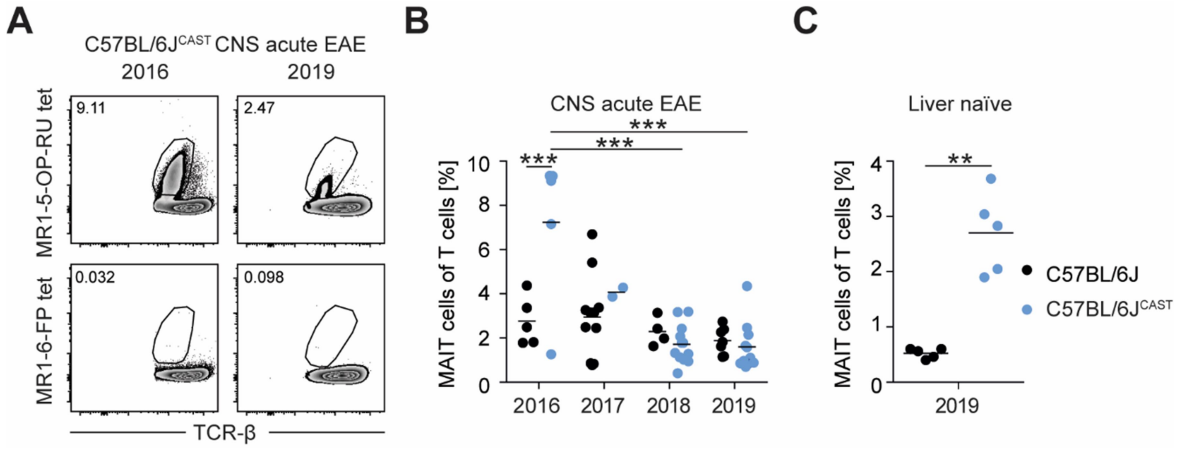

Figure 3.3: Decreased MAIT cell infiltration in the CNS of C57BL/6J^{CAST} mice in acute EAE over time. (A, B) EAE was induced in C57BL/6J and C57BL/6J^{CAST} mice by active immunisation. (A, B) CNS-infiltrating MAIT cells were quantified by flow cytometry in 2016 (n=5, n=5), 2017 (n=11, n=2), 2018 (n=4, n=12) and 2019 (n=7, n=11). Data from eight independent experiments are depicted. (C) MAIT cell frequency in the liver of C57BL/6J and C57BL/6J^{CAST} mice in 2019 (n=5, n=5). MAIT cells were identified by gating on living CD45^{+}CD11b^{-}CD45R^{-}TCR-β^{+}MR1-5-OP-RU-tetramer^{+} cells and the staining specificity was controlled by MR1-6-FP tetramer staining. Statistics: Mann-Whitney-U-test, two-way-ANOVA; $^{**}p < 0.01$, $^{***}p < 0.001$.

3.2 Characterisation of CNS-infiltrating MAIT cells in EAE

Establishing a model to specifically investigate MAIT cells in EAE, enabled for the first time to perform a functional characterisation and to study MAIT cell subsets, their activation pathways and effector functions in the CNS. For this purpose two different methods were used, flow cytometry and transcriptome analysis. Flow cytometry enables to investigate the protein level of markers of interest by antibody stainings. For the transcriptome data, a bulk mRNA sequencing of isolated MAIT cells from the spleen of naïve mice (MNS) and the spleen and CNS of mice in acute EAE (MES and MEC, respectively) was performed. The MNS, MES and MEC datasets consisted of four biological replicates each comprising pooled MAIT cells from five mice. The transcriptome of these samples was analysed by a principle component analysis (PCA), which reduced dimensionality and complexity of large datasets and enabled to estimate and visualise general differences between samples. The PCA showed that the MAIT cell samples within the MNS, MES and MEC datasets were similar and clustered together, whereas the different datasets were separated (**Fig. 3.4 A**). Comparing MAIT cells isolated from the spleen of mice in acute EAE (MES) with MAIT cells from the spleen of naïve mice (MNS) revealed differentially expressed genes (DEG) altered by EAE induction. The expression of 839 genes was different between the MES vs MNS datasets including 639 up- and 230 downregulated genes (**Fig. 3.4 B**). The differences of MAIT cells in the CNS compared to the spleen of mice in acute

EAE was analysed in the MEC vs MES datasets uncovering 1515 increased and 825 diminished genes (**Fig. 3.4 C**). Directly comparing the MEC and MNS datasets included both differences, CNS vs spleen and EAE vs naïve, and led to 3482 DEG including 2319 up- and 1163 downregulated genes (**Fig. 3.4 D)**, which were possibly the summed-up changes of the two other comparisons.

In summary, these analyses revealed massive differences between the datasets indicating that MAIT cells strongly changed after EAE induction and in the inflamed CNS. The DEG of MES vs MNS and MEC vs MES were used for further investigations to better disentangle either EAE induced or CNS specific changes in the datasets.

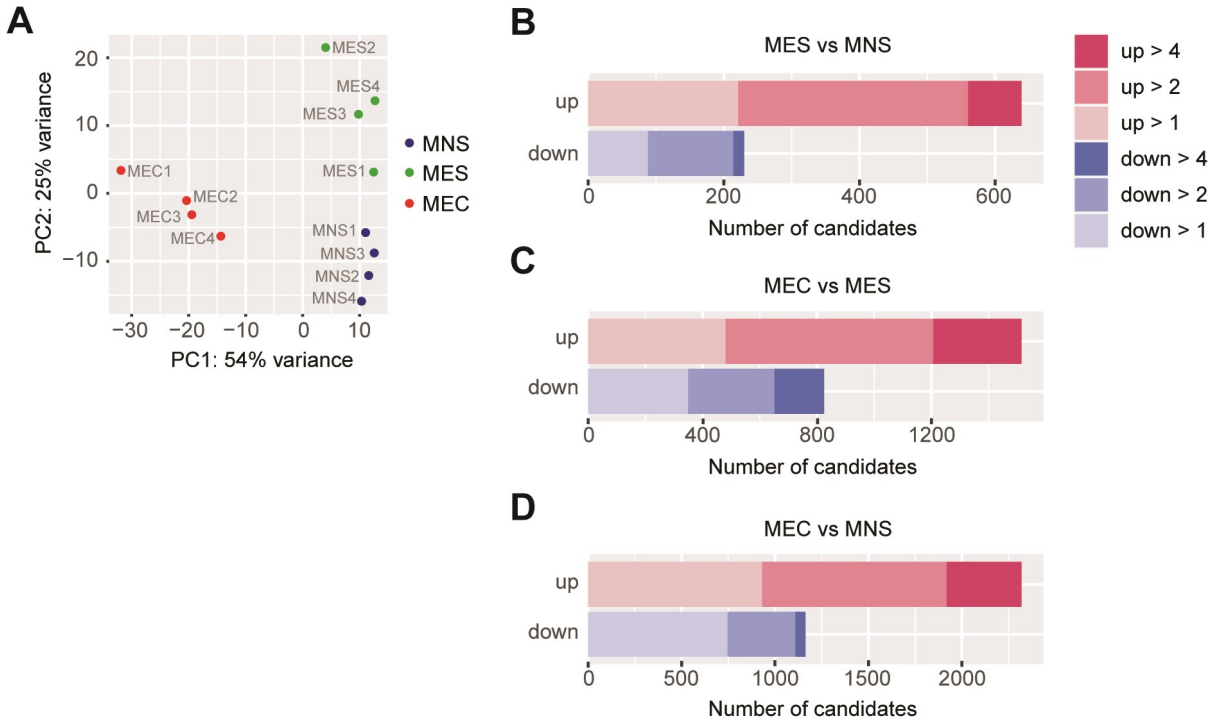

Figure 3.4: Strong differences in the MAIT cell transcriptome between MNS, MES and MEC datasets. Transcriptome data from MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively) were analysed. (A) A principal component analysis (PCA) was performed. (B-D) The differentially expressed genes between MES vs MNS (B), MEC vs MES (C) and MEC vs MNS datasets were shown as log2 fold changes and separated into up- and downregulated genes.

3.2.1 Activation of CNS-infiltrating MAIT cells in EAE

Activation of T cells is a required step for fulfilling their effector function. The frequency of activated MAIT cells represents and estimates how many MAIT cells potentially contribute to the later addressed effector functions of MAIT cells in EAE. Therefore, the expression of the early activation marker CD69 was investigated by flow cytometry (**Fig. 3.5 A-C**). The fluorescent minus one (FMO) sample lacking the CD69 antibody controlled for data spreading due to the use of multiple fluorochromes possibly altering the measured CD69 signal (**Fig. 3.5 A**). CD69 expression by MAIT cells was compared to non-MAIT CD44$^+$ T cells, since MAIT cells have a memory phenotype indicated by CD44 expression. The activation of MAIT and non-MAIT CD44$^+$ T cells was increased in the inflamed CNS in acute and chronic EAE compared to the CNS of naïve mice. Furthermore, MAIT cells in the CNS were already activated during EAE onset compared to the CNS of naïve mice (p=0.0032). During acute EAE, MAIT

cells (mean 81.7% CD69^{+}) were activated to a higher extent than non-MAIT CD44^{+} T cells (mean 58.0% CD69^{+}) (p<0.0001) (**Fig. 3.5 B)**. Moreover, this strong activation of MAIT cells was specific for the CNS and was not observed in LN. The frequency of CD69-expressing MAIT cells in the CNS of mice in acute EAE was significantly higher compared to MAIT cells from LN of naïve mice (p<0.0001) or mice in acute EAE (p<0.0001) (**Fig. 3.5 C**). In line with the flow cytometry data, the transcriptome data showed that the highest expression of CD69 by MAIT cells occurred in the CNS in acute EAE (**Fig. 3.5 D**).

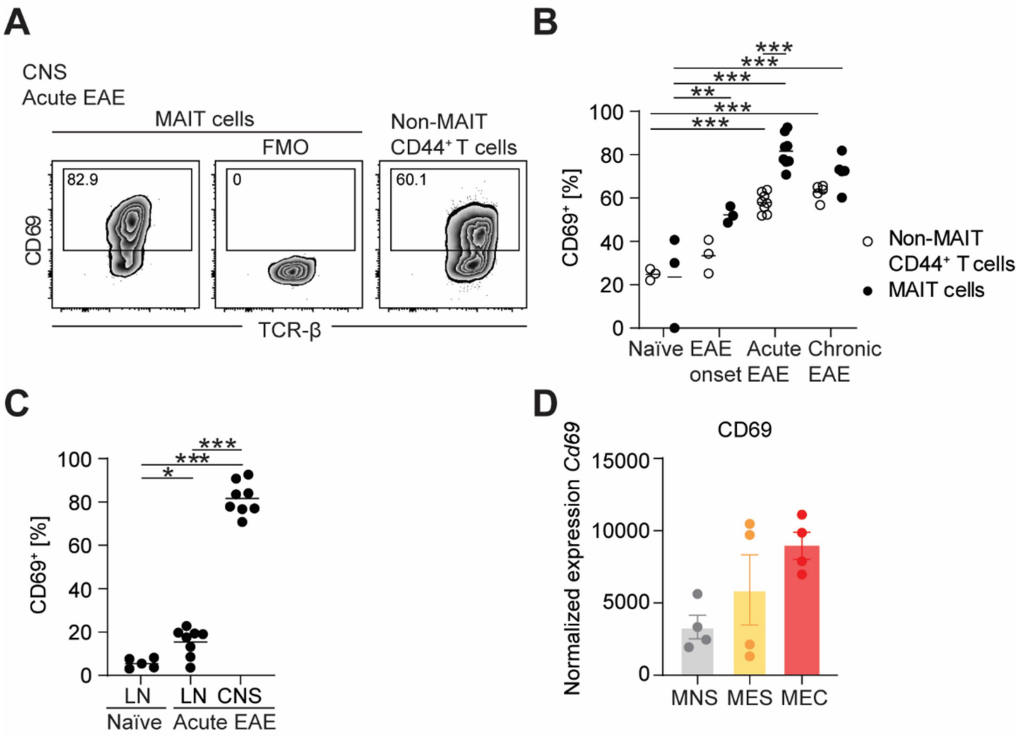

Figure 3.5: CNS-infiltrating MAIT cells were strongly activated in EAE. EAE was induced in C57BL/6J mice by active immunisation. MAIT cells were analysed by flow cytometry (A-C) or isolated by cell sorting for RNA isolation and transcriptome analysis (D). MAIT cells were identified by gating on living CD45^{+}CD11b^{-}CD45R^{-} TCR-β^{+}MR1-5-OP-RU tetramer^{+} cells. (A) During acute EAE, T cell activation reflected by CD69 expression was compared between MAIT and non-MAIT CD44^{+} T cells and a fluorescent minus one (FMO) control of the CD69 antibody was used for exclusion of false positive signals. (B) CD69 expression of non-MAIT CD44^{+} T cells and MAIT cells was quantified in naïve mice (n=3) and in mice at EAE onset (n=3), in acute EAE (n=8) and in chronic EAE (n=5). For CNS analyses of naïve mice and mice at EAE onset two independent experiments were performed and five animals were pooled per data point. (C) The frequency of CD69-expressing MAIT cells was quantified in CNS (n=8) and lymph nodes (LN) (n=8) in acute EAE and in LN of naïve mice (n=5). (D) Transcriptome data represent normalized expression of *Cd69* by MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively). Statistics: one-way-ANOVA, two-way-ANOVA; *p < 0.05, **p < 0.01, ***p < 0.001.

To clarify whether the strong activation of CNS-infiltrating MAIT cells in acute EAE resulted from TCR-dependent activation, cytokine-dependent activation or a combination of both, the transcriptome data was compared with published datasets of differntially activated MAIT cells. The DEG of the MES vs MNS and MEC vs MES datasets were compared to published gene lists containing transcriptome data of human MAIT cells activated *in vitro* either via their TCR or cytokines or both and compared to unstimulated MAIT cells[146]. Gene set enrichment analysis (GSEA) revealed that DEG of all three

activation conditions were enriched in MAIT cells from the periphery during EAE compared to the healthy state (MES vs MNS) (**Fig. 3.6 A**) and MAIT cells from the inflamed CNS in comparison to the periphery (MEC vs MES) (**Fig. 3.6 B**) having an enrichment score of about 0.6. Therefore, MAIT cells in EAE, from the CNS and spleen showed signs of TCR as well as cyokine mediated activation (**Fig. 3.6**).

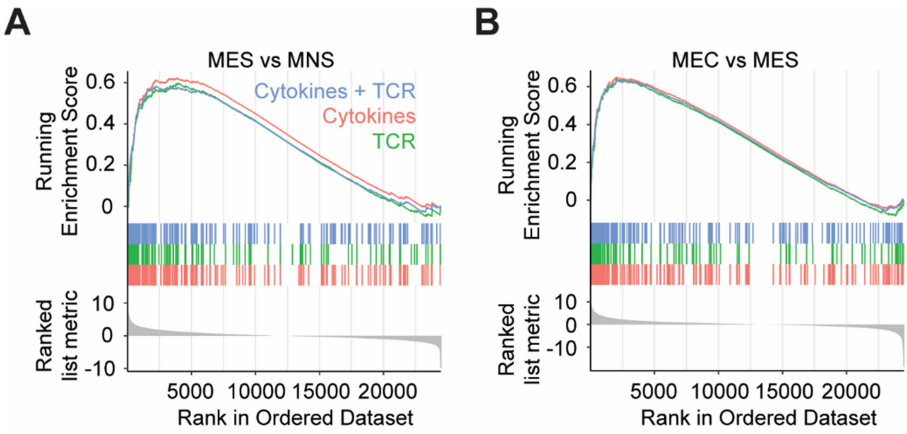

Figure 3.6: MAIT cells in EAE showed signs of TCR and cytokine-mediated activation. Transcriptome data from MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively) were analysed. Gene lists containing differentially expressed genes (DEG) between MES vs MNS (A) as well as MEC vs MES (B) were compared by gene set enrichment analyses (GSEA) to published gene sets containing DEG of human MAIT cells activated via their TCR, by cytokines or by both stimuli in comparison to unstimulated MAIT cells[146].

To further investigate TCR-mediated activation of MAIT cells, transgenic nuclear hormone receptor 77 GFP (Nur77GFP) reporter mice were analysed, which transiently expressed GFP after TCR-dependent activation, but not in response to other inflammatory stimuli like LPS and cytokines[314,315]. To verify that Nur77GFP-expressing MAIT and non-MAIT CD44$^+$ T cells could be identified by flow cytometry and to confirm that the Nur77GFP signal is induced after a TCR-dependent activation, an *in vitro* activation experiment was performed. For this purpose, single cell suspensions from LN and spleens from naïve Nur77GFP reporter mice were analysed for Nur77GFP expression by flow cytometry either directly *ex vivo* or after two days of *in vitro* culture (**Fig. 3.7**). Only 5.13% of MAIT and 12.3% of non-MAIT CD44$^+$ T cells expressed Nur77GFP *ex vivo*. In both cell types, the frequency of Nur77GFP expression was reduced to either 4.98% or 10.1% after two days in culture without activation. This background level of Nur77GFP could be mediated by a TCR-dependent activation of APCs still present in the lymphocyte culture as well as remaining signal from the *ex vivo* situation. In contrast to the strong upregulation of Nur77GFP expression in MAIT (90.9%) and non-MAIT CD44$^+$ T cells (86.7%) after the TCR-dependent activation via anti-CD3 and anti-CD28 antibodies, only a slight upregulation was observed for MAIT (11.3%) and non-MAIT CD44$^+$ T cells (19.9%) after the cytokine-dependent activation via IL-12 and IL-18 (**Fig. 3.7**). The origin of the Nur77GFP signal after cytokine activation remained elusive and can be addressed by repeating the experiment with sorted MAIT and non-MAIT CD44$^+$ T cells. However, the Nur77GFP signal of MAIT and non-MAIT CD44$^+$ T cells activated via anti-CD3 and anti-CD28 antibodies showed that Nur77GFP$^+$ and Nur77GFP$^-$ cells could be clearly distinguished by flow cytometry. Nur77GFP reporter mice showed a strong GFP signal after

in vitro TCR-dependent activation, not observed by cytokine-dependent activation. Therefore, Nur77GFP reporter mice were used for further EAE experiments to disentangle MAIT cell activation in EAE.

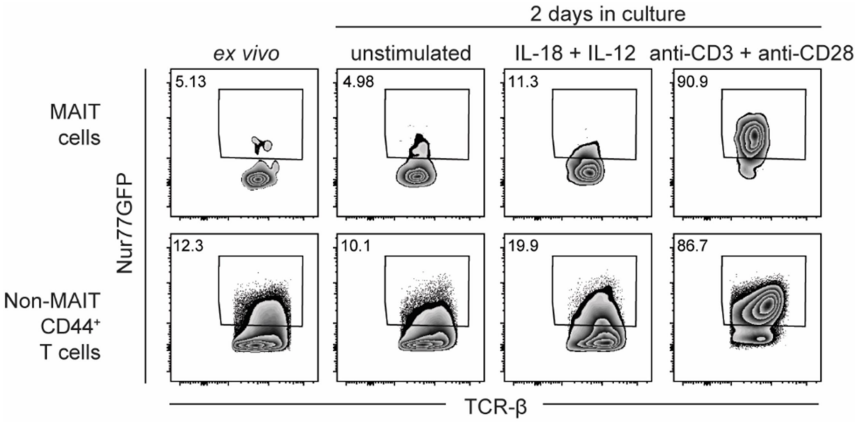

Figure 3.7: Nur77GFP expression of T cells after TCR-dependent activation *in vitro*. Single cell suspensions from pooled lymph nodes and spleens from two Nur77GFP reporter mice were analysed for Nur77GFP expression via flow cytometry either directly *ex vivo* or after two days of *in vitro* culture. The cells were left unstimulated or were activated either by IL-12 (PeproTech, 10 ng/ml) and IL-18 (MBL, 12.5 ng/ml) or with anti-CD3 (BioLegend, clone: 145-2C11, 0.125 µg/ml) and anti-CD28 antibodies (BioLegend, clone: 37.51, 0.125 µg/ml). MAIT cells were identified by gating on living $CD45^+CD11b^-CD45R^-TCR\text{-}\beta^+MR1\text{-}5\text{-}OP\text{-}RU\text{-}tetramer^+$ cells.

EAE was induced in Nur77GFP reporter mice to analyse if EAE induction was sufficient to cause a detectable Nur77GFP signal comparable to the *in vitro* activation and if CNS-infiltrating MAIT cells were activated via their TCR. The verification of the Nur77GFP signal was done by including non-transgenic littermate controls (+/+) (**Fig. 3.8 A**). The Nur77GFP signal in CNS-infiltrating non-MAIT $CD44^+$ T cells increased with EAE severity being significantly upregulated in acute EAE compared to naïve mice (p=0.0091) (**Fig. 3.8 B**). In contrast, the highest Nur77GFP signal of CNS-infiltrating MAIT cells was observed in preclinical EAE significantly upregulated compared to naïve mice (p=0.0033) and mice in acute EAE (p=0.0119). Furthermore, the frequency of Nur77GFP-expressing MAIT cells from the CNS of mice in preclinical EAE was higher compared to non-MAIT $CD44^+$ T cells (p=0.0292) indicating that TCR-dependent MAIT cell activation mainly occurred in the early phase of EAE. TCR-dependent activation of MAIT cells most likely did not take place in the LN, since the frequency of Nur77GFP-expressing MAIT cells in LN of naïve mice was low (mean 4.8%) and did not increase at any measured time point during EAE (**Fig. 3.8 C**). In contrast, the frequency of Nur77GFP-expressing non-MAIT $CD44^+$ T cells in LN was increased in preclinical EAE compared to naïve LN (p=0.0165) and decreased significantly in acute EAE compared to preclinical EAE (p<0.0001) or naïve mice (p=0.0241). This fits to the common conception that classical MOG-specific T cells get TCR-dependently activated in LN during the early phase of EAE and leave the LN to infiltrate the CNS, while the frequency of $Nur77GFP^+$ non-MAIT $CD44^+$ T cells decreased in LN after preclinical EAE and increased in the CNS with ongoing EAE (**Fig. 3.8 B, C**). Directly comparing the Nur77GFP frequency of MAIT and non-MAIT $CD44^+$ T cells in LN and CNS in acute EAE uncovered a strong increase in Nur77 signal in the CNS in both cell types (**Fig. 3.8 D**). This finding was further strengthened by a high

expression of the *Nr4a1* gene (Nur77) in CNS-infiltrating MAIT cells in acute EAE (MEC) revealed by the transcriptome data (**Fig. 3.8 E**).

In summary, CNS-infiltrating MAIT cells in EAE were strongly activated shown by significantly increased CD69 expression in acute EAE compared to non-MAIT CD44$^+$ T cells or MAIT cells from LN (**Fig. 3.5**). Nur77GFP mice and the transcriptome data uncovered that the activation of MAIT cells in the inflamed CNS was partially induced via their TCR (**Fig. 3.8)**. Moreover, the difference between the high frequency of CD69-expressing MAIT cells in the CNS of mice in acute EAE (mean 81.7%) (**Fig. 3.5**) and the lower Nur77GFP signal (mean 21.69%) (**Fig. 3.8**) indicated that next to the TCR-dependent also the cytokine-dependent activation possibly contributed to MAIT cell activation in EAE further supported by the results of the GSEA (**Fig. 3.6**).

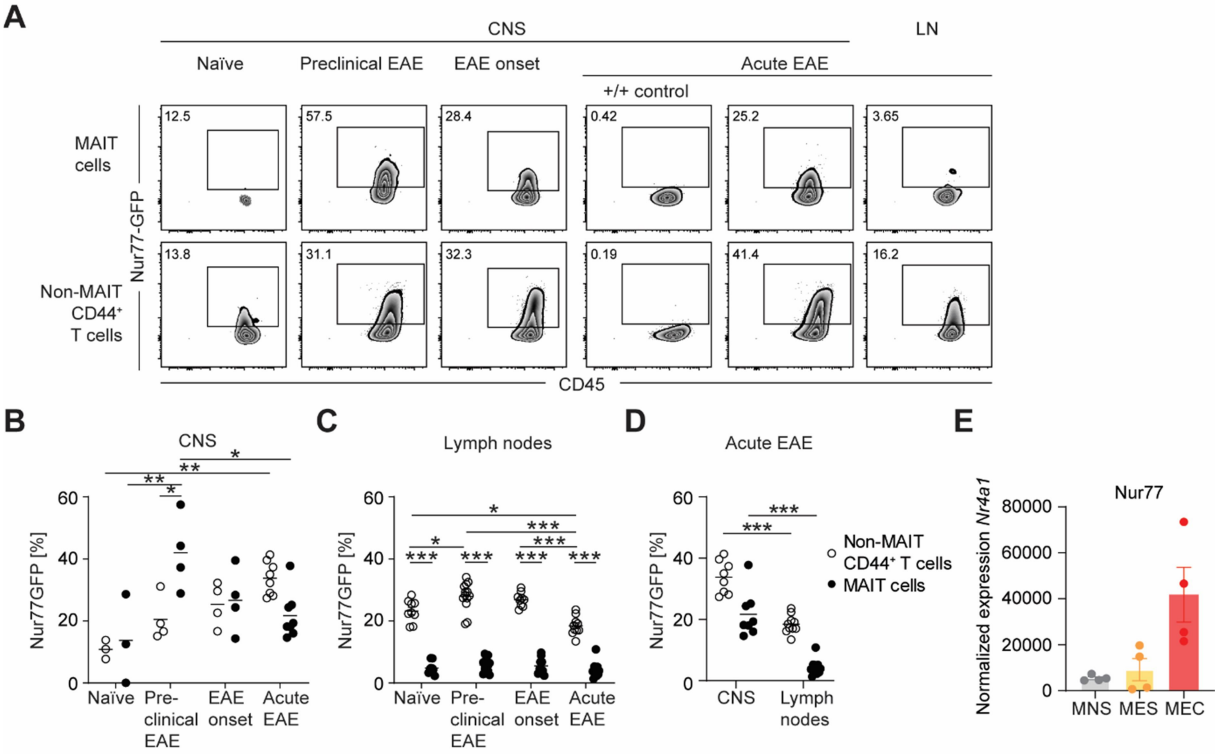

Figure 3.8: CNS-infiltrating MAIT cells were partially activated via their TCR. EAE was induced in Nur77GFP reporter mice and wildtype (+/+) littermates by active immunisation. (A-D) MAIT and non-MAIT CD44$^+$ T cells isolated from the CNS and lymph nodes (LN) were analysed for Nur77GFP expression by flow cytometry. Data from three different experiments were pooled. Isolated cells from five naïve mice and from three mice in preclinical EAE were respectively pooled for a CNS sample. (B-C) Nur77 expression by MAIT and non-MAIT CD44$^+$ T cells from the CNS (B) and LN (C) was quantified in naïve mice (n=3, n=9) and in mice in preclinical EAE (n=4, n=13, EAE disease score 0), at EAE onset (n=4, n=9, EAE disease score 0.5-1.75) and in acute EAE (n=8, n=10, EAE disease score 2.5-3.5). (D) Direct comparison of MAIT and non-MAIT CD44$^+$ T cells isolated from the CNS (n=8) and LN (n=10) in acute EAE. (E) Transcriptome data represent the normalized expression of *Nr4a1* between MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively). Statistics: two-way-ANOVA; * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$

3.2.2 Analysis of specific MAIT cell subsets in the inflamed CNS

Next, CNS-infiltrating MAIT cells subsets were investigated to uncover if a MAIT cell subset was specifically enriched in the CNS of mice in acute EAE or if MAIT cells independently of their subclassification accumulated in the CNS. Published studies classified MAIT cells by expression of the

TCR-β chains Vβ6 and Vβ8, the TCR coreceptors CD4 and CD8 and the transcription factors T-bet and RORγt[126,134,135].

Since the amount of MAIT cells in the CNS of naïve mice was too low for further classifications, only CNS-infiltrating MAIT cells from mice in acute EAE were analysed by flow cytometry. In line with published studies[134], the majority of MAIT cells was CD4 and CD8 DN (**Fig. 3.9 A, B**). Moreover, EAE induction did not differentially affect the ratio between CD4 and CD8 expression of MAIT cells from LN and spleen (**Fig. 3.9 B**). MAIT cells from the spleen had a higher frequency of $CD4^+$ and a lower frequency of DN MAIT cells compared to MAIT cells from LN and CNS, independent of whether the cells were isolated from naïve mice or mice in acute EAE. However, MAIT cells from the CNS showed the same composition with respect to CD4 and CD8 expression compared to MAIT cells from LN and all three, DN, $CD4^+$ and $CD8^+$, MAIT cell subsets were present in the inflamed CNS showing that not only one specific MAIT cell subset based on classification by CD4 and CD8 infiltrated the inflamed CNS (**Fig. 3.9 B**).

The majority of MAIT cells expressed the TCR-Vβ8 chain and only some MAIT cells used the TCR-Vβ6 chain. This distribution did not change between tissues of naïve mice and mice in acute EAE. Therefore, the composition of MAIT cell subsets with respect to TCR-Vβ expression was unaltered by EAE induction and CNS infiltration (**Fig. 3.9 C, D**).

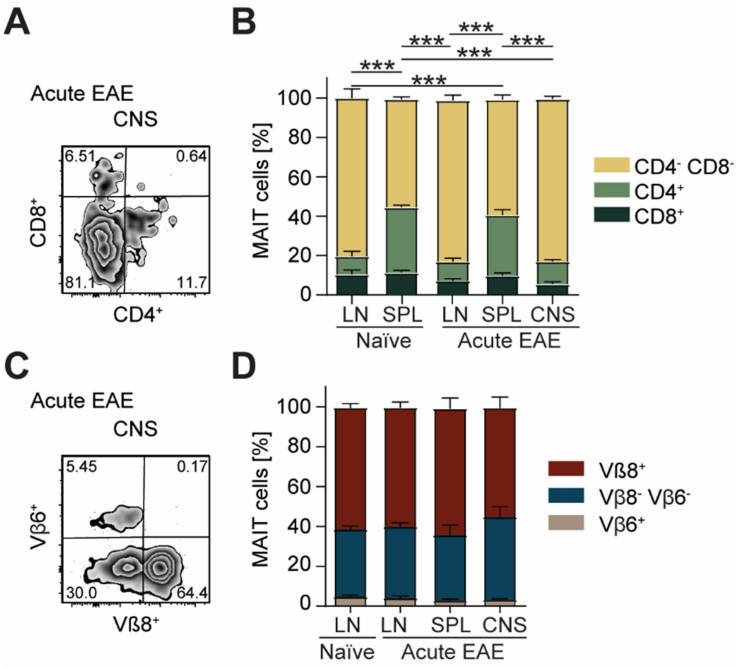

Figure 3.9: Unaltered MAIT cell subtypes based on TCR-β chain or CD4/CD8 expression in the inflamed CNS. EAE was induced in C57BL/6J mice by active immunisation. (A, B) Lymphocytes from the central nervous system (CNS) (n=4), lymph nodes (LN) (n=5) and spleen (SPL) (n=5) were isolated from naïve mice or mice in acute EAE and were analysed for their CD4 and CD8 expression by flow cytometry. (C, D) TCR-Vβ6 and TCR-Vβ8 chain expression of MAIT cells from CNS (n=5), LN (n=5) and spleen (n=3) of mice in acute EAE as well as from LN of naïve mice (n=8) were analysed by flow cytometry. Statistics: two-way-ANOVA; ***$p < 0.001$

To further address poossible MAIT cell functions in the inflamed CNS, we next interrogated the infiltrated subsets, T-bet^+ MAIT1 and $ROR\gamma t^+$ MAIT17 cells. $C57BL/6J^{CAST}$ mice were analysed due to their RORγtGFP transgene. In spleens and LN, either from naïve mice or mice in acute EAE,

RORγtGFP$^+$ MAIT17 and RORγtGFP$^-$ most likely T-bet$^+$ MAIT1, cells could be detected (**Fig. 3.10 A, B**). Moreover, EAE induction did not significantly change the distribution of RORγtGFP$^+$ and RORγtGFP$^-$ MAIT cells in all three tissues. Nearly all MAIT cells in the CNS expressed RORγtGFP and the frequency of RORγtGFP$^+$ MAIT cells in the CNS was significantly higher than in spleen and LN, in both naïve mice and mice in acute EAE (**Fig. 3.10 B**). Therefore, CNS-infiltrating MAIT cells represent RORγtGFP-expressing MAIT17 cells, whereas in the periphery RORγtGFP$^+$ MAIT17 and RORγtGFP$^-$ MAIT1 cells existed.

Published studies showed that MAIT cells in naïve mice expressed mutually exclusive either RORγt or T-bet[126,135], whereas in a *Salmonella* infection model RORγt$^+$ T-bet$^+$ MAIT cells were described[153]. To analyse if also RORγt$^+$ T-bet$^+$ MAIT cells exist in EAE, T-bet expression was investigated in C57BL/6J^{CAST} mice during acute EAE. The FMO sample lacking the T-bet antibody controlled for data spreading due to the use of multiple fluorochromes possibly altering the measured T-bet signal (**Fig. 3.10 C**). Non-MAIT CD44$^+$ T cells were included in the analysis and compared to MAIT cells, since RORγt$^+$ T-bet$^+$ non-MAIT T cells most likely represent pathogenic Th17 cells, which were previously shown to be important for EAE pathogenesis[72]. Only 7.96% RORγt$^+$ T-bet$^+$ MAIT cells were found in LN of mice in acute EAE, while no RORγt$^+$ T-bet$^+$ non-MAIT CD44$^+$ T cells were present. In acute EAE, RORγt$^+$ T-bet$^+$ MAIT (34.5%) and RORγt$^+$ T-bet$^+$ non-MAIT CD44+ T cells from the inflamed CNS (26.6%) were significantly enriched compared to LN (**Fig. 3.10 D**).

Taken together, a specific enrichment or infiltration of MAIT cells in the CNS of mice in acute EAE was not observed by classifying MAIT cells based on their TCR-Vβ6 and TCR-Vβ8 or CD4 and CD8 expression (**Fig. 3.9**). However, all CNS-infiltrating MAIT cells expressed RORγt and therefore belonged to the MAIT17 cell subset, while in the periphery MAIT1 and MAIT17 cells existed (**Fig. 3.10**). RORγt$^+$ T-bet$^+$ MAIT cells were found in the CNS of mice in acute EAE resembling pathogenic Th17 cells and MAIT cells from a *Salmonella* infection model[153]. It remains to be investigated if only RORγt$^+$ MAIT cells infiltrate the CNS and then partially become RORγt$^+$ T-bet$^+$ MAIT cells, like it was reported for pathogenic Th17 cells[71] or if RORγt$^-$ T-bet$^+$ MAIT cells upregulated RORγt after or during CNS infiltration.

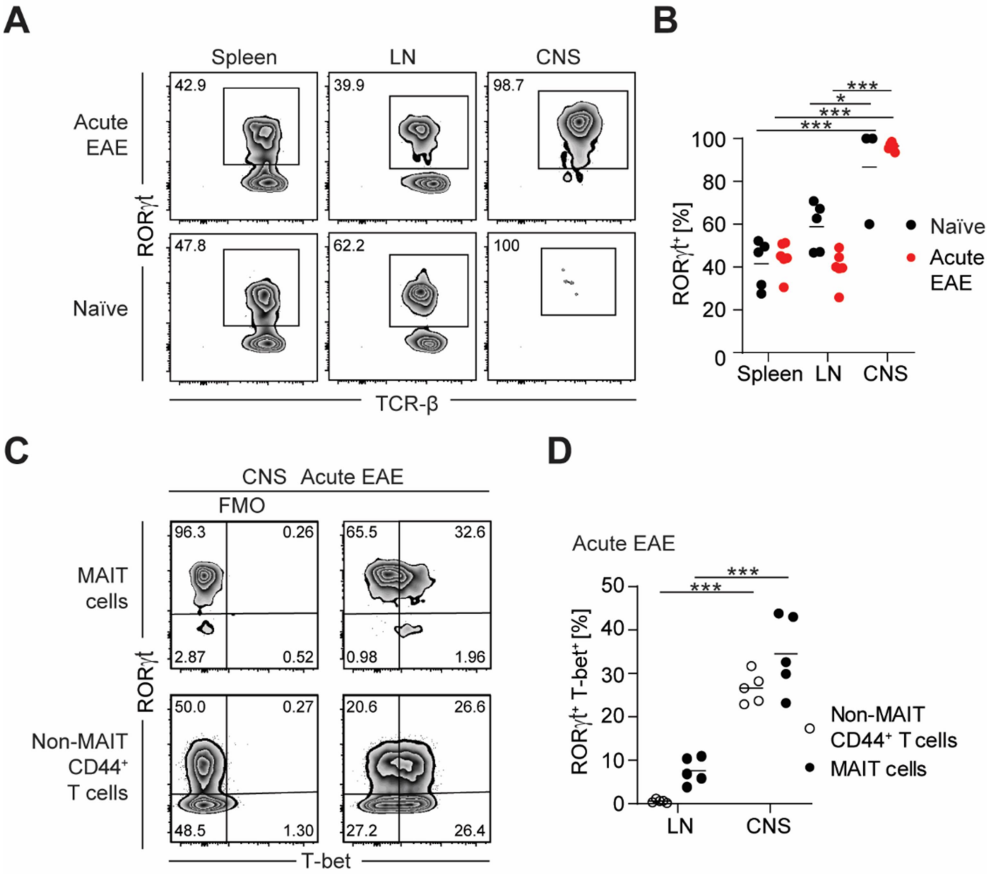

Figure 3.10: Enrichment of MAIT17 and MAIT1/17 cells in the inflamed CNS. EAE was induced in C57BL/6J^{CAST} × RORγtGFP reporter mice by active immunisation. (A, B) Lymphocytes from naïve mice and mice in acute EAE were isolated form the central nervous system (CNS) (n=3, n=6), lymph nodes (LN) (n=5, n=6) and spleen (n=5, n=6) and MAIT cells were analysed for RORγtGFP expression by flow cytometry. Data from two independent experiments were pooled. (C, D) During acute EAE, MAIT and non-MAIT CD44$^+$ T cells were isolated from LN (n=5) and the CNS (n=5) of C57BL/6J^{CAST} × RORγtGFP reporter mice and analysed for RORγtGFP and T-bet expression after intranuclear staining. Statistics: two-way-ANOVA; *$p < 0.05$, ***$p < 0.001$

3.2.3 Mechanism of CNS infiltration by MAIT cells

MAIT cells were enriched in the inflamed CNS of acute EAE and were already present in the CNS during preclinical EAE indicating a specific invasion of the CNS instead of a bystander infiltration due to the open BBB. To study the potential of MAIT cells to infiltrate the CNS, the integrins CD49d and CD11a and the chemokine receptor CCR6 were analysed by flow cytometry (**Fig. 3.11 A-C**). CD49d is a subunit of VLA-4, an integrin important for CNS infiltration of Th1 cells, whereas CD11a a subunit of the integrin LFA-1 is primarily used by Th17 for invading the CNS in EAE[268]. The chemokine receptor CCR6 mediates CNS infiltration via the choroid plexus, which is important during the early phase of EAE[271]. Since the vast majority of CNS-infiltrating MAIT cells were RORγt$^+$ MAIT17 cells, MAIT cells were compared to RORγt$^+$ and RORγt$^-$ non-MAIT CD44$^+$ T cells, which were most likely Th17/Tc17 and Th1/Tc1 cells. MAIT cells in the CNS expressed significanlty less CD49d compared to RORγt$^-$ (p=0.002) and RORγt$^+$ (p=0.003) non-MAIT CD44$^+$ T cells (**Fig. 3.11 D**). RORγt$^+$ non-MAIT CD44$^+$ T cells had the highest mean fluorescent intensity (MFI) of CCR6, which was significantly increased compared to RORγt$^-$ non-MAIT CD44$^+$ T cells (p=0.0027) and MAIT cells (p=0.0014) (**Fig. 3.11 E**). A

strong expression of CD11a was observed in RORγt$^+$ non-MAIT CD44$^+$ T cells (p=0.0001) and MAIT cells (p=0.0001) significantly higher than in RORγt$^-$ non-MAIT T cells (**Fig. 3.11 F**).

Furthermore, both integrins and the chemokine receptor were investigated in MAIT cells from LN and spleen and compared to the CNS revealing elevated expressions in the CNS (**Fig. 3.11 G-I**). Peripheral MAIT cells were analysed as RORγt$^-$ MAIT1 and RORγt$^+$ MAIT17 cells. The frequency of CD49d-expressing MAIT cells from the CNS was significantly upregulated compared to RORγt$^-$ (p=0.0006) and RORγt$^+$ MAIT cells from LN (p=0.003) as well as RORγt$^-$ MAIT cells from the spleen (p=0.0001) (**Fig. 3.11 G**). The CCR6 MFI of CNS-infiltrating MAIT cells was significantly enhanced compared to RORγt$^-$ (p=0.0018) and RORγt$^+$ MAIT cells from spleen (p=0.0016), but unaltered compared to MAIT cells from LN (**Fig. 3.11 H**). The expression of CD11a was strongly increased in CNS-infiltrating MAIT cells compared to either RORγt$^+$ or RORγt$^-$ MAIT cells from LN and spleen (**Fig. 3.11 I**). The transcriptome data revealed a discrepancy compared to the flow cytometry data and showed that mRNA levels of CD49d and CCR6 were lower in MAIT cells from the inflamed CNS (MEC) compared to the spleen (MES) (**Fig. 3.11 J-L**). Whereas the integrins and the chemokine receptor were upregulated and required during CNS infiltration, this increased expression is possibly downregulated after entry of the CNS and a reduced expression first affected the mRNA level.

In summary, diverse integrins and chemokine receptors mediate CNS infiltration and MAIT cells from mice in acute EAE expressed CD49d, CD11a and CCR6, which were elevated in the CNS compared to LN and spleen. However, which integrins and chemokine receptors were essential for the infiltration of MAIT cells in the inflamed CNS remains elusive and can be addressed by using specific blocking antibodies or knockout mice in EAE experiments. CD11a is an interesting candidate for prospective investigations due to the strong expression by MAIT cells in the CNS, which also further indicated a type-17-like phenotype of MAIT cells resembling RORγt$^+$ non-MAIT CD44$^+$ T cells.

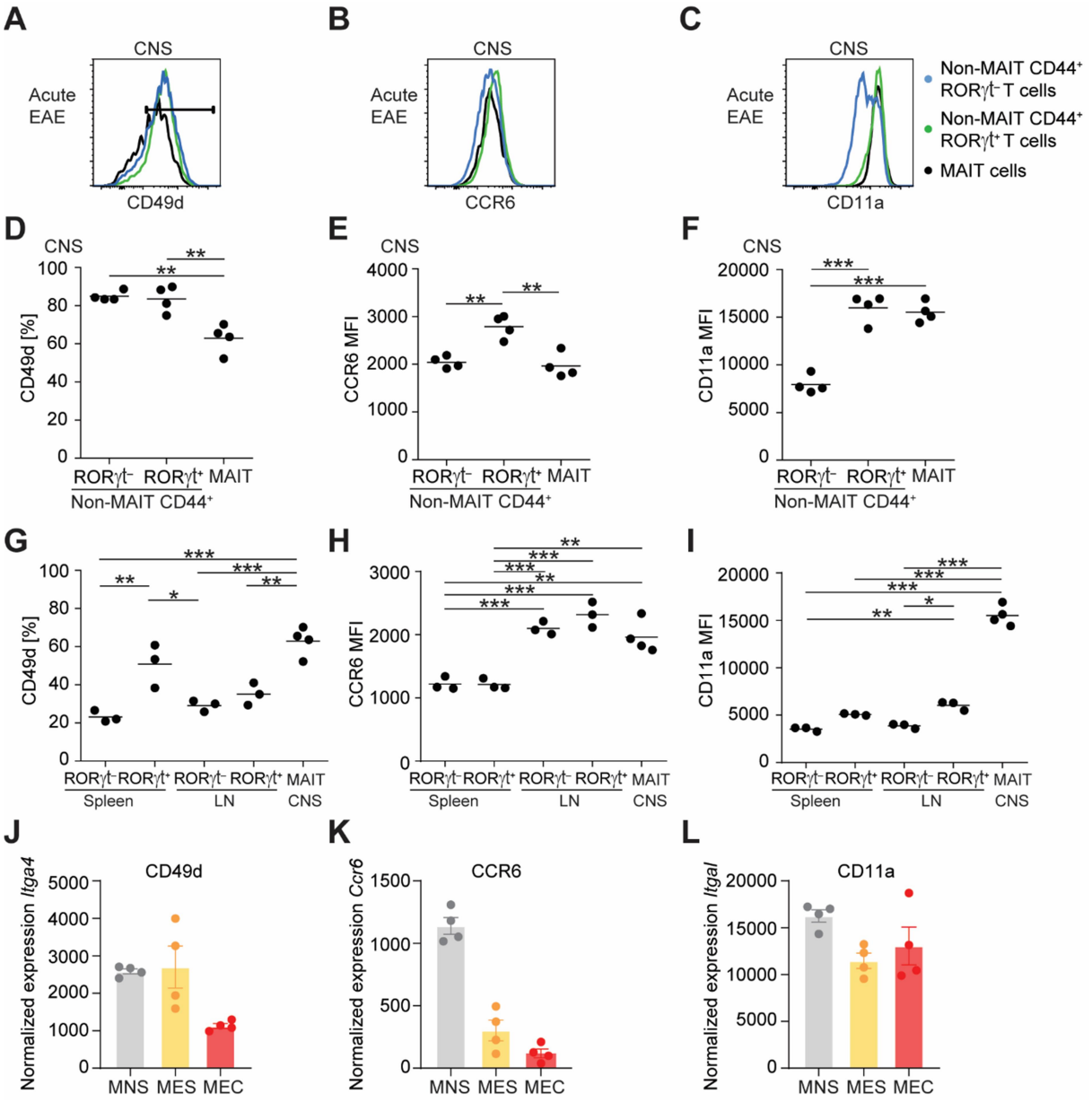

Figure 3.11: Chemokine receptor and integrin expression of MAIT cells in acute EAE. EAE was induced in C57BL/6J^{CAST} × RORγtGFP reporter mice by active immunisation. CNS-infiltrating MAIT cells and RORγt^{-} and RORγt^{+} non-MAIT CD44^{+} T cells were analysed by flow cytometry for expression of CD49d (A), C-C motif chemokine receptor 6 (CCR6) (B) and CD11a (C) and the results were quantified (D-F) (n=4). (G-I) Expression of chemokine receptor and integrins was analysed during acute EAE in MAIT cells from the CNS (n=4) and RORγt^{-} and RORγt^{+} MAIT cells from spleen and LN (n=3). (J-L) Transcriptome data represent the normalized expression of *Itga4, Ccr6* and *Itgal* between MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively). Statistics: one-way-ANOVA; *p < 0.05, **P < 0.01, ***p < 0.001

3.2.4 Effector function of MAIT cells in EAE

A broad repertoire of different effector functions has been described for MAIT cells ranging from the secretion of proinflammatory cytokines and cytotoxic molecules to tissue repair function. To investigate which effector function MAIT cells fullfil in EAE, DEG from the transcriptome data were analysed for enriched Gene Ontology (GO) terms. Many of the 50 GO terms with the highest normalised enrichment score (NES) were associated with proinflammatory and tissue repair function. Comparison

of CNS and peripheral MAIT cells in EAE (MEC vs MES) revealed many GO terms associated with proinflammatory as well as tissue repair function (**Fig. 3.12 A**), whereas only proinflammatory associated GO terms were enriched in peripheral MAIT cells in EAE in comparison to the healthy state (MES vs MNS) (**Fig. 3.12 B**). The proinflammatory GO terms from DEG of CNS and peripheral MAIT cells in EAE (MEC vs MES) were mainly a reaction to or a secretion of cytokines. In contrast, the proinflammatory GO terms resulting from DEG comparing peripheral MAIT cells in EAE with the healthy state (MES vs MNS) included primarily reactions to bacterial molecules, cell killing and cytotoxicity. However, that MAIT cells from the spleen of mice in acute EAE also upregulated tissue repair associated genes could not be excluded by this analysis. GO terms allow to get an overview about a large dataset without addressing exact functions or identifing specific molecules/genes. Therefore, more analyses were performed to further investigate either proinflammatory or tissue repair functions of MAIT cells.

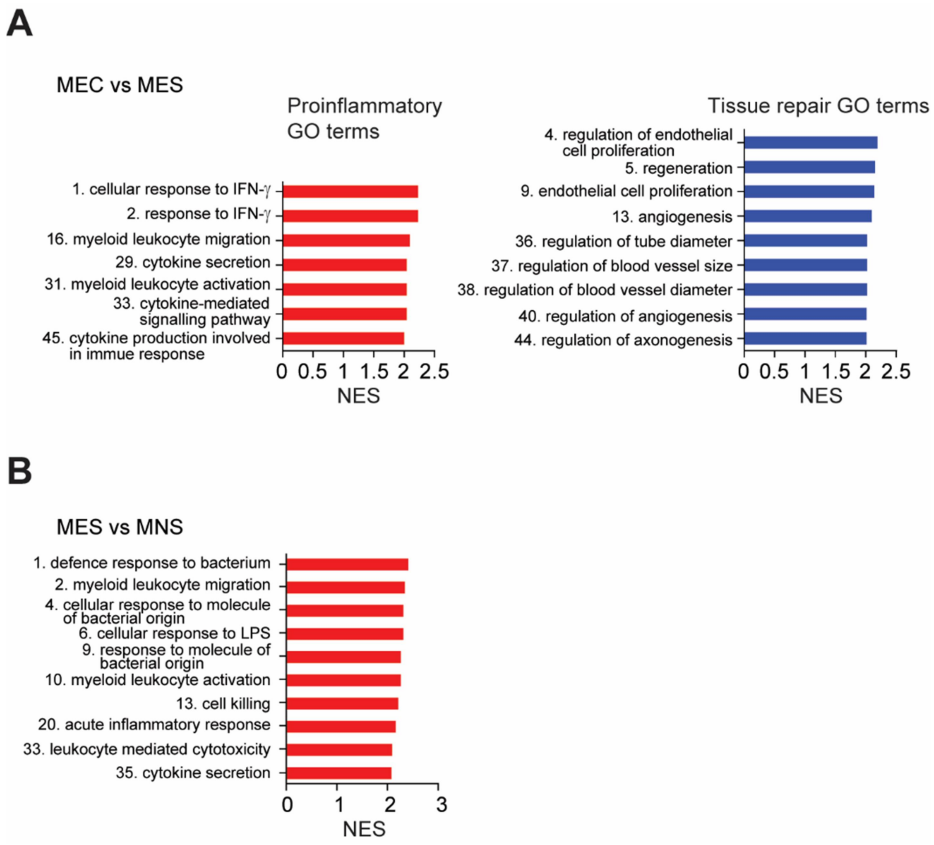

Figure 3.12: Upregulation of genes associated with proinflammatory and tissue repair GO terms by MAIT cells in EAE. Transcriptome data from MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively) were analysed. Differentially expressed genes (DEG) from MEC vs MES (A) and MES vs MNS (B) were analysed for enriched Gene Ontology (GO) terms. Many of the 50 GO terms with the highest normalized enrichment score (NES) were associated with proinflammatory and tissue repair function.

To further investigate the tissue repair function of MAIT cells, candidate genes with a known tissue repair function were analysed in the transcriptome data. AREG is expressed in different immune cells and is involved in many effector functions including wound healing and tissue repair[316]. The vascular endothelial growth factors (VEGF) VEGF-A and VEGF-B are main mediators of angiogenesis[317], an

important mechanism of tissue repair and regeneration[318]. Angiogenesis, regeneration and many similar GO terms were upregulated in the GO term analysis of the transcriptome data from MAIT cells from the CNS of mice in acute EAE compared to the periphery (MEC vs MES) (**Fig. 3.12 A**). Expression of *Vegfa* and especially *Areg* was strongly increased in MAIT cells in the CNS of mice in acute EAE (MEC), whereas *Vegfb* was also elevated in the periphery during EAE (MEC and MES) indicating an EAE-induced upregulation instead of a CNS-specific increase (**Fig. 3.13 A**).

To analyse a broader tissue repair signature of MAIT cells in EAE, GSEA were performed and the transcriptome data was compared to two published lists containing genes associated with tissue repair. The gene list Linehan *et al*., 2018[312] contains 105 genes, including *Vegfa*, *Vegfb* and *Areg*, and was already used in studies addressing the tissue repair function of MAIT cells[146,147]. The tissue repair database (TiReDB) gene list contains 319 tissue repair associated genes mainly generated by investigations using direct interventions like knockout or overexpression experiments[313]. Analysing the DEG of MAIT cells from the periphery in EAE in comparison to the healthy state revealed that also peripheral MAIT cells in EAE upregulated tissue repair genes. GSEA showed a running enrichment score of about 0.5 and an enhancement of genes from Linehan *et al*., 2018 (p=0.0033) and TiReDB (p=0.0019) (**Fig. 3.13 B**). Furthermore, tissue repair genes from Linehan *et al*., 2018 (p=0.002) and TiReDB (p=0.002) were enriched in MAIT cells from the inflamed CNS in comparison to the periphery (MEC vs MES) having a running enrichment score of about 0.6 (**Fig. 3.13 C**).

To study if the upregulation of tissue repair genes in EAE was specific for MAIT cells or also occured in non-MAIT T cells, published transcriptome data of CNS-infiltrating T cells were examined for their tissue repair potential via GSEA. Therefore, a dataset was used containing memory $CD4^+$ T cells in acute EAE, which were compared between the spinal cord and LN (**Fig. 3.13 D**)[310]. Furthermore, another study investigated Th17 cells of mice in acute EAE isolated from the cerebellum and LN (**Fig. 3.13 E**)[311]. The DEG of both published datasets showed an enrichment in GSEA of tissue repair genes from Linehan *et al*., 2018 and TiReDB (**Fig. 3.13 D, E**). Therefore, the tissue repair signature upregulated in MAIT cells in EAE was not specific for MAIT cells and occured also in other CNS-infiltrating T cells subsets. It has to be further investigated, if these tissue repair genes were upregulated by all CNS-infiltrating T and MAIT cells or if the upregulation was driven by specifc subsets.

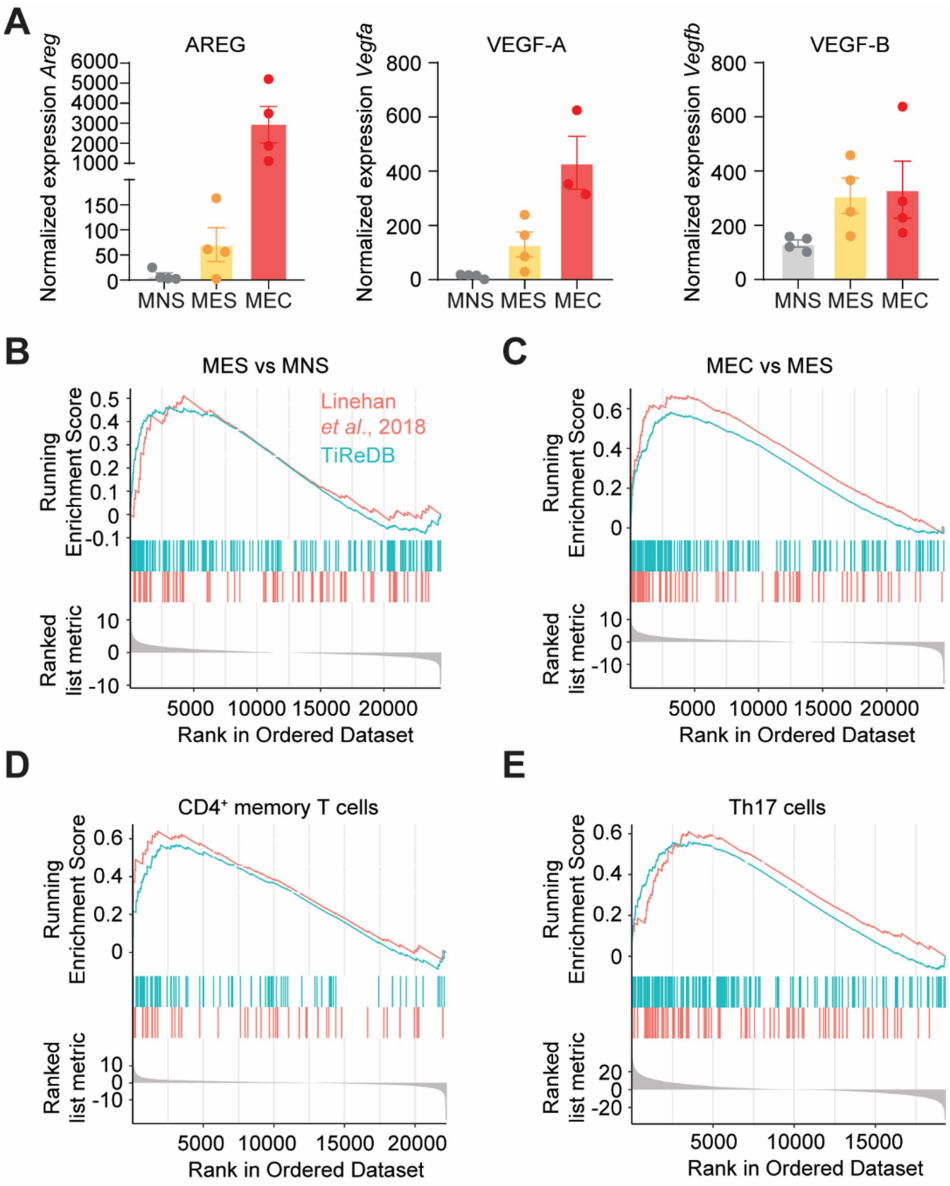

Figure 3.13: Upregulation of tissue repair genes by CNS-infiltrating MAIT and non-MAIT T cells in EAE. (A) Transcriptome data depict the normalized expression of *amphiregulin* (*Areg*), *vascular endothelial growth factors-A* (*Vegfa*) and *Vegfb* in MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively). Gene lists containing the differentially expressed genes (DEG) between MES and MNS (B) as well as MEC and MES (C) were compared by gene set enrichment analyses (GSEA) to published gene lists containing genes associated with tissue repair[312,313]. (D, E) The tissue repair gene lists from Linehan *et al*., 2018 and from the tissue repair database (TiReDB) were compared with DEG from $CD4^+$ memory T cells from cerebellum and LN in acute EAE (D)[310] as well as with DEG from Th17 cells from spinal cord and LN in acute EAE (E)[311].

Next to tissue repair function, also proinflammatory effector functions of MAIT cells in EAE were analysed. Therefore, the MAIT transcriptome dataset was compared by GSEA to DEG upregulated in pathogenic Th17 cells in comparisons to non-pathogenic Th17. Pathogenic Th17 cells were considered to be main contributors of EAE pathogenesis[72,264]. In a published study, naïve T cells were differentiated *in vitro* into either pathogenic or non-pathogenic Th17 cells, whereby only pathogenic Th17 cells were able to induce EAE after transferring these cells to recipient mice[233]. GSEA revealed that the majority of genes upregulated in pathogenic Th17 cells in comparison to non-pathogenic Th17

cells was also upregulated in MAIT cells in acute EAE either in the CNS (**Fig. 3.14 A**) or in the periphery compared to MAIT cells from periphery of naïve mice (**Fig. 3.14 B**) further indicating a pathogenic potential of MAIT cells in EAE.

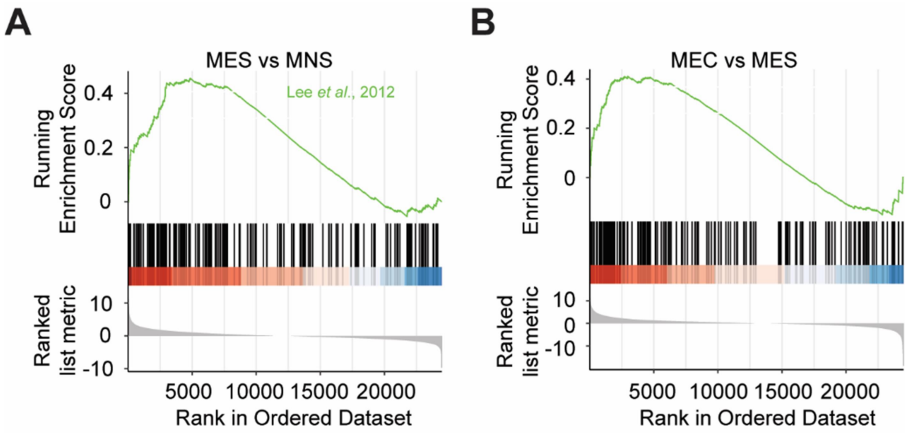

Figure 3.14: MAIT cells in acute EAE expressed genes upregulated in pathogenic Th17 cells. (A, B) Transcriptome data from MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively) were analysed. Gene lists containing the differentially expressed genes (DEG) between MES and MNS (A) as well as MEC and MES (B) were compared via gene set enrichment analyses (GSEA) to a published list of genes upregulated in pathogenic Th17 cells after *in vitro* differentiation[233].

To further and more specifically investigate the proinflammatory functions of MAIT cells, the cytokine secretion of CNS-infiltrating MAIT cells in acute EAE was analysed by flow cytometry. To better estimate the secretion levels of the cytokines IL-17A, IFN-y and GM-CSF, MAIT cells were compared to RORγt$^+$ and RORγt$^-$ non-MAIT T cells. These cytokines play different roles in EAE pathogenesis and are produced by pathogenic Th17 cells[64,70]. EAE was induced in C57BL6/J^{CAST} mice to distinguish RORγt$^+$ and RORγt$^-$ non-MAIT T cells. During acute EAE, lymphocytes from the CNS of three mice were isolated, pooled and stained either directly *ex vivo* or after four hours of *in vitro* culture. Lymphocytes in culture were either left unstimulated or stimulated with PMA and ionomycin. *Ex vivo* cytokine stainings revealed which cytokines are produced *in vivo* without a further *in vitro* activation. 22.9% of MAIT cells and 11.6% of RORγt$^+$ non-MAIT T cells produced IL-17A *ex vivo*, whereas RORγt$^-$ non-MAIT T cells did not produce IL-17A (0.9%). *Ex vivo* IFN-y and GM-CSF production was not observed in any of the three T cell subsets (**Fig. 3.15 A**). After four hours of unstimulated culturing, no cytokine production could be detected anymore. PMA and ionomycin activation strongly upregulated the cytokine production. The majority of MAIT cells expressed IL-17A (86%). In addition, 16.7% of MAIT cells co-produced IFN-y and IL-17A and 16.1% made GM-CSF and IL-17A. These cells could possibly belong to the RORγt$^+$ T-bet$^+$ MAIT cell subset (**Fig. 3.10**). The cytokine expression of MAIT cells resembled RORγt$^+$ non-MAIT T cells producing mainly IL-17A (74.1%). Furthermore, also IL-17A$^+$ IFN-y$^+$ (30%) and IL-17A$^+$ GM-CSF$^+$ (26.1%) RORγt$^+$ non-MAIT T cells were detected. These cells belong most likely to the pathogenic Th17 cells expressing the transcription factors RORγt and T-bet. In contrast, RORγt$^-$ non-MAIT T cells mainly produced IFN-y (63.61%) and only 6.64% IL-17A further indicating that these cells were Th1 cells (**Fig. 3.15 A)**.

To better estimate how many MAIT cells contributed to IL-17A production of all T cells, IL-17A$^+$ T cells were gated before MAIT and non-MAIT T cells were selected (**Fig. 3.15 B**). 17.6% of all *ex vivo* IL-17A-producing T cells were MAIT cells. This frequency decreased after *in vitro* culture to 4.92% in unstimulated conditions and to 3.55% after PMA and ionomycin activation. The lower contribution of MAIT cells to the IL-17A production after PMA and ionomycin activation was most likely caused by a decreased MAIT cell frequency. Whereas the MAIT cell frequency of all T cells was unaltered in *ex vivo* (3.86%) and unstimulated conditions (3.96%), the frequency decreased after PMA and ionomycin stimulation to 1.18% (**Fig. 3.15 B**). This was possibly caused by a downregulation of the TCR after PMA and ionomycin activation, which led to a decreased MR1-5-OP-RU tetramer signal resulting in an aberrant classification of MAIT cells as non-MAIT T cells.

Therefore, to verify the results of the cytokine production of MAIT cells after PMA and ionomycin stimulation, MAIT and RORγt$^+$ non-MAIT T cells from the CNS of mice in acute EAE were sorted and stimulated separately (**Fig. 3.15 C**). The cytokine expression of sorted MAIT cells was similar to the results obtained after culturing all CNS-infiltrating lymphocytes together. The majority of MAIT cells expressed IL-17A (86.5%), while 24.5% of these cells also produced IFN-y and 18% GM-CSF. The cytokine expression of RORγt$^+$ non-MAIT T cells changed and the majority expressed IFN-y (53.5%), whereas the frequency of IL-17A-producing RORγt$^+$ non-MAIT T cells was reduced (35.2%) (**Fig. 3.15 C**). This discrepancy could only partially be explained by subtracting the IL-17A production of MAIT cells mistakenly classified as non-MAIT T cells. Furthermore, active immunisation results in varying Th1 to Th17 ratios in the inflamed CNS most likely mainly responsible for the difference in cytokine secretion of non-MAIT T cells between the two experiments. This would further increase the contribution and importance of MAIT cells to the overall IL-17A production especially in EAE conditions resulting in a high Th1 to Th17 ratio. Investigating the transcriptome data revealed a strong upregulation of *Il-17a* and *Csf2* expression of MAIT cells in the inflamed CNS, whereas the *Ifng* levels were unaltered (**Fig. 3.15 D**).

In summary, MAIT cells in the CNS strongly expressed IL-17A and made up to 17.6% of IL-17A-producing T cells *in vivo* (**Fig. 3.15**). In addition, activated MAIT cells also expressed IFN-y and GM-CSF showing a type-17 like cytokine secretion profile resembling pathogenic Th17 cells further supported by GSEA (**Fig. 3.14**).

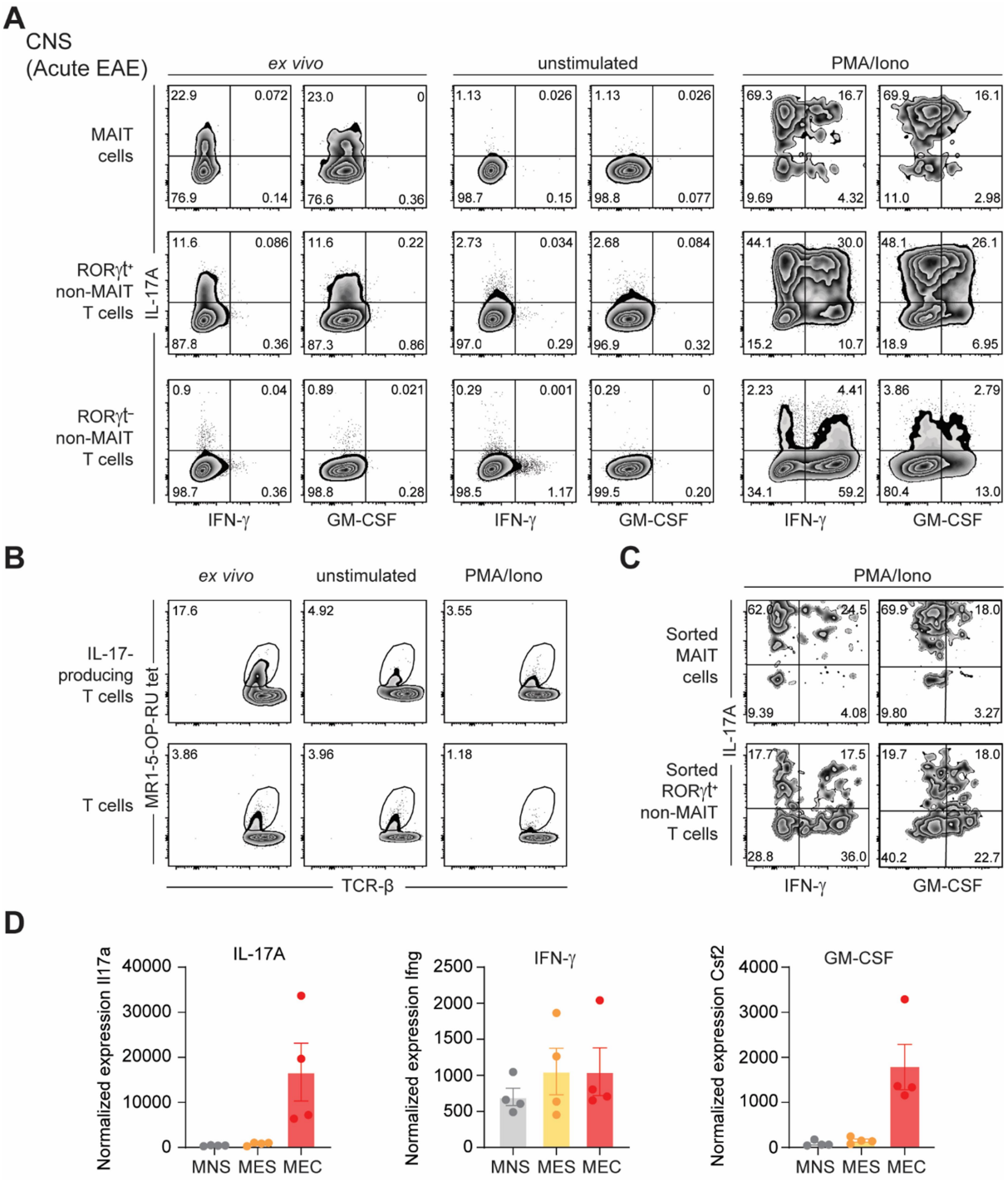

Figure 3.15: Expression of proinflammatory cytokines by MAIT cells in acute EAE. EAE was induced in C57BL/6J^{CAST} × RORγtGFP reporter mice by active immunisation. In acute EAE, lymphocytes from the CNS were isolated and analysed by flow cytometry for expression of the cytokines interleukin 17A (IL-17A), interferon γ (IFN-γ) and granulocyte macrophage colony stimulating factor (GM-CSF). (A) The cytokines were analysed in MAIT, RORγt^{-} and RORγt^{+} non-MAIT T cells directly *ex vivo* or after 4 hours of *in vitro* culture either left unstimulated or activated by phorbol 12-myristate 13-acetate (PMA) (10 ng/ml) and ionomycin (1 µg/ml). (B) The proportion of MAIT cells of IL-17A-producing T cells (upper row) and the MAIT cell frequency of all T cells (bottom row) are shown. (C) CNS-infiltrating MAIT and RORγt^{+} non-MAIT T cells were isolated by cell sorting before they were cultured, activated and analysed by flow cytometry for the expression of IL-17A, IFN-γ and GM-CSF. (D) Transcriptome data depict the normalized expression of *Il17a, Ifng* and *Csf2* in MAIT cells isolated from the spleen of naïve mice (MNS) and from the spleen and CNS of mice with acute EAE (MES and MEC respectively).

3.3 Deciphering the role of MAIT cells in EAE

The characterisation of MAIT cells in EAE revealed that MAIT cells were able to fulfil proinflammatory as well as tissue repair function. To address which role MAIT cells play in EAE, the influence of genetic *Mr1* ablation and MR1 blocking antibodies on EAE pathogenesis was investigated.

First, the effect of MAIT cells on other immune cells was analysed in the inflamed CNS. Therefore, EAE was induced in C57BL/6J and *Mr1*$^{-/-}$ mice and frequencies and absolute cell counts of immune cells during EAE onset and acute EAE were quantified by flow cytometry (**Fig. 3.16**). Only one significant difference between C57BL/6J and *Mr1*$^{-/-}$ mice was detected showing a decrease of the absolute cell number of living CD45^{+} lymphocytes in *Mr1*$^{-/-}$ mice during EAE onset (**Fig. 3.16 B**), which could not be assigned to specific immune cell population. However, the absolute numbers of living CD45^{+} lymphocytes in acute EAE were unaltered (**Fig. 3.16 D**). Therefore, the loss of MAIT cells in *Mr1*$^{-/-}$ did not influence the amount and frequencies of other immune cells in the inflamed CNS.

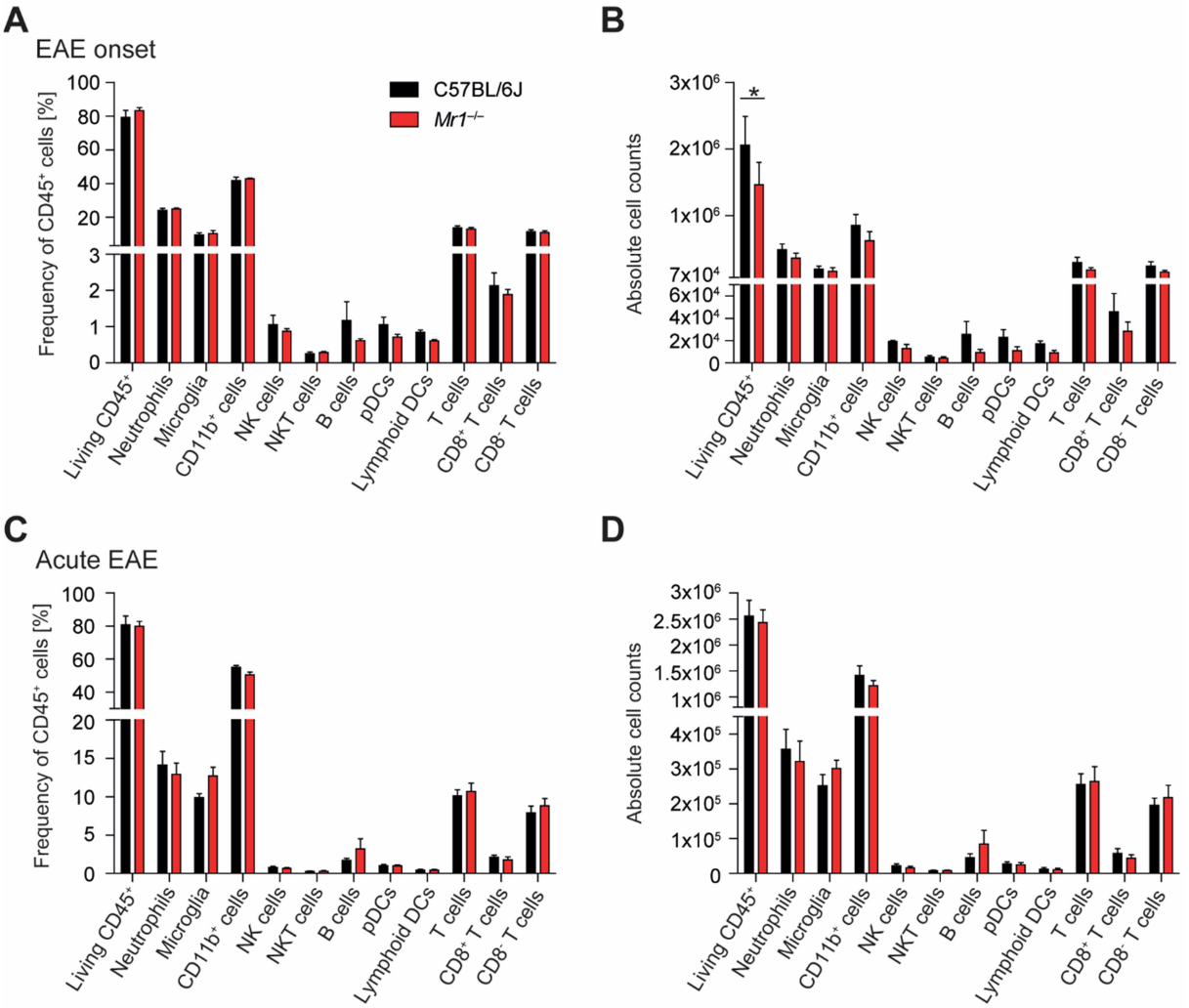

Figure 3.16: Unaltered immune cells in the CNS of C57BL/6J and *Mr1*$^{-/-}$ mice in EAE. (A-D) EAE was induced in C57BL/6J (n=5) and *Mr1*$^{-/-}$ mice (n=5) by active immunisation. Lymphocytes from the CNS were isolated during EAE onset and acute EAE and analysed by flow cytometry for frequencies and absolute numbers of immune cell populations. The gating strategy is depicted in figure 2.2. The absolute numbers of the different immune cell populations were quantified by using Trucount tubes (BD Biosciences). Statistics: two-way-ANOVA; *$p < 0.05$

To further investigate if the lack of MAIT cells in *Mr1*$^{-/-}$ mice directly affected the EAE phenotype, the EAE courses of C57BL/6J and *Mr1*$^{-/-}$ mice were neurologically scored over 30 days. The cumulative clinical score showed a trend (p=0.0831) that *Mr1*$^{-/-}$ mice developed a more severe EAE course (**Fig. 3.17 A**), whereas no difference in the mean body weight was detected (**Fig. 3.17 B**). Therefore, MAIT cells might have a protective effect in EAE possibly mediated by their tissue repair function. Recent publications indicate tissue repair function of MAIT cells only after TCR-dependent activation[146]. To address the tissue repair function of MAIT cells more specifically in EAE, C57BL/6J mice were treated with an anti-MR1 blocking antibody. The treatment timepoints 5, 10 and 15 dpi covered preclinical, onset and acute EAE and were based on the results of Nur77GFP-expression by MAIT cells in EAE revealing the strongest TCR-dependent activation during preclinical EAE (**Fig. 3.8**). Specific blockade of TCR-dependent activation of MAIT cells led to a significantly exacerbated EAE course compared to IgG isotype-treated littermates (cumulative disease score; p=0.0462) (**Fig. 3.17 C**), while the mean body weight was unaltered (**Fig. 3.17 D**).

Therefore, either genetic ablation of MAIT cells by using *Mr1*$^{-/-}$ mice or blocking the TCR-dependent activation of MAIT cells resulted in a more severe EAE course indicating a protective effect of MAIT cells in EAE possibly mediated by their tissue repair function.

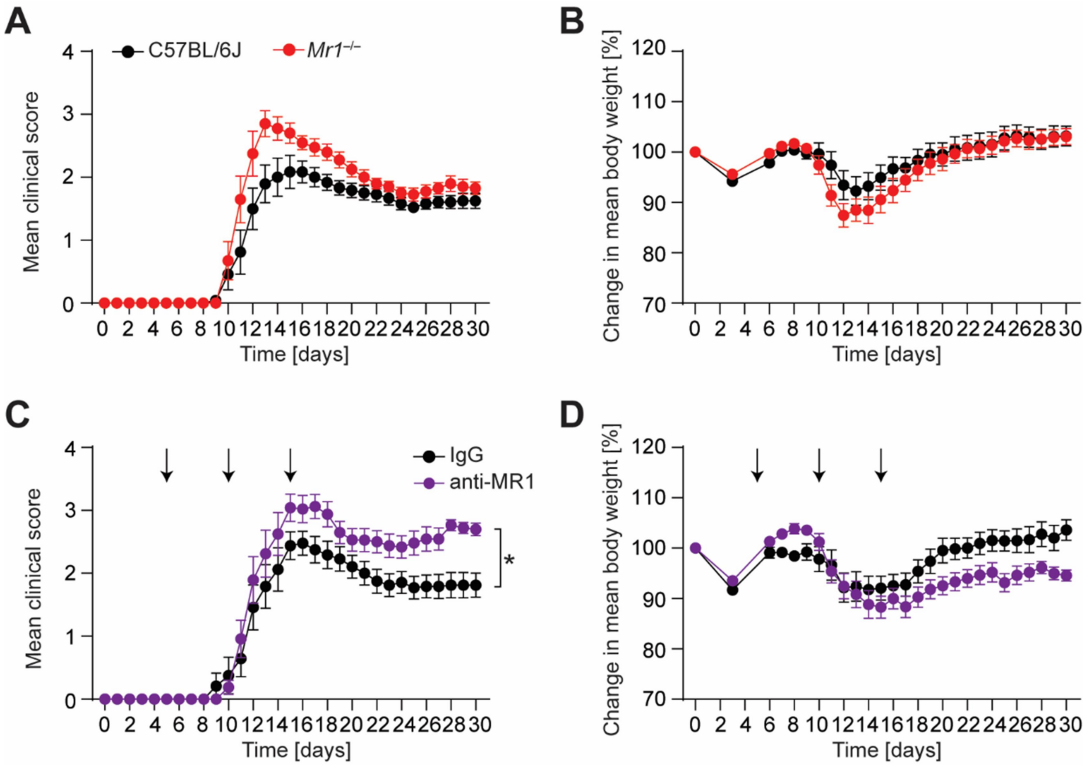

Figure 3.17: Protective effect of MAIT cells in EAE. (A-D) EAE clinical scores and body weight were assessed daily shown as mean values ± SEM. (A, B) EAE was induced by active immunisation in C57BL/6J (n=12) and *Mr1*$^{-/-}$ (n=10) littermates. (C, D) EAE was induced by active immunisation in C57BL/6J mice, which were treated on day 5, 10 and 15 post immunisation either with an anti-MR1 blocking antibody (n=12) (BioLegend, clone:26.5, 250 µg per animal i.p. injections) or with a respective IgG isotype control (n=12). Statistical differences between the groups were analysed by cumulative EAE score using Mann-Whitney U test. *p < 0.05

4 Discussion

The discovery of new immune cells populations provides interesting starting points for research hypothesis and treatment possibilities of diseases. Especially the discovery of MAIT cells and their recognition of vitamin B2 metabolites, a new class of T cell antigens, was a landmark event in immunology[120]. Many alterations of MAIT cells in different diseases have already been reported, while functional analyses and direct disease-influencing effects are rarely described, mainly due to missing data from animal models.

In this work, newly available tools to study MAIT cells in mice were combined with the EAE model (Aim 1), which enabled for the first time to characterise CNS-infiltrating MAIT cells in EAE (Aim 2) and to investigate their role in EAE pathogenesis (Aim 3).

4.1 MAIT cell accumulation in the inflamed CNS

Investigations of MS patients uncovered that MAIT cells were present in inflamed brain lesions[7,300,301], while exact frequencies, further characterisations and possible effects of these cells in the CNS remain elusive. Analysing MAIT cells in the EAE mouse model circumvent many of the experimental limitations in humans and allowed to include direct manipulation to further decipher the role of MAIT cells in MS pathogenesis. This work showed for the first time that after EAE induction MAIT cells strongly accumulated in the inflamed CNS. The frequency of MAIT cells in the CNS of mice in acute EAE was higher than all investigated tissues from naïve mice either from this work or from published studies[134,153] indicating a specific accumulation of MAIT cells in the CNS. Furthermore, this enrichment of MAIT cells in the inflamed CNS was most likely not a general EAE effect since the increase was only observed in the CNS and not in LN. Nevertheless, to verify that the MAIT cell enrichment in acute EAE was only specific for the CNS, more tissues have to be investigated for their MAIT cell frequency in EAE.

MAIT cells share many similarities with iNKT cells, another subset of unconventional T cells[319]. However, whereas MAIT cell frequencies are higher in humans than in mice, the opposite is the case for iNKT cells with frequencies of up to 26% in the liver and above 1% in the spleen of naïve mice[319,320]. At the same time, in EAE, the frequency of CD1d-α-Gal-Cer tetramer$^+$ iNKT cells did not increase above 1% of T cells in the CNS[320,321], supporting a specific enrichment of MAIT cells in the inflamed CNS.

Infectious disease models showed a great variety of MAIT cell accumulation depending on the pathogen and inflamed tissue. The highest accumulation of MAIT cells was observed in the lung of bacterial-infected mice leading to an up to 200 fold increase of MAIT cells comprising 25-50% of αβ T cells[145,147,153], while influenza infection only lead to a 2.6-fold increase of MAIT cells in the lung[322]. Furthermore, MAIT cells compose up to 40% of αβ T cells in the skin of mice exposed to *Staphylococcus epidermidis*[131]. However, the skin and the lung are tissues comprising already a high amount and frequency of MAIT cells in naïve mice[131,134]. The increase of MAIT cells in these tissues upon bacterial infections was most likely caused by a strong TCR-dependent activation resulting in proliferation[168] instead of infiltration from the circulation or other tissues[145]. Investigations of other mouse disease models on MAIT cells in tissues with a low MAIT cell frequency are rare. For example,

in pancreatic islets of diabetic NOD mice MAIT cells increased only to a frequency of about 0.2%[155], further indicating an unusual strong enrichment of MAIT cells in the CNS of mice in acute EAE.

4.2 Genetic and environmental factors influencing MAIT cell frequencies

MAIT cell frequencies in naïve C57BL/6J and C57BL/6J^{CAST} mice revealed that these were comparable to published studies[134,135,153,154] revealing that the microbiome in our animal facility was not promoting or restricting the development of MAIT cells. First, C57BL/6J^{CAST} mice, which have higher MAIT cell frequencies in the naïve state[154], also showed increased frequencies of CNS-infiltrating MAIT cells in acute EAE. However, this high infiltration decreased over the years, whereas the general higher MAIT cell frequency in naïve C57BL/6J^{CAST} mice persisted. Possible explanations are based on genetic and environment factors influencing MAIT cells. The microbiome has a strong impact on MAIT cell development shown by their absence in GF mice[135] and by a large variability of MAIT cell frequencies between distinct mouse cages, while MAIT cell frequencies within a cage were similar[131]. The exchange of the mouse food in the end of 2016 at the UKE animal facilities might have impacted the microbiome leading to a reduced CNS infiltration of MAIT cells in EAE. However, a reduced CNS infiltration of MAIT cells in C57BL/6J was not observed at the same time. Next to environmental, also genetic factors might have led to the reduction. The congenic C57BL/6J^{CAST} mice differ from C57BL/6J mice in a 600 bp long region on chromosome 13 encompassing the 3′ end of the Vα locus, TCR-δ, Jα, and Cα segments[154]. This region can be identified by two SNPs. Backcrossing the imported C57BL/6J^{CAST} mice to C57BL/6J mice from our animal facility in 2016 might have changed this region without affecting the SNPs. Furthermore, in 2016 some C57BL/6J^{CAST} mice also had a low CNS infiltration of MAIT cells in acute EAE. If such mice were selected for matings during the time of mouse line establishing and backcrossing in 2016, this might have led to the reduced CNS infiltration of MAIT cells in EAE over time. Nevertheless, the amount of MAIT cells in the CNS of C57BL/6J mice remained stable over time and provided a good model to further investigate MAIT cells in EAE.

4.3 Localisation and activation of MAIT cells in EAE

In this work, MAIT cells in the CNS were for the first time specifically characterised, revealing a low frequency and absolute number in naïve mice. Immune cells from the CNS of five naïve mice had to be pooled for analyses. The immune privilege of the CNS restricts the exchange with the peripheral immune system and limits the amount of peripheral immune cells in the CNS[323] resulting in low numbers of T and MAIT cells. It is likely that the strong accumulation of MAIT cells in the inflamed CNS resulted from infiltration of peripheral MAIT cells, like has been shown for other T cells in EAE[268]. However, CNS-specific proliferation of MAIT cells might have contributed to their expansion. The accumulation of MAIT cells already during preclinical EAE as well as the increased expression of CCR6, VLA-4 and LFA-1 on MAIT cells in the CNS compared to peripheral tissues indicated a specific CNS infiltration of MAIT cells in EAE. These integrins and chemokine receptor are important for CNS infiltration of T cells in EAE[268,271] and were also elevated on MAIT cells from the peripheral blood of MS patients compared to healthy controls[7,295]. An infiltration of MAIT cells into the inflamed tissue would be distinct compared to other disease models of MAIT cells, which depend on tissue-specific

proliferation[145,168]. Moreover, this would increase the purview of MAIT cell functions and further suggest analysing MAIT cells in diseases affecting non-mucosal tissues, which comprise low MAIT cell frequencies in naïve mice.

This work uncovered strongly activated MAIT cells in the inflamed CNS, while it remained elusive if the activation occurred in the CNS or in peripheral tissues prior to CNS infiltration. The gut lamina propria or the lung could be candidate tissues for MAIT cell activation in EAE, since MAIT cells are highly abundant and belong mainly to the MAIT17 subset in both tissues[134,135]. In addition, both tissues are possibly involved in priming or reactivation of autoreactive T cells in EAE[199,324]. One study investigated the early migration of T cells in EAE and showed that they homed to the lung and reprogramed their gene expression before infiltrating the CNS[324]. This process could also lead to an activation of MAIT cells in the lung, which then infiltrate the CNS. Another possibility is that MAIT cells in the lamina propria get activated during the early phase of EAE and then migrate into the inflamed CNS. A gut dysbiosis with increased intestinal permeability occurs at EAE onset either induced by active immunisation, passive transfer or in a spontaneous EAE model using humanized transgenic mice[325,326]. The resulting increase of bacteria and bacterial antigens might possibly cause an activation of MAIT cells contributing to infiltration of the CNS.

Intestinal permeability is also increased in MS patients[327,328,329] further underlining the importance of the microbiome and the gut-brain axis in MS as well as other brain disorders[330]. Published studies assume that MAIT cells are not only indirectly activated by a leaky gut barrier but also actively contribute to maintenance of the gut barrier integrity most likely by producing IL-17A and IL-22[331]. In addition, *Mr1*$^{-/-}$ mice have an increased gut permeability resulting in more bacterial antigens and DNA in LN[155]. It has to be disentangled if the alterations of the microbiome and gut integrity observed in many metabolic and autoimmune diseases are cause or consequence of the pathogenesis[159,332]. In this context, MAIT cells might be involved in the initiation and early phases of diseases and are interesting candidates for treatment opportunities or as biomarkers.

In the EAE model, MAIT cell activation in the lung or lamina propria might result in a direct infiltration of MAIT cells into the CNS since they already have a memory phenotype and do not require an initial activation in SLO. Early responses and strong activation of MAIT cells occurred not only in EAE, but also in other disease models. MAIT cell frequency was increased in the lung in influenza- or *Salmonella*-infected mice during early time points of the disease and MAIT cells strongly upregulated the expression of CD69, which was respectively elevated compared to non-MAIT T cells[153,322]. In line with these published studies, CNS-infiltrating MAIT cells in acute EAE were strongly activated as reflected by CD69 expression, which was significantly increased compared to non MAIT CD44^{+} T cells.

4.4 TCR and cytokine-mediated activation of MAIT cells in EAE

CNS-infiltrating MAIT cells were strongly activated and GSEA indicated that this activation was mediated by their TCR and by cytokines. The TCR-dependent activation of MAIT cells in EAE was confirmed by an increased frequency of Nur77GFP-expressing MAIT cells in the inflamed CNS. However, the exact frequency of MAIT cells activated by their TCR cannot be quantified by Nur77GFP reporter mice, since these transient reporter mice have a peak of GFP expression 12-24 hours upon

activation and the GFP signal declines afterwards[315]. Therefore, early TCR-dependently activated MAIT cells could have possibly lost their Nur77GFP signal already over time suggesting that the amount of MAIT cells activated by their TCR in EAE might be higher than measured by Nur77$^+$ MAIT cells. Nevertheless, the discrepancy between the strong MAIT cell activation in the CNS of mice in acute EAE shown by CD69 expression (almost 82%) and the substantially lower frequency of Nur77GFP$^+$ MAIT cells (around 22%) indicates that also a TCR-independent, cytokine-dependent MAIT cell activation occurred in EAE. Both activation pathways have already been described for MAIT cells in other disease models. While the accumulation and activation of MAIT cells in influenza-infected mice was modulated through cytokines, independent of MR1[142], GSEA revealed that MAIT cells from *Legionella*-infected mice were activated in a TCR- and cytokine-dependent manner[146].

So far, it remains elusive if TCR-dependent activation of MAIT cells in EAE took place in a peripheral tissue before CNS infiltration or in the CNS. MR1 is widely expressed in different cell types[117] indicating that TCR activation of MAIT cells was potentially not only mediated by one certain cell type and can most likely occur in all tissues. That distinct cell types were responsible for MAIT cell activation has already been shown in different bacterial infection models. The activation of MAIT cells from the lung of *Legionella*- or *Salmonella*-infected mice was dependent on bone marrow-derived APCs or non-bone marrow-derived cells respectively[145]. To further address the TCR-dependent MAIT cell activation, it is necessary to decipher the origin of CNS-infiltrating MAIT cells in EAE.

This work presented data supporting both possibilities, a TCR-dependent activation of MAIT cells in a peripheral tissue or in the CNS. GSEA showed that genes associated with a TCR-dependent activation of MAIT cells were not only upregulated in the CNS compared to the spleen in acute EAE (MEC vs MES), but also in MAIT cells from the spleen of mice with EAE in comparison to naïve mice (MES vs MNS). Nevertheless, even if a TCR-dependent activation of MAIT cells occurred in the periphery, a reactivation in the CNS is equally supported by this analysis. An increased frequency of Nur77GFP-expressing MAIT cells in EAE was only found in the CNS, while the frequency in LN remained unaltered between naïve mice and mice with EAE. Furthermore, MAIT cells from LN in acute EAE had a higher frequency of CD69-expressing cells in comparison to MAIT cells form naïve LN indicating that MAIT cell activation in LN was most likely caused by the cytokine milieu independent of TCR activation. Therefore, more tissues have to be investigated during early phases of EAE to uncover possibly peripheral TCR activation of MAIT cells in EAE. In addition, the TCR activation of MAIT cells in the inflamed CNS can be further addressed by *in vitro* experiments culturing MAIT cells from Nur77GFP mice and DP thymocytes together with supernatant from digested CNS of either naïve mice or mice with EAE. It has been shown that DP thymocytes were 50 times more efficient than splenocytes at activating MAIT cells in a MR1-dependent manner *in vitro*[127]. TCR-dependent activation of MAIT cells cultured with the supernatant form the inflamed CNS would further indicate a TCR activation of MAIT cells in the CNS in EAE as well as the existence of a CNS-specific autoantigen of MAIT cells. Studies have already proposed the existence of other MAIT cell-activating ligands without identifying specific molecules[124,125]. Furthermore, the antigen-binding groove of MR1 is not fully occupied by riboflavin metabolites[120,122] demonstrating a more diverse repertoire of MR1 antigens as currently identified and appreciated[152].

4.5 T-bet$^+$ and T-bet$^-$ MAIT17 cell subsets in the inflamed CNS

In this work, investigations of different molecules to classify MAIT cell subsets in EAE revealed that either T-bet$^+$ as well as T-bet$^-$ MAIT17 cells were present in the inflamed CNS, but no RORγt$^-$ MAIT1 cells. While in naïve laboratory mice either T-bet$^+$ MAIT1 or RORγt$^+$ MAIT17 cells existed[134], the formation of RORγt$^+$ T-bet$^+$ MAIT cells seems to be a general mechanism also observed in inflamed lungs of either *Salmonella*- or *Legionella*-infected mice[145,153]. Published studies revealed that these MAIT cell subsets were most likely induced by a combination of TCR- and cytokine-dependent activation. The TCR stimulation of MAIT cells leads to an induction of a type-17 phenotype marked by an upregulation of RORγt and the cytokines IL-17A, TNF and GM-CSF[168,333,334]. In addition, also MAIT17 cells from the thymus displayed an increased Nur77GFP signal compared to MAIT1 cells[127]. Next to a direct TCR stimulation, also costimulatory molecules like the inducible T cell costimulatory (ICOS) are important for RORγt upregulation in MAIT cells[145]. In contrast, cytokine-dependent MAIT cell activation induced a type-1 phenotype initiated by an increase of T-bet expression leading to elevated expression of IFN-γ, granzyme B and perforin[147,333,334].

In humans, all MAIT cells express RORγt and T-bet[8,335] and it remains elusive if the mutually exclusive expression of these transcription factors in naïve mice is a general species-specific difference or a phenomenon only observed in laboratory mice raised in pathogen-free or pathogen-reduced environments. RORγt$^+$ T-bet$^+$ MAIT cells from bacterial infection or EAE models also co-expressed, like human MAIT cells, IL-17A and IFN-γ[8,153]. Therefore, MAIT cells from infection and EAE models closer resemble human MAIT cells possibly further improving the translation of findings in animal models to human patients. Next to human MAIT cells, CNS-infiltrating MAIT cells in EAE were also similar to pathogenic Th17 cells by co-expressing the transcription factors RORγt and T-bet, secreting the cytokines IL-17A, IFN-γ and GM-CSF[72,264] and upregulating a pathogenic Th17 gene signature in GSEA[233]. Furthermore, the differentiation of naïve T cells into pathogenic Th17 cells required the IL-23-IL23R axis[233] also important for MAIT cell functions[131,145]. MAIT cells highly express the IL-23R[131] and their accumulation and activation in bacterial infection models was significantly reduced in the absence of IL-23, whereas non-MAIT T, NKT and γδ T cells were unaltered[145]. IL-23 is important for EAE induction[67] as well as elevated in MS patients[336] and might also contribute to the activation and subset formation of MAIT cells in EAE.

Taken together, RORγt$^+$ T-bet$^-$ as well as RORγt$^+$ T-bet$^+$ MAIT cells infiltrated the CNS in EAE and were most likely generated by distinct activation pathways.

4.6 Proinflammatory and tissue repair function of MAIT cells in EAE

A broad repertoire of different effector functions has been proposed for MAIT cells comprising killing of target cells by cytotoxic molecules, attracting and activating other immune cells by proinflammatory cytokines and contributing to tissue repair[131,134,150,144,8]. Disentangling these functions and their respective contribution to diseases and especially autoimmune diseases is challenging and one reason for their often still elusive role in disease pathogenesis. Here, we found evidence for both proinflammatory as well as tissue repair function of MAIT cells in EAE. Since, the tissue repair signature of MAIT cells was only upregulated after a TCR-dependent activation in another transcriptome analysis[146,147] and cytokine-dependent activation has been shown to induce T-bet and

IFN-γ[147,333,334], it is likely that the different effector functions of MAIT cells in EAE were also mediated by different MAIT cell subsets induced by distinct activation pathways.

The upregulation of tissue repair associated genes and the proinflammatory cytokines IL-17A, IFN-γ and GM-CSF was not only observed in MAIT cells in EAE, but also in MAIT cells from bacterial infection models[131,147]. In addition, the highest enriched GO terms associated with proinflammatory or tissue repair function were partially the same or similar between CNS-infiltrating MAIT cells in EAE and MAIT cells from the skin after *Staphylococcus epidermidis* exposure[131]. In contrast to bacterial infection models where both effector functions contribute to disease amelioration, proinflammatory effector functions of MAIT cells in EAE have most likely an exacerbating disease effect like it was reported for pathogenic Th17 cells[64]. The cytokines IL-17A, IFN-γ and GM-CSF are key contributors to EAE pathogenesis[337], while their exact effector function depends on the localisation in spinal cord or brain and the timepoint in EAE pathogenesis[279]. Many studies revealed that these cytokines contribute to the infiltration of other immune cells like monocytes, macrophages and neutrophils in the inflamed CNS in EAE[278,279]. There were no differences between C57BL/6J and *Mr1*$^{-/-}$ mice in the frequencies of CNS-infiltrating immune cells, indicating that the cytokines produced by MAIT cells in EAE did not induce the infiltration of specific immune cells. However, an influence on the activation status of these immune cells as well as effects on neurons, astrocytes and other cells not included in the analysis could not be excluded. In this work, EAE was induced in C57BL/6J mice using the MOG_{35-55} peptide resulting in a classical EAE predominated by infiltration of the spinal cord. CNS-infiltrating MAIT cells highly expressed LFA-1 and had a higher frequency of IL-17A-producing cells compared to Tc1/Th1 and Tc17/Th17 cells *ex vivo* as well as after PMA and ionomycin stimulation. Therefore, the proinflammatory effector function of MAIT cells might have a stronger influence in atypical EAE models primarily based on IL-17A and the infiltration of Th17 cells in the brain[293].

Analysing the EAE course of *Mr1*$^{-/-}$ mice or mice receiving an anti-MR1 antibody resulted in an exacerbated EAE revealing that the protective function exceeded the proinflammatory function of MAIT cells in the classical EAE model. *Mr1*$^{-/-}$ mice have been reported to have some developmental phenotypes possibly also affecting EAE pathogenesis. An increased gut permeability was reported in NOD *Mr1*$^{-/-}$ mice and might further boost the activation of immune cells and the elevated gut permeability observed after EAE induction[325,326]. Furthermore, a recent publication reported an increased frequency of γδ T cells in a person having a homozygous point mutation in *Mr1* resulting in the absence of MAIT cells[338]. γδ T cells have been implicated in MS pathogenesis, as they were accumulated in MS lesions[339] and had a pathogenic role in EAE[340]. An increased frequency of γδ T cells in *Mr1*$^{-/-}$ mice has not been investigated yet but vice versa the absence of γδ T cells leads to an increase of MAIT cells further indicating that these cells competed for a shared niche[131,341]. Therefore, an elevated frequency of γδ T cells might also contribute to the exacerbating EAE course of *Mr1*$^{-/-}$ mice. However, treating C57BL/6J mice with an anti-MR1 antibody most likely avoided these developmental phenotypes and specifically blocked the TCR-dependent activation of MAIT cells. Therefore, it was anticipated that this treatment would inhibit tissue repair function of MAIT cells, whereas the cytokine-mediated proinflammatory function was mainly unaltered. The anti-MR1 antibody has previously been used in bacterial infection models and blocked MAIT cell activation as well as strongly reduced their accumulation in inflamed lungs[153,168]. However, if and how this treatment

reduced MAIT cell frequencies and shifted their subset distribution in the CNS has not been analysed yet, but will be a necessary investigation to disentangle the connection between activation pathways, subset distribution and effector function of MAIT cells in EAE.

Taken together, the protective effect of MAIT cells in EAE was most likely mediated by their tissue repair function driven by TCR-mediated activation. Published studies reported that a tissue repair signature was increased in MAIT cells, but not in non-MAIT T cells after TCR-dependent activation[147]. This work uncovered that this tissue repair signature was upregulated in CNS-infiltrating MAIT cells, but also in published datasets of CNS-infiltrating Th17 and $CD4^+$ memory T cells in EAE. Therefore, the enrichment of tissue repair signatures in EAE was not MAIT cell specific. However, it has to be investigated if the upregulation of the tissue repair signatures in MAIT and non-MAIT T cells in EAE was driven by the same genes and mechanisms.

4.7 Potential molecules and mechanisms involved in MAIT cell-mediated tissue repair

The tissue repair signature of MAIT cells is a newly discovered effector function of human and murine MAIT cells[131,146,147,333]. *In vivo* tissue repair function has been shown only by one study so far revealing the contribution of cutaneous MAIT cells to wound healing[131]. No specific molecules or mechanisms mediating the MAIT cell-mediated tissue repair have been identified. Furthermore, it has to be proven if molecules contributing to tissue repair in peripheral tissues have the same function in the CNS, since the CNS is a specific tissue comprising many unique cell types and a complex structure. Neurons are mainly but not exclusively present in the CNS and also about half of the CNS volume consists of oligodendrocytes, astrocytes and microglia only located in the CNS[342]. Remyelination, glial scar formation and closing the BBB are mechanisms contributing to tissue repair of the CNS not only in MS, but also in stroke, neurodegenerative diseases and traumatic injuries[343]. Reactive astroglial scars at plaque edges seal lesions and separate the injured and inflamed from the surrounding tissue, but also prevent remyelination by inhibiting migration of oligodendrocyte progenitor cells into the lesion[344,345]. Closing the BBB by modulating endothelial cells is possibly the most similar mechanism compared to repair in peripheral tissues. BBB recovery by modulating the Wingless-related integration site (Wnt) signalling pathway in endothelial cells limits CNS-infiltration of immune cells and partially restores transcellular transport in EAE[346]. Nevertheless, genes and pathways involved in tissue repair in the periphery could also contribute to an exacerbated EAE/MS pathogenesis.

For example, GM-CSF was part of the upregulated tissue repair signature of MAIT cells in this work as well as in published studies[146,147]. GM-CSF is contributing to different processes of tissue repair by recruiting other immune cells and stimulating re-epithelisation, micro-vascular barrier integrity and angiogenesis[347,348]. Furthermore, it has been shown that local injections of GM-CSF accelerate healing of chronic wounds in patients[349] and the absence of GM-CSF in *Csf2*$^{-/-}$ mice leads to a compromised wound healing[350]. At the same time, GM-CSF is the only so far identified proinflammatory cytokine being indispensable for EAE induction[288,289,351].

Moreover, angiogenesis is a general mechanism of tissue repair and induced by different molecules like VEGF-A and VEGF-B. The GO term angiogenesis, *Vegfa*, *Vegfb* and other involved genes were upregulated by TCR-dependently activated MAIT cells[131,146,147] as well as by MAIT cells in

EAE. The role of VEGF and angiogenesis in EAE and MS is still controversial and ameliorating as well as exacerbating effects have been reported[352]. In the early phase of EAE, VEGF contributes to the break-down of the BBB[353] and increases inflammation in the CNS[354]. In contrast, in later disease phases VEGF has neuroprotective effects on neurons and neural progenitors and promotes angiogenesis and glial survival[352,355].

CNS-infiltrating MAIT cells in EAE as well as MAIT cells from *Legionella*-infected mice strongly upregulated *Areg*[147] revealing that this might be a general mechanism how MAIT cells contributed to tissue repair. AREG has not only been shown to be involved in tissue repair in peripheral tissues like muscles[102] but also in the CNS of mice after ischaemic stroke[103]. In the stroke model, brain Tregs produced AREG leading to neuroprotection by regulation of astrogliosis and neural recovery. These effects were most likely mediated via the IL-6 and STAT3 pathway in microglia and astrocytes and were not observed after transferring *Areg*$^{-/-}$ Tregs compared with wildtype Tregs. Furthermore, Ito and colleagues postulated that preliminary experiments showed that Tregs in the brain of mice with EAE had a similar phenotype. Transferring brain Tregs from the stroke model into mice with EAE suppressed EAE indicating that AREG could have a similar effect in EAE[103]. Therefore, further deciphering AREG-mediated and other tissue repair functions of MAIT cells in EAE will help to disentangle the effector function of MAIT cells and to uncover the mechanism of their protective impact on EAE as well as other diseases.

4.8 MAIT cells as target for MS treatment

Discoveries and possible treatment approaches targeting MAIT cells in EAE are likely be translatable to the human situation since MR1 and MAIT cells are evolutionarily highly conserved between mice and humans[113356]. Furthermore, discrepancies between humans and naïve mice concerning expression of cytokines and transcription factors[8,152] were reduced when investigating MAIT cells in EAE as well as in other disease models[153]. MAIT cells are neither the only cells producing proinflammatory cytokines like GM-CSF and IL-17A nor are they exclusively having a possible tissue repair function in the CNS of mice with EAE, but in contrast to many other immune cells they can be specifically modified. Blocking of MAIT cells by an anti-MR1 antibody or activation by administration of 5-OP-RU would precisely target MAIT cells without directly affecting other immune cells[145,153,322]. Furthermore, the presence of Nur77^{+} TCR-activated MAIT cells in the inflamed CNS is an indication that possibly a CNS autoantigen could exist. Identifying this autoantigen could open the possibility to affect CNS-infiltrating MAIT cells even more specifically. While nearly all treatments for MS patients broadly targeted several components of the immune system[2], a MAIT cell treatment would be more specific and could possibly reduce side effects. Moreover, activating MAIT cells via their TCR could induce tissue repair function and thereby also convert a MAIT cell subset having proinflammatory function into a subset with a protective effect. Since tissue damage either induced by immune cells, pathogens, surgeries and other causes is a general consequence of many diseases, enhancing the tissue repair function of MAIT cells might be a treatment option not only for MS patients but also in other diseases. Furthermore, even if only treating MAIT cells turns out not to be sufficient to stop disease progression, the treatment could be due to the specificity combined with other medications to turn an inflammatory into a more regenerative environment.

5 Summary

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system (CNS) affecting approximately 2.5 million people worldwide, whereas no curative treatment exists. The complex contribution of immune cells to damage and repair of the CNS in MS pathogenesis is unresolved and a major focus of MS research. MS is considered to be an autoimmune disease most likely caused by autoreactive T cells. A recently discovered T cell population, mucosal-associated invariant T (MAIT) cells, is highly abundant in humans and shares many similarities with T helper 17 (Th17) cells, a T cell subset previously implicated to play a fundamental role in MS and its animal model experimental autoimmune encephalomyelitis (EAE). Indeed, MAIT cells have been detected in inflamed brain lesions of MS patients. However, functional analyses of CNS-infiltrating MAIT cells are lacking and require a characterisation in the EAE model.

In this work, newly available tools to study MAIT cells in mice were combined with the EAE model enabling for the first time to characterise CNS-infiltrating MAIT cells in EAE and to investigate their role in EAE pathogenesis.

Few MAIT cells were found in the CNS of naïve mice, whereas MAIT cells highly accumulated in the CNS of mice with EAE. The substantial increase of MAIT cell frequencies in the CNS already during preclinical EAE as well as the upregulation of chemokine receptor and integrin expression by CNS-infiltrating MAIT cells indicated CNS infiltration of MAIT cells from the periphery, while a local proliferation of CNS-resident MAIT cells could not be excluded. Transcriptome analysis and the use of Nur77GFP reporter mice revealed that MAIT cells in the inflamed CNS were activated by cytokines and antigen-specifically via their TCR. Published studies recently showed that TCR-dependent activation of MAIT cells induced a tissue repair function, while cytokine-dependent activated upregulated proinflammatory and cytotoxic molecules. In line with these studies of other disease models, proinflammatory and tissue repair signatures as well as RORγt^{+} T-bet^{+} or RORγt^{+} T-bet^{-} MAIT cell subsets were identified in the inflamed CNS. MAIT cells in EAE closely resembled pathogenic Th17 cells and produced the cytokines GM-CSF, IL-17A and IFN-γ, which are known to contribute to EAE pathogenesis. However, analysing the EAE course of *Mr1*$^{-/-}$ mice and mice treated with an anti-MR1 antibody uncovered that the protective exceeded the proinflammatory effects of MAIT cells in EAE resulting in a more severe disease course. Using an anti-MR1 blocking antibody most likely avoided developmental phenotypes observed in *Mr1*$^{-/-}$ mice and blocked the TCR-dependent activation of MAIT cells required for the upregulation of the tissue repair signature. Specific molecules involved in MAIT cell mediated tissue repair remain elusive. Amphiregulin (AREG) might be a candidate for further investigations, since *Areg* was strongly upregulated in MAIT cells isolated from the CNS of mice in acute EAE and published studies uncovered a protective effect of AREG in a stroke mouse model and claimed a similar effect in EAE.

In summary, this work proposes that differentially activated MAIT cells infiltrate the inflamed CNS and fulfil either proinflammatory or tissue repair function, while *in vivo* experiments indicates that the protective effect exceeds. Therefore, specifically inducing the tissue repair potential of MAIT cells and blocking their proinflammatory functions represent interesting treatment opportunities for MAIT cells in EAE and MS.

Zusammenfassung

Multiple Sklerose (MS) ist die häufigste entzündliche Erkrankung des zentralen Nervensystems (ZNS) und betrifft weltweit ungefähr 2,5 Millionen Menschen. Es wird angenommen, dass MS eine Autoimmunerkrankung ist, die durch autoreaktive T-Zellen ausgelöst wird. Bei den kürzlich erst beschriebenen *mucosal-associated invariant T* (MAIT)-Zellen handelt es sich um eine T-Zell-Population, die viele Gemeinsamkeiten mit Typ17-T-Helferzellen (Th17-Zellen) aufweist, welche eine wichtige Rolle in MS und deren Tiermodell, der experimentellen autoimmunen Enzephalomyelitis (EAE), spielen. Des Weiteren wurden MAIT-Zellen in entzündeten Läsionen im ZNS von MS-Patienten nachgewiesen. Es liegen jedoch noch keine funktionellen Analysen der MAIT-Zellen im ZNS vor, was eine Charakterisierung der MAIT-Zellen im EAE Modell erforderlich macht.

In dieser Arbeit wurden neu verfügbare Methoden zur Detektion und Analyse von MAIT-Zellen in Mäusen mit dem EAE Modell kombiniert, wodurch erstmals eine Untersuchung der ins ZNS einwandernden MAIT-Zellen und deren Rolle in der EAE Pathogenese möglich wurde.

MAIT-Zellen waren in der EAE stark im ZNS angereichert und eine erhöhte Frequenz an MAIT-Zellen konnte schon während der vorklinischen Phase der EAE festgestellt werden. Da MAIT-Zellen aus dem ZNS eine erhöhte Expression von Chemokinrezeptoren und Integrinen auf der Oberfläche aufwiesen, kann von einer Einwanderung der MAIT-Zellen aus der Peripherie ausgegangen werden. Eine lokale Proliferation kann allerdings nicht ausgeschlossen werden. Transkriptomanalysen und Untersuchungen von Nur77GFP Reportermäuse haben gezeigt, dass MAIT-Zellen aus dem entzündeten Gehirn durch Zytokine und antigenspezifisch durch den T-Zell-Rezeptor (TZR) aktiviert wurden. Erst kürzlich erschienene Veröffentlichungen haben gezeigt, dass eine TZR-abhängige Aktivierung von MAIT-Zellen zu einer gesteigerten Expression von Genen führte, die an der Gewebereparatur beteiligt sind, während eine Zytokin-abhängige Aktivierung eine Erhöhung von proinflammatorischen und cytotoxischen Molekülen zur Folge hatte. Übereinstimmend mit Studien zu MAIT-Zellen aus anderen Krankheitsmodellen konnten in der vorliegenden Arbeit ebenfalls proinflammatorische und mit Gewebereparatur assoziierte Signaturen, sowie RORγt^{+} T-bet^{-} und RORγt^{+} T-bet^{+} MAIT-Zell-Subpopulationen im entzündeten ZNS nachgewiesen werden. MAIT-Zellen aus dem ZNS von Mäusen im EAE Modell wiesen viele Gemeinsamkeiten mit pathogenen Th17-Zellen auf und produzierten die Zytokine GM-CSF, IL-17A und IFN-γ, welche zur EAE Pathogenese beitragen. Die Analyse des EAE Verlaufs von *Mr1*$^{-/-}$ Mäusen und mit einem anti-MR1 Antikörper behandelten Mäusen zeigte einen verschlimmerten Krankheitsverlauf, was auf eine protektive Funktion der MAIT-Zellen schließen lässt. Durch die Verwendung des anti-MR1 Antikörpers wurden wahrscheinlich Entwicklungsphänotypen, die in *Mr1*$^{-/-}$ Mäusen beobachten wurden, vermieden und die TZR-abhängige Aktivierung blockiert, die erforderlich für die Gewebereparatur von MAIT-Zellen ist. Spezifische Moleküle, die die Gewebereparatur von MAIT-Zellen vermitteln, sind bisher nicht bekannt. Amphiregulin (AREG) ist ein Kandidat für weitere Untersuchungen, da *Areg* sehr stark von MAIT-Zellen aus dem entzündeten ZNS exprimiert wurde und Studien einen protektiven Effekt von AREG im Schlaganfallmodellen zeigten und diesen ebenfalls für das EAE Modell postulierten.

Zusammenfassend wurde in dieser Arbeit gezeigt, dass auf unterschiedliche Weise aktivierte MAIT-Zellen im EAE Modell ins entzündete ZNS einwandern und sowohl proinflammatorische als

auch gewebereparierende Funktionen haben, wohingegen die protektiven Effekte in *in vivo* Experimenten überwiegen. Folglich sind die gezielte Anregung der Gewebereparatur und das Blockieren der proinflammatorischen Funktion interessante Behandlungsansätze für MAIT-Zellen im EAE Modell und in MS-Patienten.

6 References

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Affidavit

I hereby confirm that this dissertation is my own written work and that I have used no sources and aids other than indicated.

M. Walkenhorst — Hamburg, 23.09.2020

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