THE CLOËTTA PRIZE 2022
IS AWARDED TO

PROFESSOR

DORON MERKLER

BORN IN 1974 IN RAMAT GAN, ISRAEL
DEPUTY HEAD OF THE DIVISION OF CLINICAL PATHOLOGY
AND SENIOR PHYSICIAN IN NEUROPATHOLOGY
IN THE DEPARTMENT OF PATHOLOGY AND IMMUNOLOGY
AND THE DEPARTMENT OF DIAGNOSTICS AT THE UNIVERSITY
OF GENEVA AND GENEVA UNIVERSITY HOSPITALS

FOR HIS OUTSTANDING CONTRIBUTIONS
TO BIOMEDICAL RESEARCH AND TO THE ELUCIDATION
OF THE FUNCTIONAL CHARACTERIZATION OF CD8+ T-CELLS
IN THE PATHOGENESIS OF BRAIN INFLAMMATION

GENEVA, 25TH NOVEMBER 2022

IN THE NAME OF THE FOUNDATION BOARD:

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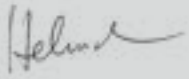

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CURRICULUM VITAE

Personal Information

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Current Position(s)

As of 2020	Full Professor, Deputy Head of the Division of Clinical Pathology, and Senior Physician in Neuropathology in the Department of Pathology and Immunology and the Department of Diagnostics, University of Geneva and Geneva University Hospitals (HUG), Switzerland

Previous Positions

2016–2020	Associate Professor and Consultant in Neuropathology, University and University Hospitals of Geneva, Dept. of Pathology and Immunology, Switzerland
2010–2016	Assistant Professor and Consultant in Neuropathology, University and University Hospitals of Geneva, Dept. of Pathology and Immunology, Switzerland

2009–2010	Consultant in neuropathology at the Department of Neuropathology (University Medical Center, Göttingen, Germany)
2003–2008	Resident in Clinical Neuropathology, University Medical Center Göttingen, Department of Neuropathology, Germany (head: Prof. W. Brück)
2002	Research fellow, Brain Research Institute, University of Zurich, and Department of Biology, Swiss Federal Institute of Technology Zurich, Switzerland

Education

2009	Venia Legendi (Habilitation) in Neuropathology at the Georg August University, Göttingen, Germany
2003–2006 & 2008	Research associate and training as a specialist in neuropathology (University Medical Center, Göttingen, Germany)
2007	Visiting scientist (scholarship from the University of Goettingen) at the Institute of Experimental Immunology (heads: Profs. R. Zinkernagel and H. Hengartner), University Hospital Zurich, Switzerland
1998–2002	MD thesis in Neuroscience with Martin Schwab, PhD, Professor of Neuroscience at the University & ETH Zurich, Switzerland
2001–2002	Postgraduate course in Experimental Medicine at the University of Zurich, Switzerland (organized by Prof. Zapf, Dept. of internal medicine, University Hospital of Zurich, Switzerland)
2000	United States Medical Licensure Examination (USMLE) steps 1 and 2
1994–2000	Medical School, State examination (University of Zürich, Medical Faculty, Switzerland)

Funding (since 2015)

2021–2025	Principal PI: SNSF Project Grant Swiss National Science Foundation (Excellence grant in Life Sciences) «Deciphering transcriptional regulators of T cell fate decisions in CNS autoimmunity.»
2020–2022	Co-PI (P. Walker & D. Merkler) Fondation HUG STARTER translational research project «The Role of the DNA-Binding Factor TOX in CD8-mediated immunity to Glioblastoma.»
2020–2025	Principal PI – ERC Consolidator Grant «Molecular pathology of anti-viral T cell responses in the central nervous system.»
2019–2023	Principal PI – SNSF Project Grant Swiss National Science Foundation (Projects Life Sciences), «Drivers and signatures of neuronal dysfunction in neuroinflammation.»
2017–2018	PI – Swiss MS society grant «Analysis of functional and transcriptional landscape of brain-resident memory T cells in a mouse model of multiple sclerosis.»
2017–2021	Principal PI – SNSF Project Grant Swiss National Science Foundation, «Compartmentalized T cell memory in brain viral infection and autoimmunity.»
2016–2019	Co-PI (M. Trajkovski & D. Merkler), Fondation HUG Confirm Grant «Protective role of brown fat induction in multiple sclerosis.»
2016–2018	Consortium (4 groups: PI: Mikael Simons), Klaus Tschira Stiftung Gemeinnützige GmbH «The molecular signature of microbes in the immunology and neurobiology of multiple sclerosis.»
2016–2019	Principal PI – Helmut Horten Foundation «In vivo barcoding of cytotoxic T cell for the identification of novel biomarkers and therapeutic targets in CNS autoimmunity.»

2016–2018	Consortium (4 groups, Co-applicant) Sinergia Grant, Swiss National Science Foundation, «The alarmin interleukin-33 in infection, immunity and autoimmunity»

Fellowships and Awards

2022	Cloëtta-Prize for outstanding contributions to biomedical research
2010–2016	Stipendiary professorship of the Swiss National Science Foundation
2009	Award for best «Habilitation» of the Faculty of Medicine, UMG, Göttingen, Germany
2007	Award for outstanding publication, Göttingen Research Council, Göttingen, Germany
2006	Research Scholarship, University Göttingen, UMG, Göttingen, Germany
2001	Scholarship for a Postgraduate Course in Experimental Medicine and Biology, Swiss National Science Foundation

Supervision of 9 postdoctoral fellows, 7 graduate students (since 2008)

Teaching Activities (summary)

- Problem-based learning series in «Défense and Immunity» and Lecturer «Immunohistopathology» for 3rd, year MD students at the Medical Faculty, University of Geneva, Switzerland
- Lecturer first year MD students «Dysfunction of immune responses: autoimmunity and cancer»
- Courses in a Clinical Setting (AMC) for medical students in neuropathology
- Teaching in neuropathology for medical interns for FMH

Organisation Of Scientific Meetings

2018	Member of the program organization committee for the annual meeting of the Swiss Society of Allergology and Immunology (SSAI) (about 200 participants), Interlaken, Switzerland

Institutional Responsibilities

As of 2020	Scientific Coordinator of the Geneva Centre for Inflammation Research (https://www.unige.ch/medecine/gcir/en/about-us/)
As of 2019	President of the animal facility commission, University of Geneva
As of 2017	Co-organizer of the Departmental seminar series, University of Geneva, Switzerland
As of 2016	Responsible for medical interns in the division of clinical pathology and specialist training in Neuropathology in Geneva
As of 2012	Responsible for biosafety level 2 animal facilities, University of Geneva, Switzerland
As of 2012	Member of the commission for the MD PhD Program, Medical Faculty of Geneva

Reviewing Activities

As of 2010	Ad hoc reviewer for Brain, Annals of Neurology, Experimental Neurology, Acta Neuropathologica, J. Neuroscience, Nature Immunology, European Journal of Immunology, and others
As of 2012	Reviewer for: Swiss National Science Foundation (SNF), German Research Council (DFG), ARESEP (French Multiple Sclerosis Foundation), ERC, Swiss MS Society

As of 2019	Member of the Editorial Board of Acta Neuropathologica

Memberships Of Scientific Societies

As of 2018	Member of the Commission of Experimental Immunology of the Swiss Society of Allergology and Immunology (SSAI), Switzerland
As of 2019	President of the Swiss Society of Neuropathology, Switzerland
As of 2019	Member of the Scientific Committee of ARSEP Foundation (France)

Patents

Patent application (EP3218504A1; US 16/922,489): Tri-segmented arenaviruses as vaccine vectors Patent application (WO 2013098264 A1; US 20140378548 A1) Inhibitor of trpm-4 ion channel for treating or preventing neurodegeneration

Publication Record

As of August 2022: h-index: 57; >11.9 k citations (Source: Google Scholar)

Complete List: https://scholar.google.ch/citations?user=EMV2SU%20MAAAAJ&hl=en&user=lidwIScAAAAJ

SELECTED PUBLICATIONS

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Jafari M, Schumacher AM, Snaidero N, Ullrich Gavilanes EM, Neziraj T, Kocsis-Jutka V, Engels D, Jürgens T, Wagner I, Weidinger JDF, Schmidt SS, Beltrán E, Hagan N, Woodworth L, Ofengeim D, Gans J, Wolf F, Kreutzfeldt M, Portugues R, **Merkler D***, Misgeld T*, Kerschensteiner M*. Phagocyte-mediated synapse removal in cortical neuroinflammation is promoted by local calcium accumulation. ***Nat Neurosci.*** 2021 Mar;24(3): 355–367. * shared last-authorship

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Page N, Klimek B, De Roo M, Steinbach K, Soldati H, Lemeille S, Wagner I, Kreutzfeldt K, Di Liberto G, Vincenti I, LIngner T, Salinas G, Brück W, Simons M, Murr R, Kay J, Zehn D, Pinschewer DD, **Merkler D**. TOX expression governs the encephalitogenic potential of microbe-induced autoreactive CD8+ T cells. ***Immunity.*** 2018 May 15;48(5): 937–950

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Steinbach K, Vincenti I, Kreutzfeldt M, Page N, Muschaweckh A, Drexler I, Pinschewer D, Korn T, **Merkler D**. Brain-resident memory T cells represent an autonomous cytotoxic barrier to viral infection. ***J Exp Med.*** 2016 July 4

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THE DOUBLE-EDGED SWORD OF IMMUNE RESPONSES IN THE CENTRAL NERVOUS SYSTEM

Doron Merkler [1,2]

Summary

With a well-functioning immune system, our body successfully fights most of the different types of infectious threats that challenge us daily. Especially in adult mammals' central nervous system (CNS), where neurons have a limited regenerative capacity, the immune system faces a major challenge in performing the desired task without causing irreversible damage to our host. *How does that work?* Ideally, CNS infections should be prevented as much as possible or detected early while avoiding excessive inflammatory responses. Therefore, it is generally believed that the anatomical and functional barriers of the CNS, compared with other organs, limit pathogen access to this vital organ and minimize immune surveillance under physiological conditions. In recent decades, both experimental models and observations in humans have led, however, to the view that these barriers in place can be constantly breached by pathogens, which are rapidly eradicated in most cases by continuous immune surveillance of the CNS. Accordingly, the CNS is not exempt from immune responses that can also become harmful and persist chronically in the CNS, as observed in autoimmune and infectious disease conditions. *So, what went wrong in these situations? What are the causes and consequences of a misdirected immune response that can lead to devastating outcomes seen in people with neuroinflammatory diseases?* Understanding the role of immune system sentinels, how they are activated, and the nature of interactions with other cells in the CNS in health and disease is key to answering these still incompletely understood questions.

[1] Department of Pathology and Immunology, Medical Faculty, University of Geneva.
[2] Diagnostic Department, Division of Clinical Pathology, University Hospitals Geneva.

In this review, I summarize how my laboratory has contributed to understanding immune surveillance and pathology in the CNS. Inspired by observations of human pathology in infectious and autoimmune conditions, I have been pursuing several of the above mentioned questions. For this purpose, I harnessed different experimental model systems, mainly revolving around the roles and functioning of cytotoxic T cells in the context of the CNS's protective and deleterious immune responses.

Introduction

Inflammatory responses within the Central Nervous System (CNS) generally signify the interplay of two essential organ systems with fundamentally different properties. On the one hand, the immune system mainly comprises mobile cell types and macromolecules that circulate in our body and can act systemically or locally whenever needed. On the other hand, the cells of the adult CNS, such as neurons, oligodendrocytes, or astrocytes, form highly interconnected but immobile functional cellular networks. Moreover, under physiological conditions, the CNS is separated by anatomical and biochemical barriers from the rest of the body, including circulating immune cells. In this regard, the seminal Medawar's observation in the 1940s that the CNS does not show typical inflammatory responses to allografts has led to the concept of the CNS as an "immune privileged" organ. However, this originally somewhat dogmatic concept has been put into perspective over the years.

Contrary to initial observation, the CNS displays a draining lymphatic system in the meninges and can trigger an inflammatory response. However, unlike most other organs, the CNS shows certain peculiarities in regulating immune responses [1]. The fact that the regenerative capacity of CNS cells, especially neurons, is limited fuels the notion of tightly controlled immune responses by the tissue microenvironment. Accordingly, inflammatory responses in the CNS always face a delicate balance between protecting this vital organ from various types of infections and the risk of causing irreversible tissue damage that can lead to long-term functional impairment, as seen in chronic autoimmune and viral diseases of the CNS.

What these peculiarities in the CNS contain has accompanied me in my scientific questions. Probably since I took my first step in research in the field of neuroscience with a focus on axon regeneration after traumatic injury [2] and only later dove into the immunological world, I have always kept an eye on the "other" side of the picture, namely cells originating in the CNS, apart from my focus on the immune system.

CNS viral infections

Although anatomical barriers reduce the access of virus entry into the CNS, a wide range of neurotropic viruses are indeed capable of infecting this vital organ. Such infections can become medical emergencies associated with significant morbidity, mortality, or long-term sequelae and can have devastating outcomes [3]. Thereby, disease patterns of CNS viral infections can be sporadic, endemic, epidemic, or pandemic [4]. Viruses that enter the human CNS include, among others, Enteroviruses, Arboviruses, and Herpesvirus (for an extensive list, see, e. g. [5]). Most viral CNS infections result from hematogenous dissemination and are initially confined to the CNS coverings [6] and use different routes of entry across CNS barriers, including transcellular or paracellular transport or infection of leukocytes entering the CNS as a Trojan horse [7,8]. Depending on whether the viruses spread mainly in the meninges or the parenchyma, such infections are classified as meningitis, encephalitis, or a combination of both [9].

The exact incidence of CNS viral infections is difficult to estimate and depends on the cohort studied, which varies in geography, age groups, case definition, and the immunological status of investigated individuals [10–15]. Overall, the estimated annual incidence ranges from 2–10 per 100 000 for all ages [11], and according to the United States Centers for Disease Control and Prevention, ~20 000 cases of clinically manifest infections occur each year in the United States. In addition, and well beyond these numbers, many common viral infections affect the CNS without or only mild manifestations that do not need diagnostic or therapeutic interventions and are thus not included in the above statistics [16]. In the era before vaccination, various viruses frequently affected the CNS, resulting in infection of the meninges or parenchyma. For exam-

ple, an estimated 50% of infected individuals with Mumps were often accompanied by meningitis but mostly resolved without complications or sequelae [17,18]. Similar frequencies of CNS involvement were observed during acute systemic infection with measles [19,20]. As in measles and mumps, equal mild CNS involvement is also suspected for other viruses such as influenza [21] or coxsackie B virus [22].

While innate immune cells, including CNS-associated macrophages at CNS barriers (comprising the meninges, perivascular space, and ventricular system), can prevent further spread into the subjacent parenchyma, the control and elimination of most viral CNS infections depend on the adaptive immune system, which includes distinct T lymphocyte subsets. To study immune mechanisms involved in viral clearance from the CNS, we primarily relied on infection models with lymphocytic choriomeningitis virus (LCMV), the prototypical member of the arenavirus family [23]. LCMV has been a primary workhorse for immunologists for a decade, and it has also been explored during infection of the CNS in adult and neonatal mice to study the dual roles of the antiviral immune response in host protection and immunopathogenesis [24–26]. LCMV is a natural pathogen of mice but is also suspected to be an underestimated cause of aseptic meningitis in humans [27,28]. As in humans, LCMV can infect the meninges of adult mice, from where it gradually spreads to the parenchyma [29]. After intracerebral administration, the virus replicates in the leptomeninges, choroid plexus, and ependymal cells [30,31]. In addition, part of the inoculum enters the circulation [32] and elicits a vigorous antiviral immune response. Of note, LCMV is not cytolytic in mice, so subsequent CNS disease is caused solely by the resulting immunopathology driven by the adaptive antiviral immune response in the CNS. Thereby, CD8+ T cells have long been identified as critical players in the resultant choriomeningitis disease [33,34], which could be prevented by depleting T cells [34].

Autoimmune diseases of the CNS

More than 80 different autoimmune diseases in humans are known, affecting 3–5% of the general population [35–37]. Among autoimmune diseases affecting the CNS, demyelinating diseases such as multiple sclerosis (MS) and neuromyelitis optica spectrum disorders (NMOSD), but also paraneoplastic and other autoimmune encephalomyelitis have been best studied. These diseases typically show a chronic clinical course and are associated with disabling outcomes with important socio-economic consequences for the affected individuals and their families. In general, the extent of unrecoverable neurological impairment in CNS autoimmune diseases is related to the extent of neuro-axonal damage and irreversible neuronal loss, as these cells cannot be replaced in the adult CNS. Therefore, to curb disease progression, it is essential to understand what underlies the process of neuronal damage in various inflammatory disorders.

MS is the prime example of a chronic inflammatory demyelinating disease of the CNS that clinically presents as a relapsing-remitting (approximately 75% of cases) or progressive (about 25% of cases) disease course. In MS, several therapeutic approaches that interfere with different subsets of immune cells, including T or B cells, have shown positive results in reducing disease relapses [38]. Thus, different immune cells are likely involved at the various stage of pathogenesis. Histopathologically, active MS lesions are characterized by macrophage-rich demyelination in which axons are relatively preserved and are accompanied by lymphocytic infiltrates of variable extent [39]. This lymphocytic infiltrate is composed mainly of T cells and, to a lesser extent, B cells and plasma cells [40]. Among the distinct T cell subsets found in MS lesions, cytotoxic CD8+ T cells (CTLs) constitute the majority [41] and are clonally expanded [42]. Furthermore, CTLs persist in the CSF and peripheral blood [43,44], altogether providing valuable arguments that CTLs are important players in MS lesion formation and likely subsequent neuronal damage. Beyond MS, CTLs are particularly suspected of mediating neuronal alterations in viral infections [45], autoimmune encephalitis [46], and paraneoplastic neurological disorders [47].

The etiology of most autoimmune CNS diseases remains elusive, but they are thought to result from an interaction of genetic and environmental factors [48]. Among environmental factors, infectious agents have traditionally been suspected, but a causal relationship between a given infectious agent with an autoimmune disease remains elusive [49]. In particular, a history of viral infection is considered to increase the risk of developing the autoimmune disease [50,51]. In the same line, population migration studies have shown that in areas with high MS prevalence, the risk of developing MS increased with migration to high-risk areas before the age of 15 [49]. Thus, there may be a considerable time lag between the first exposure to the environmental factor (e. g., a viral infection) and the precipitation of manifest clinical MS. Several structurally unrelated microbes have been associated with the onset or exacerbation of MS. In this regard, Epstein-Barr virus (EBV) and other viruses have been studied most intensively [52–54]. It is believed that in certain constellations, infectious triggers may override the various immune tolerance mechanisms in place and steer the immune system toward an autoimmune response in genetically susceptible individuals. To mechanistically explain how viral infections can break immune tolerance mechanisms, several concepts have been elaborated in the past, primarily based on experimental model systems. These concepts include, e. g., molecular mimicry [55], epitope spreading [56], and bystander activation [57]. Molecular mimicry signifies the presence of T cells that exhibit cross-reactivity between a CNS antigen and a pathogen they have encountered. Epitope spreading and bystander activation refer to mechanisms by which the inflammatory environment created by an infection can facilitate the accidental priming of an autoreactive T-cell response [55].

In my various studies of autoimmune CNS diseases, I have been intrigued by the infection hypothesis as a precipitating or predisposing factor for autoimmunity, a hypothesis that remains difficult to prove. In this regard, I have investigated how viral infections, possibly during a critical time window in life, can deviate immune responses to favor autoimmune processes.

CD8+ T cell (CTL) differentiation and immunological memory

CTLs are crucial in protecting our host from intracellular infections and in the pathogenesis of various chronic autoimmune diseases. In various autoimmune diseases, including multiple sclerosis (MS [48]), type 1 diabetes (T1D [58]), polymyositis [59], and Hashimoto thyroiditis [60], CTLs can promote tissue destruction. Upon the first encounter with their cognate antigen in secondary lymphoid organs, naive CTLs expand and form different subsets of effector T cells with distinct properties (Figure 1.). This differentiation is guided by extrinsic signals from the tissue microenvironment and mediated by T cell-intrinsic transcription factors associated with chromatin remodeling events [61–63]. Following T cell priming, so-called memory progenitor effector cells (MPECs) are formed on the one hand, which show a low expression of cytotoxic proteins but a high potential to generate long-lived memory T cells with self-renewal capacity [64]. On the other hand, so-called short-lived effector T cells (SLECs) are terminally differentiated and express large amounts of cytotoxic effector molecules such as perforin and granzyme B but have little capacity for memory formation [62]. Phenotypically, SLECs and MPECs can be distinguished based on prototypic surface markers: SLECs express the killer cell lectin-like receptor KLRG1 [65], and MPECs express IL-7 receptor α chain CD127 [66]. Transcription factors involved in CTL differentiation into SLECs include B lymphocyte-induced maturation protein 1 (Blimp-1), T-box transcription factor 21 (T-bet), and inhibitor of DNA binding 2 (Id2) [67–69]. Transcription factors such as Eomesodermin (Eomes) and the high mobility group (HMG) transcription factor T Cell Factor-1 (TCF-1) promote the formation of functional memory CTLs [70,71]. In particular, several studies have provided evidence that TCF-1 is crucial for the proliferative burst upon anti-PD1 therapy and is critical for viral control [72–74].

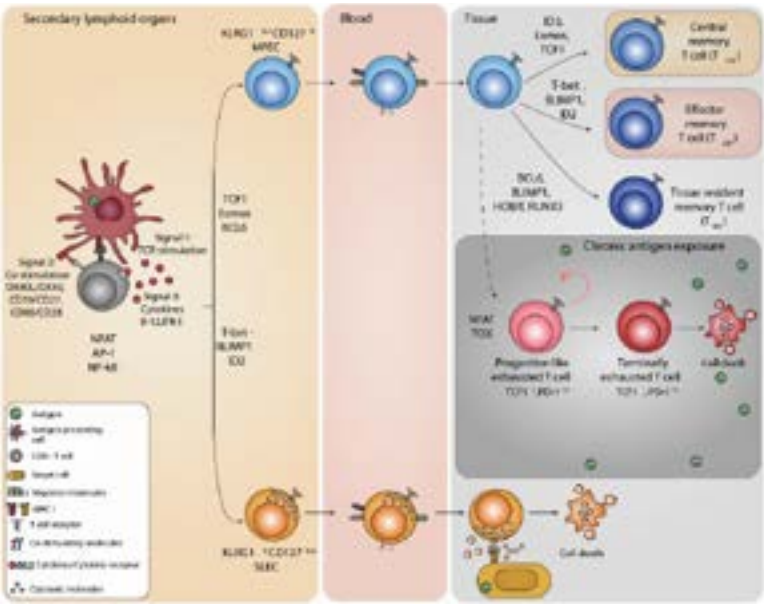

Figure 1 CD8+ T cell response to acute infection. *CD8+ T cells get primed in the secondary lymphoid organs (SLOs) by professional antigen-presenting cells (APCs) capturing the antigen. Three signals during priming are essential for an efficient T cell priming and subsequent differentiation: Signal 1 consists of the TCR stimulation by the MHC class I-peptide complex. Signal 2 is provided by co-stimulatory molecules expressed on the APCs that bind to their receptors expressed on the T cell. Signal 3 are cytokines that the APCs release. Upon priming, CD8+ T cells expand and differentiate into KLRG1^{hi}CD127^{low} short-lived effector cells (SLECs) and KLRG1^{low}CD127^{hi} memory precursor effector cells (MPECs). This differentiation is controlled by distinct transcription factors determining the cells' fate. CD8+ T cells egress from SLOs and migrate via the blood to the site of infection and other tissues for patrolling. The T cells start expressing distinct adhesion molecules and chemokine receptors to egress from the blood into the target tissue. In the inflamed organ, SLECs recognize the antigen presented on infected cells and kill them by different mechanisms. When the pathogen has been eliminated and the antigen cleared, SLECs rapidly die, while MPECs differentiate into different types of memory CD8+ T cells with distinct phenotypic and migratory properties. We can distinguish between central memory T cells (T_{CM}), which reside in the SLOs, effector memory T cells (T_{EM}) which recirculate between blood, the lymph, and non-lymphoid tissues, and tissue-resident memory T cells (T_{RM}), which reside in the previously infected tissue. When the antigen persists, such as during autoimmunity, cancer, or chronic viral infection, CD8+ T cells remain chronically exposed and become exhausted. Exhausted CD8+ T cells upregulate co-inhibitory receptors such as PD-1 and*

become less responsive to the antigen stimulus. We can differentiate between early exhausted and terminally exhausted CD8+ T cells. Early exhausted TCF-1+PD-1int cells are progenitor-like with stemness properties, which give rise to the terminally exhausted TCF-1-cells with low proliferative capacities and higher levels of co-inhibitory receptors. Adapted from B. Klimek's doctoral thesis, DOI: 10.13097/archive-ouverte/unige: 131624

While the functioning and regulation of these transcription factors were mainly studied in the context of acute and chronic viral infection, little was known about transcriptional programs that govern the tissue-destructive capacity of self-reactive CTLs in autoimmune disease conditions, which was the focus of our laboratory.

Following an accomplished microbe elimination, the adaptive immune system remembers to be better prepared against future infection with the same or structurally related pathogen, which is referred to as "immunological memory." Immunological memory is characterized by a faster and more efficient response to already known pathogens. This way, memory responses protect against infections that can otherwise lead to disease or even death in immunologically naive hosts. In this regard, memory CD8+ T cells play a critical role in rapidly recognizing and eradicating intracellular pathogens, such as viruses. Based on the migration pattern, anatomical location, and functional specialization, distinct subsets of memory T cells (TM) have been described [75] (see Figure 1.). Initially, TMs were divided into central memory T cells and effector memory T cells [76]. Central memory T cells (T_{CM}) reside mainly in secondary lymphoid organs, show a high proliferative capability following re-encounter of a cognate antigen, and serve as a self-replicating pool from which other memory T cell subsets emerge [77]. Conversely, effector memory T cells (T_{EM}) recirculate through the body and can provide immediate effector function [76]. In the last decade, an additional subset of memory T cells referred to as tissue-resident memory T cells (T_{RM}) have been identified in rodents and humans [78–82]. T_{RM} do not recirculate but mainly persist at sites of the previous infection in non-lymphoid border organs such as skin and mucosal tissues [83,84]. T_{RM} from different organs, including the brain, show overlapping transcriptional profiles characterized by a common transcriptional signature [85]. This signature is distinct from the circulating memory T cell counterpart [86,87]. Specific adhesion mole-

cules mediate their persistence in organs, such as CD103 (Integrin αE; [78,86,88] and loss of tissue egress receptors from the cell surface [89,90]. Bona fide T_{RM} have been described to express CD69, which antagonizes the tissue egress receptor sphingosine 1-phosphate receptor 1 (S1P1; [89]). The surface expression of CD103 seems specific for T_{RM}, but not all T_{RM} express the molecule. Long-lived CD103− T_{RM} have been described in secondary lymphoid organs [91], gut [92], and the female reproductive tract [93]. CD103 expression has been associated with tissue retention [81,86,88], epithelial localization [78,94], and function [81,92,95]. In various research projects in my laboratory, we demonstrated the role of T_{RM} in the CNS during viral infection and autoimmune conditions, as reviewed in more detail in the following chapters.

Mechanisms of viral clearance and tissue-resident memory T cells' role in protective immunity in the CNS.

How the immune system can cope efficiently with viral infectious threats of the CNS has been the focus of various studies in my laboratory. In the context of CNS viral infection, I investigated how the immune system, particularly CTLs, can efficiently eliminate viruses from the CNS and how consequently, immunological memory is established in this organ.

As mentioned above, the mouse wild-type LCMV infection model is a highly versatile and valuable tool for studying virus-host balance in mice. However, its use to study CNS virus clearance is hampered due to invariable fatal outcomes following intracranial infection in immunocompetent adult mice [96]. Thus, in our efforts to uncover the immune-mediated mechanism involved in CNS viral clearance, we primarily relied on intracranial infection with reverse genetically engineered, attenuated LCMV variants (rLCMV) that share many immunological properties with the wild-type LCMV but due to its attenuated spread allowed us to investigate the mechanism of successful CNS viral clearance [97]. In many studies, we harnessed the recombinant LCMV variant expressing the surface glycoprotein of vesicular stomatitis virus (rLCMV/INDG) instead of its own glycoprotein. Unlike wild-type LCMV, such as the Armstrong strain, rLCMV/INDG does not cause overt disease following intracerebral infection in adult immunocompetent mice [98–100]. Another impor-

tant difference compared to wild-type LCMV is that rLCMV/INDG replicates only in the CNS. Even in mice deficient for the recombination activation gene (RAG), which lack T and B cells, the interferon type I response prevents virus replication in other tissues [99]. In this initial study, we noted that distinct effector mechanisms and cells become crucial for virus clearance depending on the infected cell type in the CNS [97]. In particular, we noted that viral elimination from ependymal cells (the cells layering the ventricles) was achieved in a T cell-dependent manner but independently of major histocompatibility complex (MHC) class I and perforin. In contrast, the cytolytic mechanisms of CTLs became essential once the virus gained access to the CNS parenchyma, notably to astroglia [97]. In this regard, it is worth mentioning that immunocompetent individuals mostly eliminate viral infections from CNS coverings before substantial parenchyma infection is established and thus before the risk of significant tissue damage becomes imminent [101]. This initial work about differential MHCI and perforin dependence provided thus an explanation of how a self-limiting course of viral infection can occur in case the virus remains restricted to the coverings of the CNS.

Nevertheless, what happens once the virus is successfully eliminated from the CNS? My laboratory has strived to elucidate in subsequent studies how a transient viral infection can shape the immune system and the tissue microenvironment with regard to future CNS infections. In particular, we focused on studying the role of T_{RM} for protective immunity in the CNS. As indicated above, T_{RM} had been found in humans and mice at various border organs [78,86,102,103]. However, at the time we initiated our study, it remained incompletely understood if and how T_{RM} are implicated in protective responses against future infections of the CNS.

In our study using the rLCMV/INDG infection model of adult mice, T_{RM} were shown to persist for at least several months in the CNS after viral infection in anatomical niches behind the blood-brain barrier (BBB) and resisted antibody-mediated depletion by intravascular administration of anti-CD8 T cell antibodies [104] (Figure 2). Thus, following the administration of depleting antibodies for the circulating CD8 T cell pool, we could specifically investigate the role of T_{RM} in combating virus spread in the CNS. These investigations revealed that resting T_{RM} are maintained by homeostatic proliferation and can rapidly expand within a few days

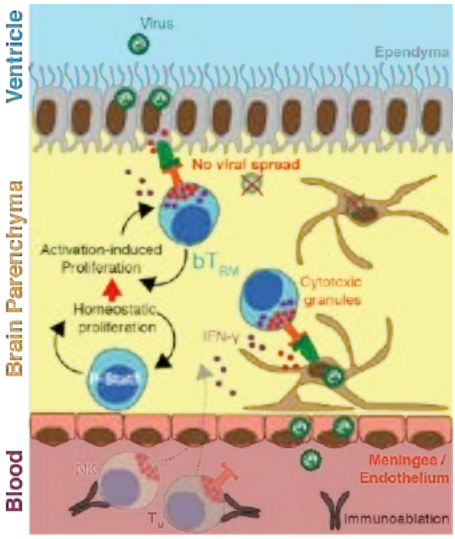

Figure 2 Schematic summary of T_{RM} functioning during CNS viral reinfection. *T_{RM} are maintained in the CNS by homeostatic proliferation. After local viral reinfection, T_{RM} rapidly acquire cytotoxic effector function and prevent viral spread from the CNS covering into the parenchyma, thus protecting the host from a fatal disease. Presentation of cognate antigen on MHC-I is essential for T_{RM}-mediated protective immunity, which involves both perforin- and IFN-γ–dependent effector mechanisms.*

following CNS infection with wild-type LCMV (Figure 2). Thereby, they differentiate into effector CTLs preventing the viral spread into the adjacent CNS parenchyma and protecting the host from developing lethal choriomeningitis (Figure 2). In line with our previous study [97], T_{RM}-mediated virus clearance relied on both IFN-γ expression and perforin-mediated cytotoxicity. Altogether this work provided evidence that T_{RM} form an autonomous antigen-dependent immunological barrier against viral reinfection in the adult CNS (Figure 2).

Tissue-resident memory T cells and autoimmune disease

Originally, chronic autoimmune diseases were thought to require continuous recruitment of effector or effector memory T cells from the circulation into the affected organ. However, with the discovery of T_{RM} in inflammatory lesions, this concept was revised [83,105–109]. Accordingly, the involvement of T_{RM} in chronic inflammatory diseases has been proposed for barrier tissues such as psoriasis [102], asthma [110], or fixed drug eruption [111] and in non-barrier tissues such as type 1 diabetes [112,113], lupus nephritis [114], and multiple sclerosis [115].

But how is the generation of T_{RM} following virus infection related to CNS autoimmunity? My research group has addressed this fundamental question in various studies over the last few years. The interest in creating knowledge here was motivated by the range of evidence speaking for viral infections that are associated with autoimmune disease precipitation or exacerbation [49,116–118]. At the same time, the causal mechanistic link between infection and autoimmunity remained obscured [119]. In this regard, it has been postulated that pathogen-induced inflammatory changes in the tissue microenvironment may create a long-lived "fertile field" that favors future autoimmune attacks [120]. However, it was not yet known whether and how a transient viral infection of the brain might permanently alter its microenvironment and thereby predispose the organ to develop autoimmune lesions.

We addressed this question in an experimental model in which we sequentially exposed mice to a transient viral infection before the transfer of autoreactive T cells (2D2) used to precipitate experimental autoimmune encephalomyelitis (EAE), which is a model of MS. In the first experiments, we noted that the age of an initial transient intracranial infection with rLCMV/INDG strongly impacted the susceptibility of mice to develop autoimmune lesions in the brain later in the life of these virus-experienced mice (Figure 3a). Indeed, mice transiently exposed to viral infection before weaning (but not later in life) tended to develop autoimmune lesions in the brain (Figure 3b–c) and associated clinical symptoms in the EAE model long after viral clearance (Figure 3d–e). We also found that the tissue micromilieu in which viral infection occurred during early life maintained a long-lasting pro-inflammatory signature which was not the case in those mice infected in adulthood (Figure 3f). This pro-inflammatory signature was characterized by a persistent expression of the chemokine CCL5 (Figure 3f), the primary cellular source of which we could trace back to T_{RM} in the brain of these mice (Figure 3g). Accordingly, pharmacological blockade of the corresponding receptor CCR5 by treatment of mice with the CCR5 antagonist 5P12-RANTES prevented autoreactive myelin-specific 2D2 cells from inducing CNS inflammation and disease in areas where T_{RM} persisted (Figure 3h).

Analogously to mice, we furthermore observed that CCL5-expressing T_{RM} accumulated in the non-demyelinated, normal-appearing white mat-

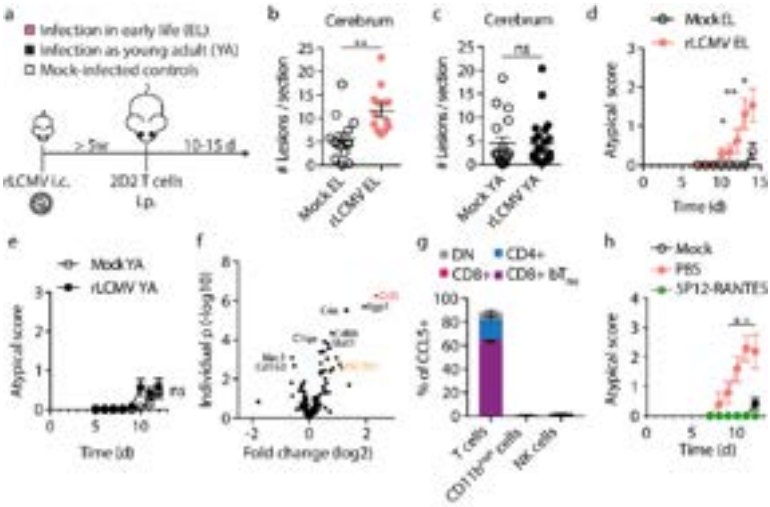

Figure 3. T_{RM} generated early in life predispose to developing autoimmune lesions in the brain. *(**a**) At 1w (early life, EL) or 3–4w of age (young adults, YA), WT mice were injected intra-cerebrally (i. c.) with rLCMV or vehicle, respectively. At least five weeks later, autoreactive 2D2 T cells were transferred into mice to induce EAE. Quantifying EAE lesions in (**b**) EL or (**c**) YA compared to age-matched mock-infected controls. Scores of brain-related symptoms for mice infected in (**d**) EL or (**e**) YA in comparison to age-matched mock-infected controls. (**f**) Volcano plot from transcriptome analysis of 256 inflammatory genes in EL compared to the pool of both mock-infected and YA groups. (**g**) Percentage of indicated leukocyte subsets among Ccl5+ cells in brains of EL mice >5 weeks after infection. (**h**) Scores of atypical EAE in 5P12-RANTES–treated YA mice compared to PBS-treated littermates or mock-infected controls. Symbols represent individual mice, except for (d), (e), and (h), where data represent means ± SEM. b–e, h: n=12–23; f: n=3–5; g: n=6. *P < 0.05, **P < 0.01, ns: not significant. Adapted from Steinbach et al., Sc. Trans. Med., 2019.*

ter (NAWM) of MS brain samples (Figure **4**). However, these cells were not randomly distributed in NAWM but were preferentially found in areas characterized by microglial activation, which previous studies have termed preactive MS lesions [121] thought to represent lesion-prone areas in the CNS of MS patients.

Overall, our study provides an explanatory approach for a possible link between preceding infections and the precipitation of autoimmune disease with some implications: First, it may explain how a potentially

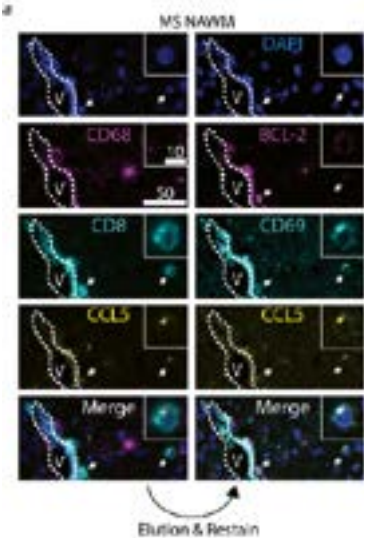

Figure 4. T_{RM} express CCL5 in preactive MS lesions. *(**a**) Representative multiplex immunofluorescence images illustrating the identification of CCL5+ T_{RM} (defined as CD8+CD69+ BCL-2+) in pre-active NAWM areas using an elution and restain approach. V= ventricle. Scale bars: 50 μm, inset: 10μm. Adapted from Steinbach et al., Sc. Trans. Med., 2019.*

preceding viruses infection in a particularly critical time window of life, even with structurally unrelated viruses can be associated with increased disease risk [51], and second how predisposing viral infections can evade detection as causative agents of autoimmune disease despite several lines of indirect evidence for such an association. Thus, demonstrating how prior viral infection can lead to a persistent inflammatory signature mediated by T_{RM} in the CNS represent a step toward understanding the predisposing role of infections in MS and potentially other autoimmune diseases.

Our findings furthermore suggested that T_{RM} with antiviral specificity facilitated the recruitment of circulating autoreactive T cells to the CNS by acting as bystanders. *However, what about T_{RM}'s role in driving an immune-compartmentalized inflammatory response in the CNS?* In MS and other chronic human neuroinflammatory conditions [105], T_{RM}-like cells have been observed within lesions [115,122]. Furthermore, clonally expanded and activated CD8+ T cells with phenotypic similarities to T_{RM} were found in the cerebrospinal fluid of MS patients early after disease onset [123]. This indicated that T_{RM} may also target a cognate self-antigen in the CNS and actively participate in tissue destruction. This would

also provide a plausible explanation for why therapeutic approaches aimed at preventing the recruitment of T cells from circulation to the CNS fail to halt disease progression in some instances.

To gain insight into the ability of T_{RM} to trigger compartmentalized inflammation in the CNS, we developed a preclinical model in which resting T_{RM} in the CNS can be re-exposed to their cognate antigen in a time- and cell-specific manner [124]. For this purpose, we crossed glial fibrillary acidic protein (GFAP)–Cre^{ERT2} mice [125] expressing a tamoxifen-inducible Cre-recombinase under the GFAP promotor with Stop-GP^{flox} mice [126] (for details about the construct, see Figure 5a). Using this mouse line, we examined how resting CD8+ T_{RM} that had colonized the CNS after transient infection with rLCMV responded to re-exposure to the LCMV glycoprotein expressed as cognate neo-self antigen in glial cells (Figure 5b). Upon exposure to the neo-self antigen, T_{RM} rapidly re-expanded without additional inflammatory stimuli (Figure 5c). Moreover, the resulting tissue damage and disease could be induced by T_{RM} without circulating CD8+ T cells (Figure 5d). However, while CD8 T_{RM} initiated CNS inflammation, the differentiation of CD8+ T_{RM} into disease-driving effector cells required the help of CD4+ T cells (Figure 5e–g), suggesting cooperative activity between these T cells subsets.

Assuming that compartmentalized inflammation is gaining further importance in patients with advanced CNS autoimmune diseases [127], we examined how tissue-resident phenotypes are related to the lesion stage of MS patients. We performed multiplexed immunofluorescence stainings on histological sections of acute active and chronic active MS lesions with prototypic T_{RM} markers, including CD8, CD69, BCL2, CD103, and GZM-B (Figure 6a). This analysis revealed that among the different memory T cell subsets, T_{RM} are the predominant CD8 phenotype found in more advanced lesion stages (Figure 6b). To visualize the source of T cells with stem cell-like properties in the spatial context, we performed co-staining with TCF-1. Similar to our finding in the animal model, we found TCF-1+ CD8+ T cells preferentially situated around blood vessels and often in close vicinity to CD4+ T cells (Figure 6c) in MS lesions. This suggests that CD8 T cells with renewable properties are located in perivascular niches and may be able to perpetuate the lesion without the need to recruit circulating cells.

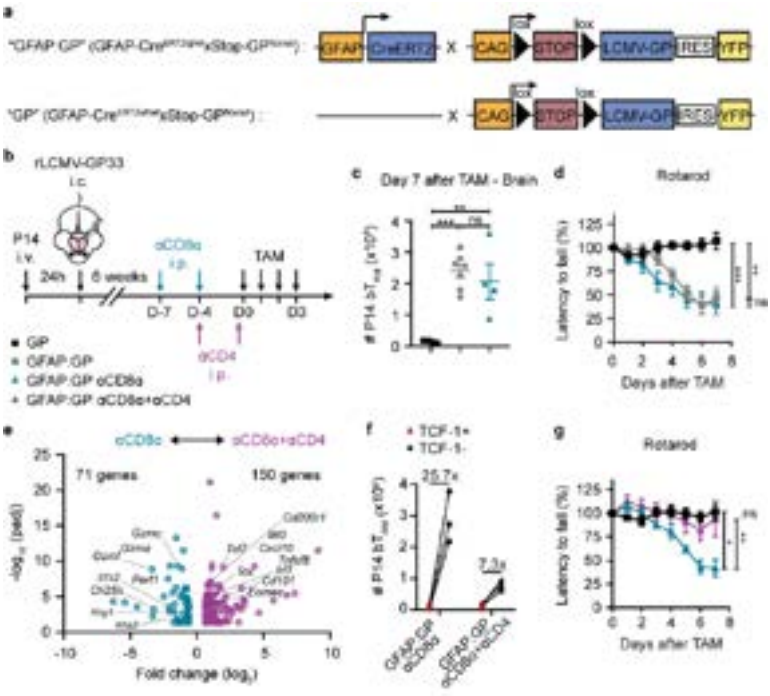

Figure 5. T_{RM} cooperate with CD4+ T cells to drive compartmentalized immunopathology in the CNS. *(**a**) GFAP:GP (GFAP-CreERT2 tg/wt:Stop-GPflox/wt) mouse line expresses tamoxifen-inducible Cre-recombinase (CreERT2) under the control of the astrocyte-specific promotor (GFAP). Binding of tamoxifen (TAM)-metabolites to ERT2 mediates the translocation of the Cre recombinase to the nucleus and thus the conditional expression of LCMV glycoprotein (GP) and reporter gene YFP in astrocytes. (**b**) T cell receptor transgenic (TCR) P14 cells (recognizing the viral H2-Db-restricted GP33 epitope of LCMV) were adoptively transferred into adult GFAP:GP mice and mice were subsequently intracranially infected with rLCMV-GP33 to generate P14 T_{RM} in the CNS. At least 6 weeks later, circulating T cells were depleted by administration of αCD8α–depleting antibody (cyan arrows), or αCD8α + αCD4–depleting antibody (purple arrows) or isotype control. One week after depletion (D0), mice were treated with TAM i.p. (black arrows) to induce expression of the cognate P14 epitope as a neo-self antigen in glia cells. (**c**) Quantification of P14 cell numbers in the brain. (**d**) Rotarod performance of indicated groups after TAM administration. (**e**) Volcano plot illustrates differential expression of transcripts in P14 cells in αCD8α-treated versus αCD8α + αCD4–treated GFAP:GP mice. (**f**) Numbers of P14 cell stratified according TCF-1+ (red circle) or TCF-1− (black triangle) expression in*

*αCD8α-treated versus αCD8α + αCD4–treated GFAP:GP mice. Fold increase is indicated. (**g**) Locomotor performance (as measured by Rotarod test) of indicated groups after TAM administration. Symbols represent individual mice, and bars represent means ± SEM, except for (e), where data represent individual transcripts, and for (d) and (g), where data represent means ± SEM. c: n=4–7; d: n=6–7; e: n=3; f: n=3–4; g: n=3–5. *P < 0.05, **P < 0.01, ***P < 0.001, ns: not significant. Adapted from Vincenti et al., Sc. Trans. Med., 2022.*

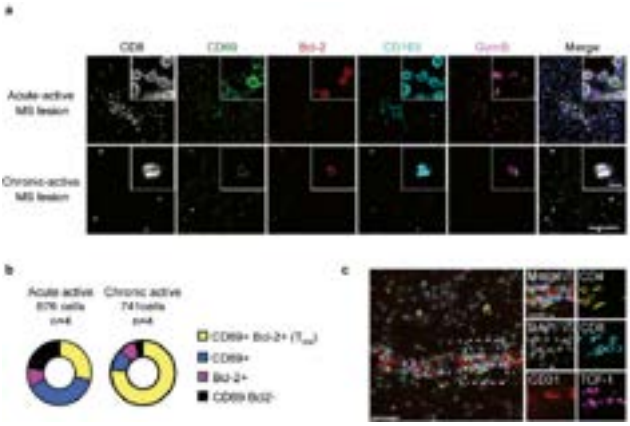

Figure 6. TRM accumulate in chronic active MS lesions. *(**a**) Representative immunostainings illustrate TRM (CD8+ CD69+ BCL2+) co-expressing CD103 and/or GZM-B with DAPI nuclear counterstaining (blue). Top: Acute active MS lesion. Bottom: Chronic active MS lesion. Scale bars:100 μm and (inset) 10 μm. (**b**) Quantification of CD8+ cells (TRM: CD69+ BCL2+; non-TRM: CD69+, BCL2+, and CD69-BCL2−) from acute active and chronic active MS lesions. (**c**) Representative image of multiplex immunofluorescence staining showing CD8+ T cells, TCF-1, CD4+ T cells, CD31+ vessels, and DAPI nuclear counterstaining of acute demyelinating lesion. Scale bars: 50 μm and (inset) 20 μm. Adapted from Vincenti et al., Sc. Trans. Med., 2022.*

Overall, our studies contributed to understanding how virus-generated TRM may be involved in the pathogenesis of autoimmune diseases of the CNS. On the one hand, we found evidence that TRM predispose the tissue microenvironment to autoimmune lesions through persistent chemokine expression and thus can act as a facilitator for immune cell recruitment from the circulation. On the other hand, we could show that TRM also directly contribute to the development and perpetuation of inflammatory

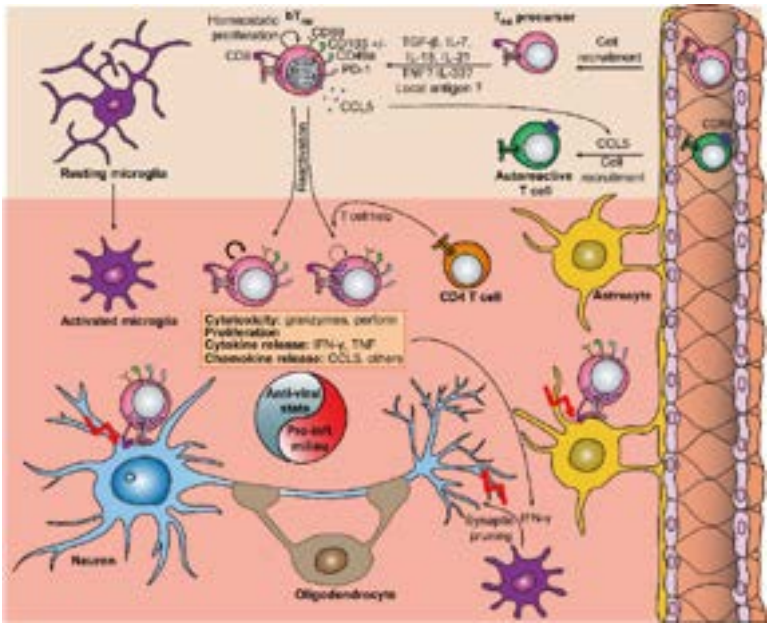

Figure 7 Schematic outline summarizing CD8 T_{RM} in CNS inflammation. *T_{RM} precursor cells recruited within the CNS differentiate into brain T_{RM} (bT_{RM}) upon sensing microenvironmental cues. The core signature of bT_{RM} includes the expression of CD69, CD49a, PD-1, and transcription factors Hobit, Blimp-1, Bhlhe40, and Runx3. In addition, both CD103+ and CD103–bT_{RM} subsets exist. Resting bT_{RM} undergo homeostatic proliferation and constitutively produce the chemokine CCL5, attracting CCR5+ autoreactive T cells within the CNS. Upon bT_{RM} reactivation, the heterogeneous pool of bT_{RM} progeny consists of cells with a high proliferative capacity and cells with a high cytotoxic capacity. CD4 T-cell help is necessary for the acquisition of the highly cytotoxic program. Activated bT_{RM} release pro-inflammatory mediators such as IFN-γ and TNF and chemokines such as CCL5. Altogether, bT_{RM} induce a damaging pro-inflammatory milieu that can be protective in case of viral reinfection. bT_{RM}-derived IFN-γ induces synaptic pruning by activated microglia, leading to neuronal damage. Activated autoreactive bT_{RM} can directly attack astrocytes, neurons, and possibly other cells such as oligodendrocytes. Adapted from Merkler et al., Curr. Op. Immunol., 2022.*

processes and thus provide an explanation of how compartmentalized inflammation can be maintained in chronic CNS autoimmune disease conditions (Figure 7).

The role of CD8+ T cell differentiation and its implication for CNS autoimmune disease precipitation

CD8+ T cells undergo functional reprogramming following activation and during their further response, which is reflected in the remodeling of their chromatin landscape. *However, what regulates the functional adaptation program of autoreactive CD8+ T cells during T cell priming in secondary lymphoid organs and during the effector phase in the inflamed organ?* A previous study found evidence that the microbial inflammatory context influences CTL differentiation after its activation [128]. For example, during CTL priming, the cytokine microenvironment modulates the transcriptional landscape of CTLs, leading to alternative fates of CTLs [129]. We thus hypothesized that the inflammatory microenvironment during T cell priming could alter the transcriptional network and chromatin landscape of T cells, impacting the destructive tissue potential of autoreactive CTLs in CNS autoimmune disease condition. In doing so, we compared how distinct pathogens and the associated inflammatory milieu impact autoreactive T cell transcriptome. Using this approach, we identified the transcription factor TOX as a crucial regulator and its role in the epigenetic remodeling of autoreactive T cells.

To address the above hypothesis, we made use of mouse models referred to as ODC-OVA mice [130] or MOG-GP mice [126], respectively, in which defined CD8 T cell epitopes are expressed as neo-self-antigens in myelin-forming oligodendrocytes. For both models, well-defined T cell receptor transgenic CD8+ T cells are available, referred to as OT-1 (specific for ovalbumin antigen in the context of H2Kb) or P14 cells (specific for LCMV glycoprotein antigen in the context of H2Kb). In the ODC-OVA model (expressing ovalbumin as neo-self-antigen in oligodendrocytes), we mainly compared the properties of two different pathogens that express full-length OVA: lymphocytic choriomeningitis virus (LCMV-OVA) and Listeria monocytogenes (Lm-OVA). We noted that the two microbes were similarly able to induce expansion of OT-1 cells despite inducing distinct inflammatory signatures in lymphoid organs [131,132]. While OT-1 cells similarly infiltrated the CNS following activation by either pathogen (Figure 8a), only ODC-OVA mice primed with LCMV-OVA but not LM-OVA developed CNS autoimmune disease (Figure 8b). On further transcriptome analysis of OT-1 cells sorted from

inflamed CNS, we found that DNA-binding factor Tox was strongly induced in CTLs from LCMV-OVA-primed and diseased animals, in contrast to OT-1 cells after priming with LM-OVA. Of note, TOX was initially identified as a DNA-binding factor required for the development of CD4+ T cells in the thymus [133] and for the development of innate lymphoid cells in the bone marrow [134], which includes NK cells [135] but was found dispensable for thymic CD8 T cell development. However, *Tox* was an essential hub gene in differential network analyses comparing memory CTLs from acute and chronic infections [136].

We identified that the cytokine IL-12 (which is induced after LM-OVA infection) represses TOX by regulating T cell-intrinsic transcription factors, including T-bet and Eomes. T-bet acts as a repressor of *Tox* by directly binding to its promoter (data not shown). In a functional assay, we showed that TOX-competent CTLs formed more stable immunological synapses with antigen-expressing oligodendrocytes in the CNS (Figure 8c), providing an explanation for why TOX was necessary for the tissue destructive and encephalitogenic properties of autoreactive CD8+ T cells. Furthermore, we investigated how TOX is implicated in the differentiation of autoreactive T cells: Gene expression profiling of CNS-infiltrating TOX-competent and -deficient CTLs revealed that TOX repressed genes such as *Klrg1, Gzma*, and *Klra5*, which are known to be associated with terminal effector differentiation of CTLs. In contrast, genes related to stemness function, like *Tcf7* (encoding TCF-1), were induced in TOX-competent CTLs. Furthermore, we noted TOX-dependent differential expression of the checkpoint receptor 2B4 (CD244, [126]). We furthermore investigated the relation between DNA binding of TOX and gene expression by performing chromatin immunoprecipitation followed by DNA sequencing (ChIP-seq) and transcriptomic cross-referencing with the transcriptome. This approach identified TOX binding sites of the gene *Id2*, which is essential for terminal-effector differentiation [137]. Together with further phenotypic analyses, we have described that DNA-binding factor TOX functions as a transcriptional regulator of T-cell differentiation, influencing the susceptibility of autoreactive T cells to checkpoint signaling and, ultimately, the encephalitogenic properties in CNS autoimmunity.

However, what determines the functional adaptation of autoreactive CD8+ T cells in the inflamed organ needed further investigation. In subsequent studies published by three independent research groups and in which our laboratory was involved, TOX was identified as a critical regulator of T-cell exhaustion during viral infections and of the antitumor immune response [138–140]. It became apparent that the adaptive program relies on chronic stimulation of the T cell receptor (TCR), which is associated with changes in the epigenetic and transcriptional landscape [141]. T cells with an exhausted phenotype showed increased expression of multiple inhibitory receptors such as PD-1 and exhibited a progressive loss of effector functions (e. g., the ability to produce various cytokines) in chronic viral infections and cancer. As a result, T cells appeared impaired in their ability to defend against persistent viruses or tumors. Nevertheless, such cells retain residual effector functions [142–144], which could be further reinvigorated by immune checkpoint inhibitors [145] and which may resemble the situation in chronic autoimmune diseases. In a follow-up study, we thus investigated the epigenetic and transcriptional landscape of autoreactive T cells in CNS inflammation [146].

To this end, we examined chromatin remodeling events in CD8+ T cells infiltrating the brain under autoimmune conditions in the MOG-GP mice

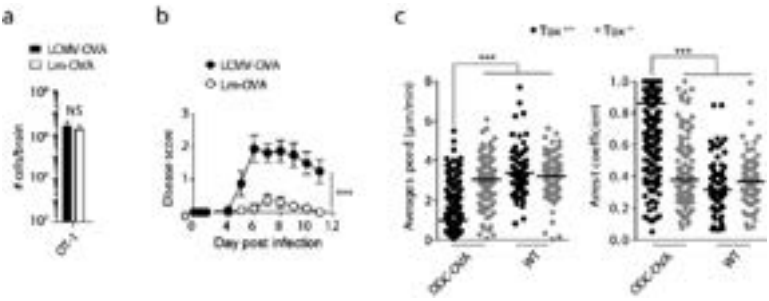

Figure 8. LCMV-OVA-primed OT-1 cells acquire Tox-dependent encephalitogenic capacity in ODC-OVA mice. (***a***) *Flow-cytometric enumeration of CNS-infiltrating OT-1 cells 7dpi in indicated groups. (n = 6 mice).* (***b***) *EAE disease course (n = 8 mice per group).* (***c***) *Average speed (μm/min) and arrest coefficient of Tox+/+ and Tox-/-OT-1 individual cells incubated onto WT or ODC-OVA slices. Horizontal lines indicate the median. a: n=6; b: n=8; c: n=80. ***P < 0.001, NS: not significant. Adapted from Page et al., Immunity, 2018.*

(as introduced in the previous section), in which the glycoprotein of LCMV is expressed as a cognate CD8 T cell neo-self-epitope in oligodendrocytes mice [126,146]. We compared the remodeling events with those following acute CNS infection (Figure 9a).

To assess the differences in genome-wide chromatin accessibility in autoimmune versus infection conditions, we performed an assay for transposase-accessible chromatin using sequencing (ATAC-seq) of T cells (Figure 9b). While virus-derived and autoreactive T cells exhibited significant differences in the landscape of chromatin accessibility, most chromatin remodeling occurred in CD8+ T cells that differentiated over time in the inflamed CNS (Figure 9c).

To partition the regions whose accessibility changed over time during CD8+ T cell differentiation, we performed unsupervised clustering at different time points after the onset of CNS disease (Figure 9d). Among the different modules identified, we could corroborate that increased accessibility occurred at the late time points in the locus encoding for TOX (Figure 9d–e). We further found that most of the chromatin accessible regions (ChARs) showed increased chromatin accessibility in autoimmune condition at later timepoints (Figure 9f). To determine which transcription factor networks account for the specific differentiation states of CD8+ T cells during CNS autoimmunity, we tested the presence of transcription factor binding motifs (Figure 9g). Consistent with the described function of nuclear factor of activated T-cells (NFAT) in driving T-cell exhaustion [147], we observed a strong enrichment for NFAT binding motifs in the ChARs that gained accessibility at a late time point in autoimmune condition compared to transient viral infection. This indicated that autoimmune CD8+ T cells acquire an epigenetic landscape reminiscent of exhaustion, which was further corroborated in the transcriptome of these cells (data not shown). In line with these observations, autoreactive CD8+ T cells displayed a reduced ability to degranulate and co-produce IFN-γ and TNF, which was paralleled by an increased expression of multiple inhibitory receptors such as PD-1, TIM-3, CD244, LAG-3, and TIGIT (Figure 9h). Collectively, this suggests that autoimmune CD8+ T cells induce TOX in the CNS and acquire a gene program that distinguishes them from memory T cells after transient viral infection.

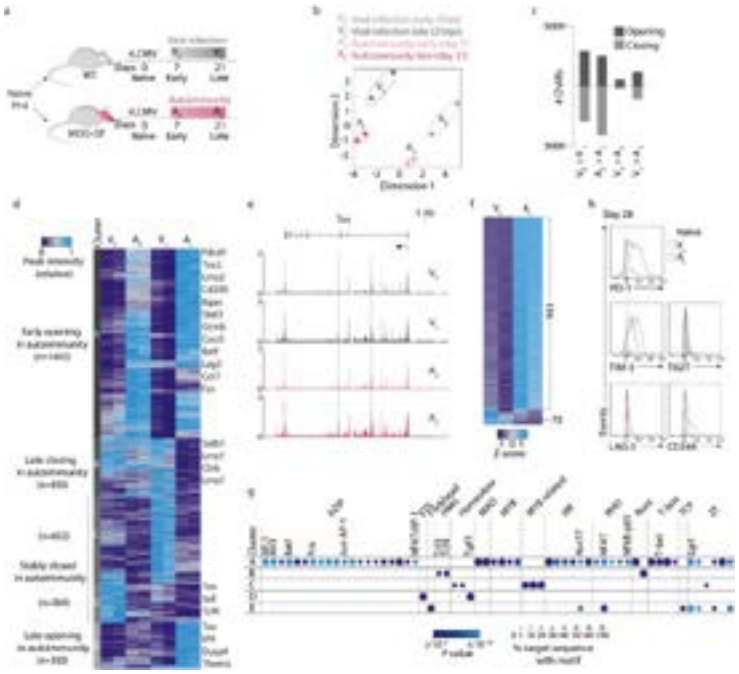

Figure 9. Chromatin accessibility changes in self-reactive CD8+ T cells. *(**a**) P14 cells were adoptively transferred into WT and MOG-GP mice. One day later (day 0), mice were challenged i. c. with rLCMV. Brain infiltrating P14 cells were submitted to ATAC-seq 7 and 21 days after i. c. infection. (**b**) Multidimensional scaling (MDS) plot of chromatin accessibility from A_E, V_E, A_L, and V_L P14 cells. The similarity of chromatin accessibility is proportional to the distance between samples. (**c**) Number of differentially accessible ChARs in each different comparison (Log_2 FC ≥1; FDR < 0.05). (**d**) Heatmap of the normalized peak intensity for ChARs displaying differential accessibility in at least one of the comparisons (V_E vs. A_E) or (V_L vs. A_L) (Log_2 FC ≥1; FDR < 0.05). Hierarchical clustering indicates grouping of samples by ChARs behavior during CNS autoimmunity. Key genes proximal to loci with differential accessibility are indicated for each cluster. Each column represents a biological replicate. (**e**) ATAC-seq track of Tox locus for V_E, V_L, A_E, and A_L. Differentially accessible ChARs (FDR < 0.05) are highlighted in gray. (**f**) ATAC-seq Z-score of significantly differentially accessible ChARs (FDR < 0.05) at exhaustion-associated regions. (**g**) Enrichment of all known transcription factor (TF) motifs within each cluster of differentially accessible ChARs as defined in (d). (**h**) Representative flow cytometry histograms of inhibitory receptor expression in A_L and V_L P14 cells 28 days post i. c. rLCMV infection. Adapted from Page et al., Nat. Comm., 2021.*

However, why is TOX in T cells essential for CNS autoimmune disease precipitation while restraining effector T cell differentiation into short-lived effector T cells?

TOX is dispensable for T cell expansion and contraction following transient viral infection [146]. Similarly, the initial expansion was not affected in Tox-deficient autoreactive T cells in our study, but these cells underwent a more rapid culling in CNS autoimmune disease conditions (Figure 10a). These results suggest that TOX deficiency leads to an intrinsic disadvantage for the survival of autoreactive CD8+ T cells that invade the CNS during autoimmune disease.

When analyzing the transcriptome, the expression of TCF-1, which is crucial for chronically stimulated T cells with stemness-like properties T cells [148], was nearly ablated in the absence of TOX in self-reactive CD8+ T cells, a finding that could also be corroborated on the protein level (Figure 10b). Thus, this suggests that TOX is critical for maintaining TCF-1-expressing self-reactive T cells during CNS autoimmunity. To get insights into the molecular mechanism by which TOX controls the presence of TCF-1-expressing cells during CNS autoimmunity, we interrogated our ATAC-seq dataset by evaluating the changes in expression of genes that became more or less accessible in the absence of TOX (Figure 10c). This analysis revealed that most TOX-dependent epigenetic changes were functionally relevant since the chromatin openness correlated with the gene expression level (data not shown). Given the role of TCF-1 in coordinating chromatin accessibility changes upon binding [149], we reasoned that TOX-dependent control of gene expression through chromatin remodeling would mainly affect TCF-1 bound genes. When we examined whether these changes in expression affected genes that had TCF-1 binding events from a previously published TCF-1 ChiP-seq dataset, we found that approximately 50% of the identified genes associated with differentially accessible ChARs were TCF-1-bound genes (Figure 10c). This indicated that TOX expression is required to recapitulate partly the epigenetic and transcriptional programs invoked by TCF-1 and thus favors the maintenance of the reservoir of autoimmune TCF-1-expressing cells.

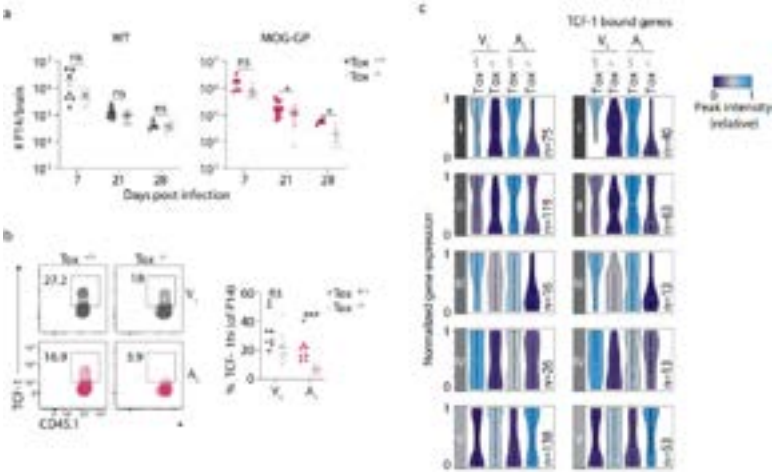

Figure 10 TOX predominantly preserves the pool of self-reactive TCF-1^{hi} CD8+ T cells. *(**a**) Flow cytometric enumeration of CNS infiltrating P14 cells at days 7 post-infection. (n = 4–14). (**b**) Frequency of TCF-1-expressing cells in V_L and A_L cells 21 days after infection (n = 8–9). Representative flow cytometry plot (left) and summary data (right). Horizontal lines represent the mean. (**c**) Violin plots illustrating normalized expression of genes found proximal to differentially accessible ChARs (maximum distance to gene = 100 kb) for each grouping of samples by TOX-dependent ChAR behavior. The bounds of the boxes indicate the 25th and 75th percentiles, the center (dot) reflects the median, the lower whisker indicates the minimum, and the upper indicates the maximum of normalized gene expression and violin colors indicate the average peak intensity of ChAR-gene pairs within each module. Genes showing at least one TCF-1 binding event are depicted within each module. Adapted from Page et al., Nat. Comm., 2021.*

Our studies shed light on how TOX-dependent fate determination favors self-reactive CD8+ T cells to promote chronic inflammation in the CNS. While TOX is associated with an exhaustion transcriptional signature, this represents an essential adaptive program for autoreactive T cells enabling them to chronically persist antigen stimulation in the CNS by reducing their differentiation into short-lived effector cells. This is notably achieved by preserving the pool of TCF-1-expressing progenitor cells in cooperation with various transcription factors. Deciphering the molec-

ular mechanisms controlling the longevity of self-reactive CD8+ T cells in chronic autoimmune diseases may have implications for future therapeutic interventions.

Neurons as an immunological target in autoimmunity

One of my long-standing interests, which has accompanied me throughout my research career, is to understand better how inflammatory processes result in neuronal damage in the CNS. To gain insight into the underlying mechanisms, I led and participated in several studies that focused on the aspect of neuronal changes mediated by immune processes in model systems and humans [150–162]. Neurons in the adult CNS are postmitotic cells, and it is therefore not surprising that the degree of neuronal damage, be it in the context of infections or autoimmune diseases, is associated with the extent of irreversible functional impairments of affected individuals. Therefore, it is evident that preventing neuronal damage in inflammatory CNS diseases is critical for any therapeutic intervention to reduce irreversible clinical decline. It should be kept in mind, however, that neurons represent highly polarized cells that transmit information to other neurons through their, partly very long, cytoplasmic projections (so-called axons) and receive signal input from other cell projections through their dendrites and corresponding synapses. Therefore, alterations in inflammation can occur along these neuronal processes and transmission sites, with far-reaching functional consequences but not necessarily equating to irreversible cell death. These changes can lead to altered synaptic inputs impacting neuronal excitability, resulting in seizures and a decline in intellectual and motor performance [163]. However, such alterations also represent a potential for reversibility and could be addressed therapeutically if the underlying molecular mechanisms were better elucidated.

Already during my early research endeavor, we established a mouse model that is particularly suited to study the interaction between CTLs and neurons of the CNS in vivo, a model that we referred to as "viral déjà vu" [99]. This model system relates to the phenomenon that immunogenic but non-cytolytic viral infections in the neonatal period are often not eliminated by the immune system and may persist lifelong in the

CNS. Mice with neonatal infection (within 24 hours after birth) with the attenuated LCMV strain (rLCMV/INDG) are healthy because the virus does not behave cytolytically but persists selectively in CNS neurons without induction of an antiviral CTL response. When these so-called "virus carrier mice" become infected later in life with wild-type LCMV genetically related to the persisting virus in the CNS, a CTL-mediated immune response is triggered against both viruses, including rLCMV/INDG persisting in neurons. The CNS disease that ensues in these carrier mice is driven by an antiviral immune response against neurons and is histopathologically dominated by CD8+ T cells (Figure 11a illustrates the experimental readout of the *viral déjà vu* model system). As a consequence of CTL neuron interaction, neurons show synaptic loss (Figure 11b), which correlates with CTL neuron contact (Figure 11c) during acute disease stages. This model recreates histopathological features

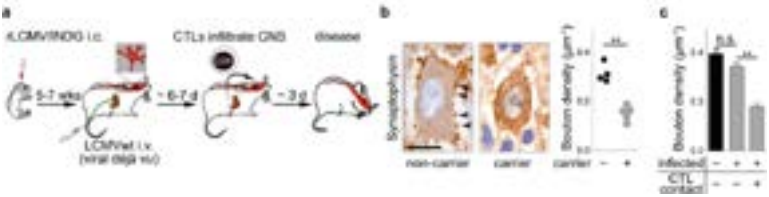

Figure 11: Schematic description of the viral déjà vu model. *(**a**) Neonatal infection with an attenuated variant of Lymphocytic chorio-meningitis virus (rLCMV/INDG) causes a persisting infection in neurons of the CNS but not in peripheral organs.*
*Note that the rLCMV/INDG is not cytolytic; therefore, the infection does not damage infected cells. Moreover, carrier mice are clinically healthy and do not show disease. However, when these carrier mice are infected in adulthood with wild-type LCMV (LCMVwt) intravenously (i.v.), they mount a vigorous CD8+ T cell response within 6–7 days which also targets persisting infected neurons in the CNS, causing disease. We refer to the phenomenon that a secondary infection triggers a response against a persisting infection in the CNS as viral déjà vu. (**b**) Left: representative histological section stained for synaptophysin+ perisomatic boutons (arrowheads) in the DCN of carrier and noncarrier mice 10 d after LCMVwt challenge. Right: quantification of perisomatic bouton density. (**c**) Perisomatic bouton density quantification in LCMV-NP+ neurons in juxtaposition to infiltrating T cells 8 d after LCMVwt challenge. Symbols represent individual animals, except in (c), where data represent mean + SEM. b: n= 4–5; c: n = 4.*
***P < 0.01, ns: not significant. Adapted from Merkler et al., J. Clin. Inv., 2006 and Kreutzfeldt et al., J. Exp. Med. 2013.*

that resemble Rasmussen's encephalitis (RE), a rare but devastating inflammatory disease of the human CNS. This disease, which typically affects children under the age of 15, is characterized by drug-resistant epilepsy and progressive neurological decline [164]. Analogous to the viral déjà vu model, in which CD8+ T cells cluster around rLCMV-infected neurons, RE lesions are dominated by infiltrating CD8+ T cells that show disease-specific clonal expansions [165] and are found close to neurons [164].

At the beginning of my endeavor in this research field, it was still unclear whether CD8+ T cells could directly interact with neurons in vivo [166]. If so, little was known about the cellular and molecular bases of how a CD8+ T cell-mediated anti-neuronal response results in the observed synaptic pathology. What was known at that time was that microglia, the brain-resident phagocytes, are essential orchestrators of synaptic refinement and maintenance [167], and in the context of CNS inflammation, microglia, and brain-infiltrating monocyte-derived macrophages, can promote pathological synaptic loss [168,169]. Activated microglia engulf synaptic terminals in CNS inflammatory conditions through an interferon-alpha-dependent mechanism [170] and complement component C3 cleavage products [171]. In the first studies, using the viral déjà vu model, we noted that CD8+ T cell-derived interferon-g (IFN-g) triggers an acute loss of axosomatic synaptic connections clinically manifested by impaired motor coordination and balance [156]. *But how was ensuing synaptic removal mechanistically linked to a targeted CD8+ T cell immune attack?*

We hypothesized that there must be a interaction between neurons under a CD8+ T cell attack with phagocytes resulting in synaptic pathology. To address this hypothesis, we tested whether the neuronal interferon gamma signaling and downstream signal transducer and activator of transcription 1 (STAT1) represent a disease-relevant pathway in neurons. For instance, we infected neonatal $Stat1^{fl/fl}$ mice with rLCMV encoding for the Cre recombinase (rLCMV-Cre). This allowed us to conditionally ablate STAT1 in persistently infected neurons in the CNS without interfering with this essential pathway in other cells (Figure 12a), including immune cells. $Stat1^{fl/fl}$ rLCMV-Cre carriers were protected from viral déjà vu disease (Figure 12b), and synaptic loss (Figure 12c), commonly seen in diseased wild-type carrier animals. The importance of this pathway also for

potential therapeutic interventions was further confirmed by pharmacological inhibition of Janus kinases (Figure 12d–e) in the viral déjà vu setting. To investigate how neuronal JAK/STAT1 signaling results in synaptic alterations, we profiled the neuronal translatome of rLCMV-cre infected neurons in the déjà vu model by exploiting "RiboTag mice" which harbor a modified allele of ribosomal protein L22 (Rpl22HA/+, Figure 12f) and that allows for pulldown of ribosomal bound RNA following Cre recombinase in a cell-specific manner [172]. This approach allowed us to uncover differentially up- and down-regulated transcripts in neurons under CD8 T-cell attack in vivo and in response to STAT1 signaling. The resulting network analysis revealed that neuronal STAT1 upregulated connected enriched gene sets with roles in immune response and downregulation of gene sets for synaptic activity. These included signatures of chemokine signaling, antigen processing and presentation, and complement and coagulation cascades, all of which depend on neuronal STAT1 signaling (Figure 12g). Among the chemokines induced upon viral déjà vu, we noted strong upregulation of the Ccl2 and Cxcl10 transcripts (Figure 12h–i). We thus speculated that STAT1-induced expression of chemokines or complement factors in neurons instructed phagocytes to engulf synapses. Analogous experiments in loxP-flanked Ccl2 mice ($Ccl2^{fl/fl}$) corroborated that neuronal CCL2 is essential for phagocyte recruitment, subsequent synapse elimination, and viral déjà vu disease precipitation. Accordingly, when animals were treated with minocycline that interferes with phagocyte activation and recruitment ameliorated viral déjà vu and synaptic loss (Figure 12j–k).

We further corroborated identified signaling signatures in a cohort of Rasmussen Encephalitis patients. Similar to the mouse model, JAK1/2-STAT1 signaling and CCL2 expression were associated with synaptic alterations in the diseased human CNS samples (Figure 13a–d).

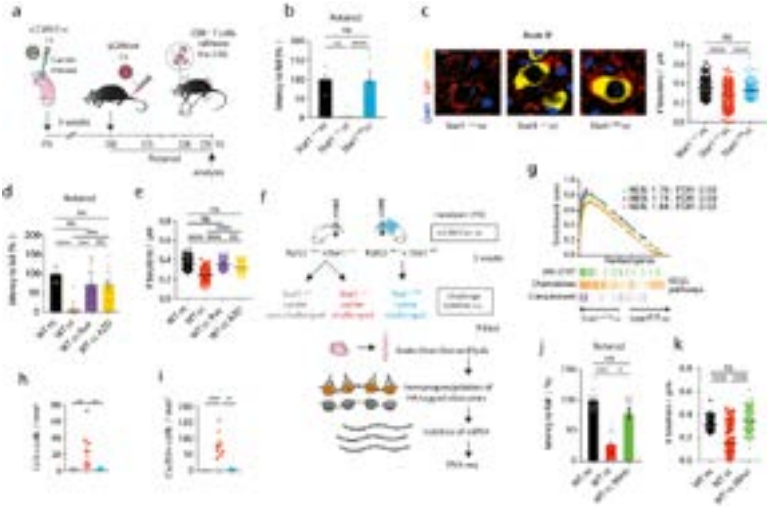

Figure 12. Neurons under T cell attack coordinate phagocyte-mediated synaptic stripping. *(**a**) Stat1+/+ or Stat1$^{fl/fl}$ mice were infected intracranially (i. c.) with attenuated LCMV encoding for the Cre recombinase (rLCMV-Cre). At around 5 weeks of age, rLCMV-Cre carrier mice were challenged (cc) i. v. with LCMVwt to trigger a CD8+ T cell response. (**b**) Rotarod performance of the indicated groups. (**c**) Representative immunostainings for synaptophysin (SYP), LCMV-nucleoprotein (LCMV), and nuclei (DAPI) and histological quantification of perisomatic synaptic bouton in deep cerebellar nuclei (DCN) neurons in indicated groups. (**d**) Rotarod performance on day 10 after LCMVwt i. v. of the indicated groups. (**e**) Histological quantification of perisomatic synaptic bouton in DCN neurons in indicated groups. (**f**) The translatome of infected neurons of Rpl22HA/+ rLCMV-Cre carrier mice was analyzed by next-generation RNA sequencing in the viral déjà vu setting (day 9 after LCMVwt i. v.). (**g**) GSEA with KEGG modules of transcripts highly expressed in Rpl22HA/+xStat1+/+ versus Rpl22HA/+xStat1$^{fl/fl}$ challenged mice. Normalized enrichment score (NES) indicates the cumulative enrichment, and false discovery rate (FDR) indicates the adjusted q value. Lines over the distribution of expression profiles mark the occurrence of the signature transcripts. Quantification of in situ hybridization of (**h**) Ccl2 and (**i**) Cxcl10 in brain sections of the indicated groups. (**j**) Rotarod performance at the peak of disease (day 10). (**k**) Histological quantification of perisomatic synaptic bouton in DCN neurons in indicated groups. Symbols represent one individual mouse, and bars represent means ± SEM, except for (**c**)(**e**) and (**k**), where symbols represent neurons and bars represent means, and, except for (**g**), where symbols represent individual transcripts. b: n=8–11; c, e, k: n=30; d: n=8–16; h, i: n=5–8; j: n=11. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, ns: not significant. Adapted from DiLiberto et al., Cell, 2018.*

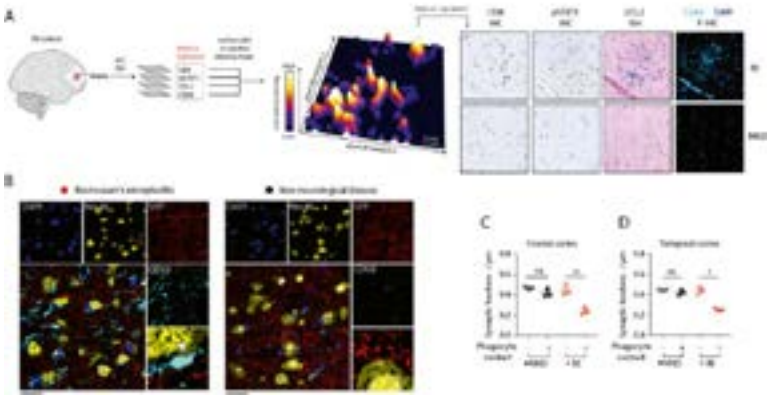

Figure 13. RE lesion correlates with the déjà vu inflammatory signature. *(**a**) Adjacent brain sections of a representative RE biopsy stained for CD8, pSTAT1 (IHC), or CCL2 (ISH) or CD68 and DAPI by fluorescence immunohistochemistry staining (F-IHC) and digitally aligned for coregistration. Positive cells for each marker were detected, and 2D signal density maps were generated. Individual 2D maps were stacked and visualized as a 3D surface plot. White peaks correspond to regions enriched in all markers. Scale bar, 1 mm in surface plot and 50 mm in IHC and ISH. (**b**) Representative images of RE and non-neurological disease (NND) co-immunostained for neurons (NeuN), synaptophysin (SYP), activated phagocytes (CD68), and DAPI. The inset on the left shows a phagocytic process interposed between neuronal somata and synaptic terminals (arrowhead). Scale bars, 20 µm. (**c and d**) Quantification of perisomatic bouton density in RE and NND matched for age, frontal (c) and temporal (d) brain region (n = 40 neurons evaluated per patient) and stratified according to the presence (+) or absence (–) of contact with CD68+ cells. Adapted from DiLiberto et al., Cell, 2018.*

Altogether this work unrevealed the tripartite interaction between CD8+ T cells, neurons, and phagocytes (Figure 14). While in our initial work, we confirmed in RE disease samples this signature, several publications have meanwhile shown that this signaling pathway was also observed in other human CNS inflammatory disease contexts, including in HIV CNS involvement [150] but also autoimmune diseases [173–175] attesting the broader relevance of our findings.

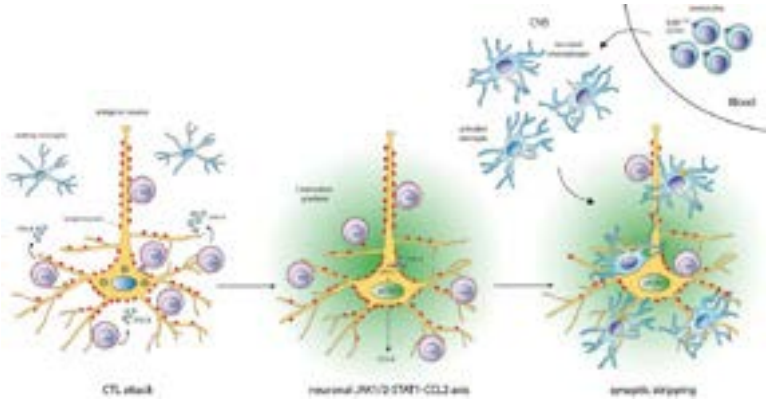

Figure 14. Schematic representation of viral déjà vu. *Upon recognition of their cognate antigen on neurons, activated CTLs secrete IFN-γ. IFN-γ signal transduction in neurons leads to the activation of the Janus kinases 1 and 2 (JAK1/2) and ultimately phosphorylation of the transcription factor STAT1, which triggers the production of CCL2 chemokine. The gradient of CCL2 generated by attacked neurons rapidly recruits macrophages and microglia that together initiate synaptic stripping.*

In future studies, we are interested in deciphering the molecular underpinnings of the long-term consequences of immune cell neuron interactions that we believe are related to epigenetic remodeling and metabolic changes of neurons that may be harnessed to develop novel therapeutic approaches aiming at preventing long-term neurodegeneration.

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