Local antigen acquisition by B cells in the brain and its potential to cause an autoimmune response

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Abbreviations

AICD	activation-induced cell death
ALCAM	activated leukocyte cell adhesion molecule
APC	antigen presenting cell
BBB	blood-brain barrier
BCR	B cell receptors
CLN	cervical lymph node
CNS	central nervous system
CSF	cerebrospinal fluid
CTV	cell trace violet
DC	dendritic cell
EAE	experimental autoimmune encephalomyelitis
EBV	Epstein-Barr virus
ELISpot	enzyme-linked immunospot
FDC	follicular dendritic cell
GC	germinal center
GFAP	glial fibrillary acidic protein
HA	hemagglutinin
i.p.	intraperitoneal
iBALT	inducible brochus-associated lymphoid tissue
ILN	inguinal lymph node
MAG	myelin associated glycoprotein
MBP	myelin basic protein
MHC	major histocompatibility complex
MHV	mouse hepatitis virus
MOG	myelin oligodendrocyte glycoprotein
MP4	MBP-Proteolipid protein fusion protein
MRI	magnetic resonance imaging
MS	multiple sclerosis
PBS	phosphate-buffered saline
PFA	paraformaldehyde
PI3K	phosphatidylinositol-3 kinase
PML	progressive multifocal leukoencephalopathy
PPMS	primary progressive multiple sclerosis
RRMS	relapsing-remitting multiple sclerosis
s.c.	subcutaneous
SCS	subcapsular sinus
scTranscriptomics	single-cell transcriptomics
Spl	spleen
TCR	T cell receptor
TLS	tertiary lymphoid structure
WT	wildtype

Summary

Background and rationale

The exact pathogenesis of multiple sclerosis (MS) is still under investigation. The recent success in treatment with monoclonal antibodies – natalizumab and anti-CD20 monoclonal antibodies – suggests that infiltration of lymphocytes, particularly naïve or memory B cells, is closely related to neuroinflammation in MS. As a central nervous system (CNS)-restricted antigen acquisition by B cells is a pre-requisite of several potential mechanisms how B cells might contribute to MS, we examined the local antigen acquisition by B cells in the brain and its potential to cause an autoimmune response in the brain.

Results

We first established B cell infiltration in the brain using a viral vector, VSVΔG. The VSV infected neurons and caused B cell infiltration and accumulation in the perivascular spaces starting from day 2 post-injection. The B cells entered the brain parenchyma after day 5 post-injection. We could observe that the anti-viral B cells closely interact with the infected cells using histology and intravital imaging, yet the interactions were not significantly higher than in wildtype controls. We demonstrated that the B cells could capture their cognate antigen directly from the antigen-expressing cells in vitro, which happened within the first hour of co-culture. Due to the difficulty of controlling the dynamics of B cell infiltration in the brain, we switched the paradigm to a lung infection and showed that FluBI B cells had significantly higher intimacy of contact with their cognate antigen in the influenza-infected lung compared to wildtype B cells. Finally, we demonstrated using enzyme-linked immunospot (ELISpot) that the IgH MOG B cells could acquire their cognate antigen locally in the brain. At least on this timepoint of 7 days post-injection we did not observe the migration of antigen-loaded B cells to peripheral lymphoid tissues. The IgH MOG B cells disappeared in the brain after antigen capture due to activation-induced cell death because they lacked further survival signals. When CD40L was provided, a plasmablast-like cell population was found in IgH MOG mouse brain by single-cell transcriptomics (scTranscriptomics) and demyelination in the B cell infiltrating area in IgH MOG mouse brain was observed using confocal microscopy.

Conclusion

Our results support that virus infection of the brain facilitates the infiltration of autoimmune B cells into the brain parenchyma. The infiltrating B cells acquire their target antigen directly in the brain. Upon CD40-CD40L interaction, they can evade activation-induced cell death and thereby circumvent an important immune checkpoint. Our study provides new insights into understanding the possible mechanisms of B cell pathogenesis in MS.

Chapter 1. Literature review

1.1 Multiple sclerosis

MS is the most common chronic neuroinflammatory disease affecting more than 2 million people, with the mean age of the diagnosis being approximately 30 years [1]. The patients suffer from partially or fully reversible episodes of days- or weeks-long neurologic disability and develop progressive clinical courses typically after 10 – 20 years [1].

1.1.1 Pathology

The neurologic disabilities of multiple sclerosis are due to lesions that can appear throughout the CNS. The lesions are focal areas of demyelination, inflammation and glial reactions throughout the CNS, which can be visualized with magnetic resonance imaging (MRI).

At the early stages of the disease, early active white-matter lesions are formed as well as early grey matter lesions [2, 3]. The active white-matter lesions can be classified into four different patterns of demyelination based on complement and immunoglobulin deposition, loss of specific myelin proteins, plaque topography and destruction of oligodendrocytes [2].

The most frequent pattern is pattern II. It shows T cell/macrophage-associated demyelination and parallel loss of all myelin proteins [2]. Most importantly, pattern II suggests humoral mechanisms may have a pathogenic role as the lesions typically involve immunoglobulin and complement deposited along myelin sheaths, which are also found within the macrophages [2]. Pattern III is the second most frequent lesion type characterized by apoptotic oligodendrocytes and preferential loss of the myelin-associated glycoprotein (MAG) [2]. Selective loss of MAG is considered a marker for metabolically stressed oligodendrocytes, leading to a dying-back oligodendrogliopathy [2]. MAG loss is also shown in several contexts, including progressive multifocal leukoencephalopathy and hypoxic and ischemic conditions [2]. The next frequent

lesion type is the pattern I lesion. The lesions are similar to pattern II lesions except for the immunoglobulin and complement depositions [2]. Pattern IV is rare with nonapoptotic oligodendrocyte death in periplaque nondemyelinated white matter [2]. There is a single dominating immune effector mechanism in each person despite several different patterns of demyelination, suggesting the mechanisms of the disease may differ among the patient subgroups [2].

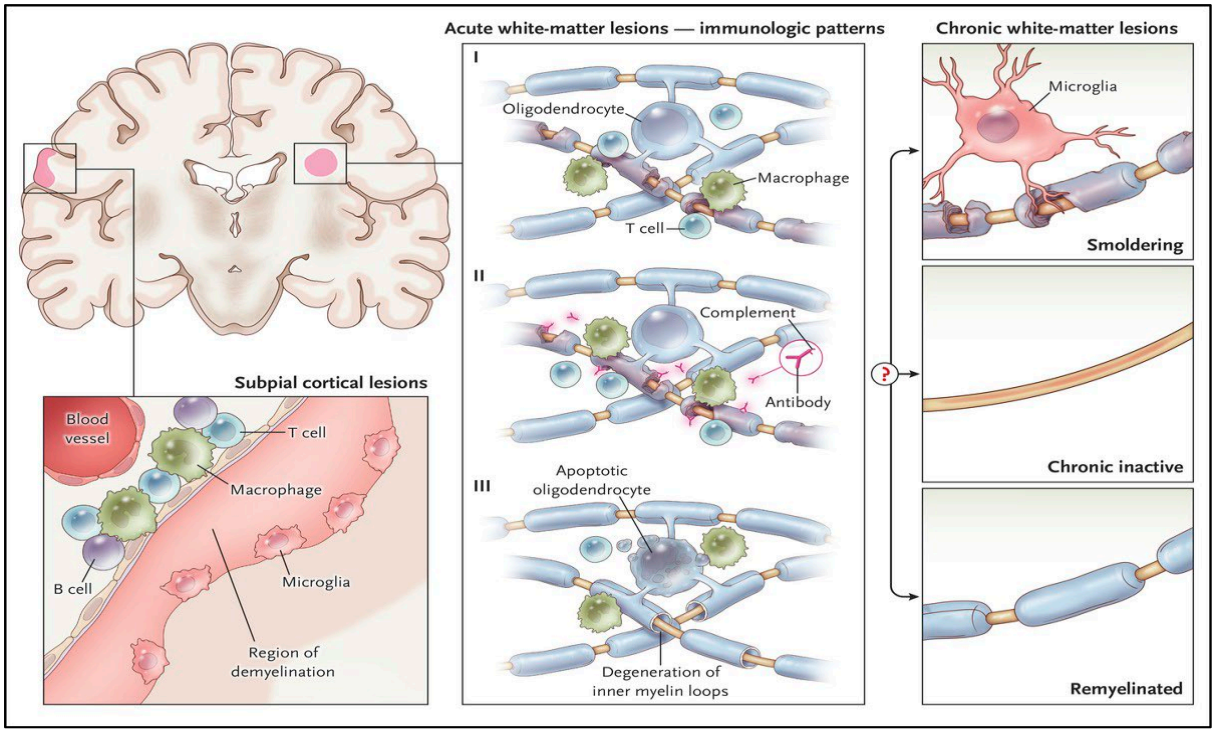

Figure 1-1. MS pathology. Several types of lesions are observed in MS pathology – Subpial cortical lesions, active white matter lesions, and chronic white-matter lesions. Active white matter lesions are classified into four patterns. Reproduced with permission from [4]. Copyright Massachusetts Medical Society.

Even though the grey matter is not heavily myelinated, this area of the brain is also affected. Different regions of the cortex are affected. Half of the cortical lesions are perivascular, either in the central cortical layers or in the leukocortical junctions. The lesions in leukocortical junctions affect both cortex and juxtacortical white matter. When comparing the density of several immune cell infiltrations in cortical lesions to white matter lesions, fewer major

histocompatibility complex (MHC) class II positive cells, fewer CD3 positive lymphocytes and fewer CD68 positive microglia/ macrophages are observed in the cortical lesions[5].

The other half of the cortical lesions are in the brain's pial surface, and those found in autopsies are pretty old and usually inactive. However, the early MS subpial lesions detected in biopsy samples are inflammatory and active [3]. Those lesions are also closely associated with leptomeningeal inflammatory aggregates [3]. The lymphoid follicle-like structures containing B cells, T cells, plasma cells and CXCL13-producing follicular dendritic cells are found in the meninges of post-mortem MS brains and spinal cord [6]. An MRI study supports a potential link between leptomeningeal inflammation and the self-sustaining tertiary lymphoid follicles [7]. Yet, the mechanism of formation of these lesions is unclear due to the lack of robust detection techniques.

1.1.2 Epidemiology

The cause of MS remains unclear and needs more investigation. Nevertheless, studies found quite a few risk factors related to MS. As similar to other autoimmune diseases, three-quarters of the patients are women [4]. The general population has a 0.1% risk of developing MS [4]. The risk is increased to 2-4% for people who are first-degree relatives and 30-50% for monozygotic twins [4]. This suggests a potential role of genetic factors in the development of MS. From the genome-wide association studies, more than 200 gene variants, including HLA DRB1*1501 haplotype, the most significant variant, are identified to be involved in MS [4]. Most of them are associated with immune-pathway genes supporting the connection between autoimmune mechanisms and MS.

A higher incidence in more temperate climates may be related to lower vitamin D levels or prevalent pathogens in these regions. Other environmental factors such as obesity, exposure to tobacco, mononucleosis and Epstein-Barr virus (EBV) infection are also associated. Although

virus infection, including EBV, has been suggested as a potential cause of MS or MS-related diseases, none have been proven to be the leading cause. However, a recent longitudinal study of 10 million young adults showed a 32-fold increase in risk after EBV infection but not after infection of other viruses [8]. This study suggests that EBV has an important role in the development of the MS [4].

1.1.3 Therapy – monoclonal antibodies

Several disease-modifying therapies are available for treating MS. Most are approved for relapsing-remitting multiple sclerosis (RRMS). Among those, Ocrelizumab is also approved for primary progressive multiple sclerosis (PPMS). Although the mechanisms of treatments are not entirely understood, there are several possible mechanisms of action, including induction of anti-inflammatory cytokines, the interference with migration of lymphocytes, and depletion of lymphocytes, namely B cells. There are many forms of disease modifying therapies. We will focus on the monoclonal antibodies – natalizumab, ocrelizumab and alemtuzumab.

Alemtuzumab targets and depletes CD52 expressing cells, including T cells, B cells, natural killer cells and monocytes [9]. Alemtuzumab effectively reduced the relapsing rate in clinical trials [10]. However, due to severe side effects it is only used for patients who are not responding to at least one therapy and still have a highly active disease. Side effects include infections, autoimmune disorders and cardiovascular symptoms [11].

Natalizumab targets lymphocytes by binding to α4/ß1 integrin expressed on the surface of the cells. α4/ß1 integrin interacts with endothelial ligand vascular adhesion molecules and mediates lymphocyte migration through the blood-brain barrier (BBB) [11]. Natalizumab therefore blocks migration of lymphocytes from the blood into the brain. It is shown to be more effective compared to many other therapies [11]. However, the use of natalizumab has to be

carefully monitored due to the high risk of developing progressive multifocal leukoencephalopathy (PML) [12] [13].

B cell-depleting monoclonal antibodies are also highly effective therapies in MS. The mechanisms of action are not clear yet, but several possible mechanisms have been proposed. The B cell depletion can impair the antigen-presenting function of B cells [14], disrupt cytokine secretion and polarization of the immune response [15], and prevent the maturation of new antibody-secreting cells. Currently, ocrelizumab and ofatumumab, anti-CD20 monoclonal antibodies, have been approved for MS. Both Ocrelizumab and ofatumumab showed a significant reduction in annualized relapse rates and new lesions in brain MRI compared to controls [16, 17].

The treatment using monoclonal antibodies contributed to extending our knowledge of MS pathogenesis. The success of natalizumab in reducing relapses indicates that infiltration of lymphocytes in the brain is an important factor in MS. Moreover, depletion of CD20 positive B cells suggests a potential role of naïve B cells or memory B cells in the relapses of MS.

1.2 B cells in MS

Although MS was traditionally thought to be a T cell-mediated disease, emerging evidence, including the success of anti-CD20 treatments, suggests the contribution of B cells in MS pathogenesis. Pattern II MS lesions with IgG and complement deposition also indicate the involvement of B cells in MS pathology.

1.2.1 Barriers of the brain

In post-mortem and biopsy brain specimens of MS patients, B cells are often observed in perivascular spaces and parenchyma in contrast to healthy brains. The healthy CNS is protected by several acellular and cellular barriers.

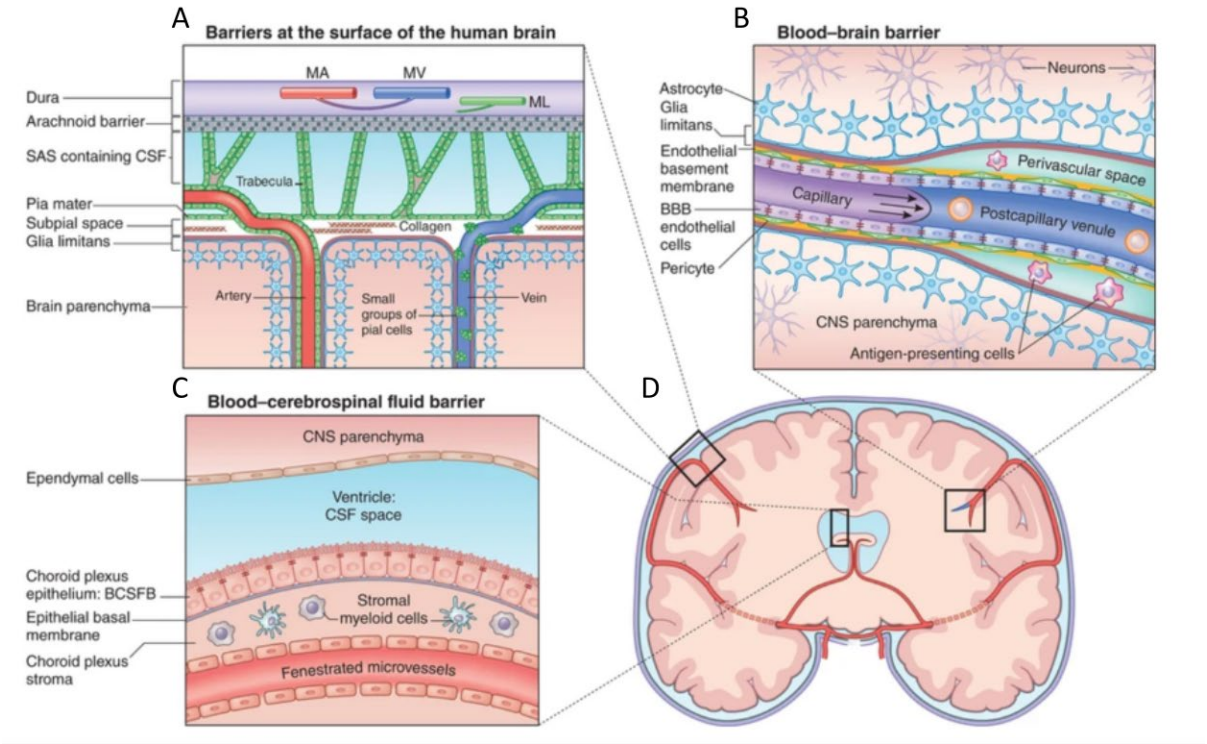

Figure 1-*2*. Barriers of the brain. (A) Barriers at the surface of the human brain (B) Blood-brain barrier (C) Blood-cerebrospinal fluid barrier (D) Schematic coronal brain section presenting the localization of the brain barriers. Reproduced with permission from [18]. Copyright © 2017, Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.

There are three layers of meninges at the surface of the human brain to separate the CNS from the cerebrospinal fluid (CSF). The outermost layer is a dense fibrous tissue called the dura mater. Arteries, veins and lymphatics are present and separated from the CNS. [19] The second layer below the dura mater is the arachnoid mater which is impermeable to fluids [19]. It consists of layers of cells expressing efflux pumps and joined by tight junctions [20]. Below the arachnoid mater is CSF-containing subarachnoid space. The pia mater is one layer of cells that coats the surface of the CNS. It is permeable to solutes and immune cells [21]. Arteries and veins in the subarachnoid space are covered by the pia mater separating the subarachnoid space from the CNS. When arteries and veins enter the surface of the CNS from the subarachnoid space, they pass through the subpial space. Arteries are still coated with pia mater without perivascular spaces, whereas a layer of leptomeningeal cells covers veins [22]. Glia limitans protect the surface of the CNS from the parenchymal side of the CNS. They are

compacted foot processes of astrocytes and overlay the parenchymal basement membrane [23]. The glia limitans allow fluid and low molecular weight tracers to pass but prevent immune cell trafficking [24].

BBB in postcapillary venules acts as a barrier between the blood and the CNS parenchyma regulating the entry of immune cells. At the postcapillary venules, BBB endothelial cells are coated by an endothelial basement membrane with embedded pericytes [18]. The glia limitans and the basement membrane at the CNS side protect the CNS surface [18]. Between the endothelial basement membrane and the glial limitans basement membrane lies the perivascular space where antigen-presenting cells (APCs) can invade [18]. The perivascular spaces are not observed in the cerebral cortex of the human [22] and mouse [25]brain but are present in the basal ganglia and white matter [26, 27].

Choroid plexuses produce CSF and are localized in the ventricles. Between the choroid plexus epithelium and CSF, choroid plexus epithelial cells form the blood-CSF barrier. As the choroid plexus stroma consists of dendritic cells (DCs) and macrophages with a dense microvascular network, the epithelial cells are connected by tight junctions and express efflux pumps to regulate the composition of CSF and passage of immune cells [28, 29].

1.2.2 B cell localization in MS brain

MS patients have B cells and plasma cells primarily in the meninges and perivascular locations, with small numbers present in the parenchyma. In the CSF of MS patients, clonally expanded and somatically hypermutated B cells and plasma cells are observed in higher numbers compared to other neurological diseases or healthy controls [30, 31].

Post-mortem studies show that B cell infiltration is highly variable in MS. Some patients rarely have B cells in the lesions, whereas others are dominated by the B cells [32]. B cells and plasma

cells are usually found near the lesions. Interestingly, B cells are more common in active lesions, in contrast to plasma cells that are present in active-inactive or inactive lesions [32].

Cortical lesions are often associated with meningeal inflammation and a large number of meningeal B cells. Extensive meningeal B cell inflammation is observed with follicle-like structures which resemble the B cell follicles in secondary lymphoid organs [33]. The structure consists of separate B cell and T cell zones with many plasma cells [33].

1.2.3 B cell recruitment and survival in MS brain

1.2.3.1 Recruitment of B cells into CNS

B cells can enter the CNS through 3 barriers – BBB, meningeal barriers and choroid plexus. Each site has unique structures; however, the overall process of entering from the blood into the CNS is similar.

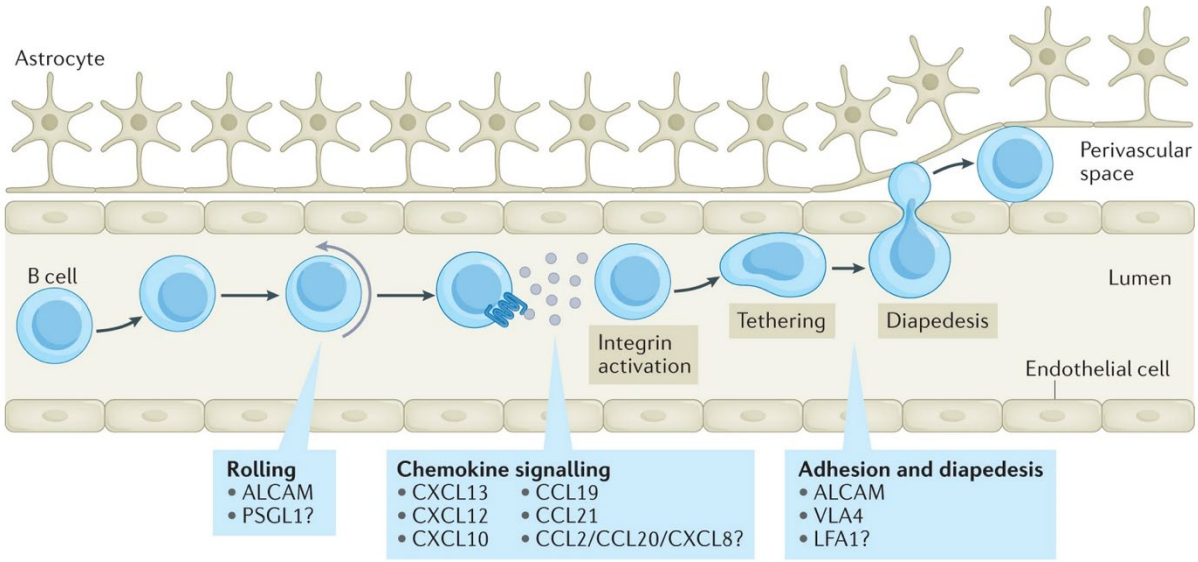

Figure 1-3. Recruitment of B cells into the CNS. B cells first attach to the blood vessel and roll along the lumen. With locally produced chemokine signaling, B cells activate integrin for high-affinity interactions and tethering to the vessel and finally migrate across the endothelial cell layer into CNS. Molecules possibly involved in the processes are presented in the boxes. Reproduced with permission from [34]. Copyright © 2021, Springer Nature Limited

Firstly, B cells are recruited into the CNS by rolling along the lumen of the blood vessel. Although L-selectin (CD62L) may be involved in the rolling stage of other immune cell [35], deletion of L-selectin did not affect the B cell infiltration into the CNS in the experimental autoimmune encephalomyelitis (EAE) model [36]. Activated leukocyte cell adhesion molecule (ALCAM) is also known to regulate the rolling stage of immune cells. It is indeed upregulated in the activated B cell subset in MS and blocking them decreases disease severity and B cell recruitment into CNS in EAE mice [37].

Many chemokines are suggested to be involved in B cell migration into CNS, including CXCL10, CXCL12, CXCL13, CCL19 and CCL21. The chemokines affect specific B cell subsets and have different expression patterns shown in table 1-1 [34].

Table 1-1. Chemokines and survival factors affecting B cells in the CNS.

Chemokine(s)/ survival factor – receptor(s)	Cells affected	Chemokine location	Chemokine expression and disease associations
CXCL10 and CXCL9 – CXCR3	Plasmablasts, plasma cells, and Tbet memory B cells.	- Found in perivascular spaces, the meninges, and the parenchyma of MS lesions. - Made by astrocytes.	- CXCL10 is increased in CSF in RRMS and variably in progressive MS.
CXCL12 – CXCR4	Germinal center B cells, memory B cells, plasmablasts and plasma cells.	- Found in perivascular spaces - Can be made by astrocytes and by oligodendrocyte precursor cells in EAE.	- Increased in CSF and MS lesions - Correlated with faster cortical thinning and relapses.

CXCL13 – CXCR5	Naïve B cells, germinal center B cells, memory B cells, T follicular helper cells.	- Found in active lesions in the meninges, perivascular spaces, and the parenchyma.	- Increased in MS lesions and CSF throughout the disease - Correlated with disease severity, relapses, new white matter lesions, new cortical lesions, increased immunoglobulin synthesis in CSF, and faster cortical thinning.
CCL19 – CCR7	Naive B cells, activated B cells, memory B cells	- Found in the meninges and to a lesser degree in perivascular cuffs and may be in the parenchyma. - Expressed by astrocytes and microglia in EAE including within the parenchyma	- Increased in CSF of RRMS and SPMS patients.
CCL21 – CCR7	Naïve B cells, activated B cells, memory B cells	- Not found in the parenchyma but small amounts of RNA are found in perivascular cuffs and the meninges. - In perivascular spaces in EAE	- Present but not increased in CSF in MS.
CCL20 – CCR6	B1 cells, Naïve B2 cells, Memory B cells	- mRNA detected in perivascular spaces and the meninges.	- Present in small amounts but not increased in CSF in MS.
CCL2 – CCR2	Naïve and memory B cells	- mRNA detected in perivascular spaces and the meninges. - Protein detected in the parenchyma - Made by astrocytes in white matter lesions but not grey matter	- Normal to decreased levels in CSF during MS.

CXCL8 – CXCR1 and CXCR2	A small portion of peripheral blood B cells	- Trend towards increased expression in white matter lesions.	- Increased in CSF of MS patients.
BAFF – BAFF-R and TACI	Naïve B cells, activated B cells, memory B cells, and plasma cells	- Found in the meninges and to a lesser extent in the perivascular spaces and parenchyma. - Made by astrocytes in lesions.	- Slightly increased to decreased in CSF, decreasing during periods of B cell expansion.
APRIL – BMCA and TACI	Memory B cells, plasmablasts, and plasma cells	- mRNA detected in perivascular spaces and to a lesser extent in the meninges. - Made by astrocytes in EAE lesions in the meninges, perivascular spaces, and the parenchyma	- Slightly increased in MS. - Correlated with relapses.

When B cells are activated by the chemokines, they express integrins and cell adhesion molecules to facilitate their migration into the CNS. ALCAM additionally plays a role in B cell migration by promoting diapedesis across brain endothelial cells [37]. Integrin LFA1 and its cell adhesion molecule ligand ICAM1 are key molecules and upregulated on activated B cells in MS patients [37]. In addition, migration of human B cells is reduced when blocking ICAM1 *in vitro* [37]. VLA4 is the most well-characterized B cell trafficking molecule. It is highly expressed on activated B cells in EAE and MS [37, 38]. B cells require VLA4 to cross brain endothelial cells *in vitro* [37]. Deletion of VLA4 on mouse B cells results in a reduction of B cell recruitment and EAE severity [39]. Natalizumab, a humanized anti-α4 integrin monoclonal antibody, increased CD19 positive mature B cells in the blood [40] and significantly decreased migration of immune cells into the CNS [41].

1.2.3.2 Survival of B cells in MS brain

BAFF and APRIL, produced by astrocytes and found in MS lesions (Table 1-1), are the main survival factors for B cells [42, 43]. Mature B cells depend on BAFF and become more dependent on APRIL as they differentiate into plasma cells [44]. Apart from BAFF and APRIL, astrocytes secret undefined factors which support human B cell survival and induce upregulation of antigen-presenting machinery *in vitro* [45]. Astrocyte-soluble factors can also support the survival and activation of B cells in MS patients, including memory B cells [45]. In addition, interaction with T cells is another crucial factor for B cell survival. CD4+ T cells can rescue activated B cells and plasmablasts from activation-induced cell death (AICD).

1.2.4 B cell pathogenesis in MS

B cells have several possible mechanisms to contribute to MS pathogenesis. Firstly, B cells can differentiate into plasmablasts and plasma cells to produce autoantibodies. Pathogenic effector mechanisms of autoantibodies can be an initiation of complement deposition, antibody-dependent cellular cytotoxicity and enhancement of B cell and T cell activation. Unfortunately, the autoantigen target for autoantibodies in MS is still unclear despite the typical oligoclonal bands in CSF indicating an intrathecal synthesis of immunoglobulins and antibody deposition in MS lesions.

Recent studies proposed a non-conventional mechanism of B cell contribution through the production of pathogenic microvesicles or exosomes. It was postulated that large secreted particles from B cells could be toxic to oligodendrocytes and neurons *in vitro* [46, 47].

B cells are particularly effective in presenting their cognate antigen to CD4+ T cells on MHC class II. Indeed, some evidence suggesting the importance of antigen presentation is available. MHC class II expression on B cells and myelin oligodendrocyte glycoprotein (MOG)-recognizing membrane-bound B cell receptors – not secreted anti-MOG autoantibodies –

influence the susceptibility of MOG-induced EAE models [48]. Antigen presentation and interaction with T cells may affect MS indirectly through various mechanisms, including reactivation of T cells in the CNS and in the periphery, which leads to the entry of the T cell into the CNS. A recent study shows that memory B cells of MS patients induce the proliferation of autoreactive CD4+ T cells in an HLA-DR dependent matter and induce these cells to enter the CNS [14].

Cytokine secretion by B cells is another important factor that can mediate the pathogenesis of MS. B cells secrete pro-inflammatory cytokines such as GM-CSF, TNF, IL-6 and lymphotoxin-α. GM-CSF induces inflammatory polarization of macrophages, promoting macrophage-driven pathology [49]. TNF is associated with microglial activation and death of neurons and oligodendrocytes [50]. IL-6 facilitates plasma cell differentiation and survival, T follicular helper cell differentiation and IL-17-producing $CD4^+$ T helper cell polarization [51]. Lymphotoxin-α is crucial for the formation of follicle-like structures in the meninges [52]. Regulatory cytokines are produced by Breg cells. TGFβ1 and IL-35 lower proliferation and inflammatory polarization of T cells and promote regulatory T cell functions [53, 54]. IL-10 has a similar function with additional effects of altering macrophage polarization and promoting remyelination [55, 56].

Potential mechanisms of B cell contribution in MS pathogenesis are generally preceded by activation of B cells through the B cell receptor. In particular, for B cells to secrete the autoantibody against CNS-restricted antigens, B cells are required to see the autoantigen that is expressed in the brain. The same is true for antigen presentation. Until now antigen recognition and capture by B cells has been mainly studied in lymphoid tissues, as described below, and it is not clear if this process could also take place inside the brain.

1.3 B cells

Together with T cells, B cells make up the adaptive arm of the vertebrate immune system. B cells contribute to the immune response by recognizing antigens through surface immunoglobulins called B cell receptors (BCRs). Those immunoglobulins are eventually secreted from B cells after maturation and differentiation. The secreted immunoglobulins are known as antibodies, the main effector of B cells in adaptive immunity.

1.3.1 Antigen acquisition by B cells in lymphoid tissues

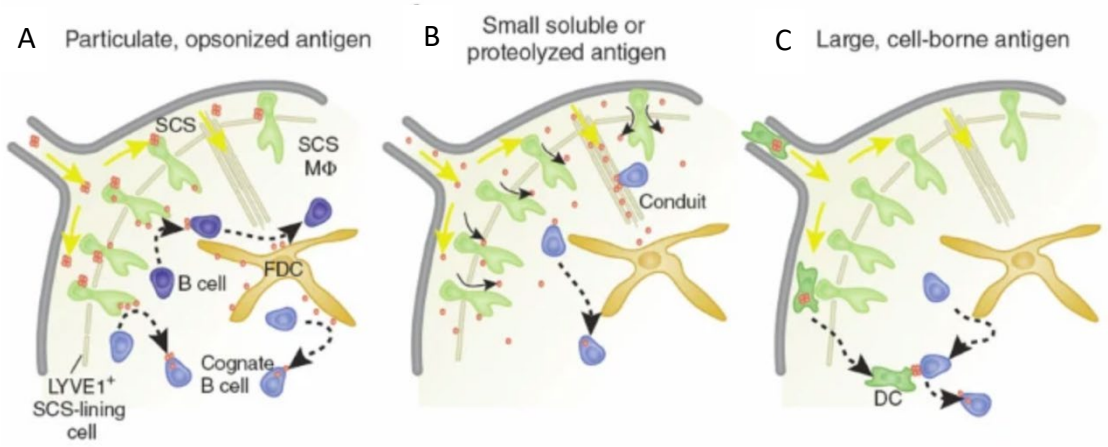

Figure 1-4. Antigen acquisition of B cells in a lymph node. (A) Acquisition of particulate antigens (B) Acquisition of soluble antigens (C) Acquisition of cell-borne antigens. Reproduced with permission from [57]. Copyright © 2010, Nature Publishing Group, a division of Macmillan Publishers Limited. All Rights Reserved.

B cells are either found in circulation or at the follicles in lymphoid tissues, including lymph nodes and spleens. The mature circulating B cells enter the lymph node from the blood through high endothelial venules and are located in the interfollicular regions. They finally migrate to the follicles and stay for around 24 hours [57].

In the lymphoid tissues, B cells can acquire small soluble antigens or antigens displayed on the surface of other cells. When acquiring antigens, immunological synapses are formed between B cells and cells with an antigen on their surface, where BCR recognizes an antigen through

the formation of a macromolecular cluster [58]. B cells then recruit and secrete MHC II-positive lysosomes at the immunological synapses in a microtubule-dependent polarized fashion [58]. The secreted lysosome mediated by the GTPase Cdc42 causes acidification of immunological synapses extracellular space and may facilitate the removal of antigens from the cells [58].

B cells acquire the antigen from antigen-displaying cells by physical extraction during the contact of 20 – 30 minutes with the cells [59]. The efficiency of this physical extraction is dependent on the affinity of the BCR-antigen interaction [60].

It is important to distinguish between antigen acquisition by B cells from antigen-displaying cells and antigen capture by B cells from antigen-expressing cells. However, the mechanisms of both processes are not well-established yet.

1.3.1.1 Acquisition of particulate antigens

Afferent lymphatic vessels drain lymph into the subcapsular sinus (SCS), where the pathogens and particles are filtered. The SCS is lined with LYVE1 positive lymphatic vascular endothelial cells, which form an anatomical and functional barrier to the free diffusion of particles into the parenchyma. SCS macrophages also populate the SCS and trap soluble and particulate antigens. B cells are in contact with SCS macrophages on the follicular side and assist the development and maintenance of the macrophages by providing cytokine lymphotoxin-α1β2 [61]. Unlike classical macrophages, SCS macrophages are not efficient at the internalization and degradation of antigens. Instead, they acquire lymph-borne antigens in the form of immune complexes [62], viruses [63], and virus particles [64]. The antigens are then attached to the surface of SCS macrophages, and B cells can acquire the intact antigens on the follicular side, as shown in figure 1-1A.

Follicular B cells routinely migrate and interact with SCS macrophages in the subcapsular region. When antigen-specific B cells acquire their cognate antigen from SCS macrophages, they subsequently migrate with reduced velocity and localize at the T-B border. The antigen-acquired B cells further show the downregulation of BCR and upregulation of CD86 [62, 64].

1.3.1.2 Acquisition of soluble antigens

Unlike particulate antigens, small soluble antigens can bypass the SCS macrophage layers and directly enter the follicle quickly within 2 hours of injection [65].

The lymph node contains fibroblast reticular cells forming an interconnected network called the conduit system. The loosely packed collagen-rich fibers have holes around a 5.5 nm radius which allows molecules approximately smaller than 70 kDa in size to pass through. The conduit system serves as structural support for the lymphocytes and APCs and as a molecular sieve to filter the bigger antigens in the lymph fluid. The smaller soluble antigens can travel rapidly from the lymph to high endothelial venules B cells may acquire the soluble antigens directly or through the DCs [66]. Another study suggested that the soluble antigens can enter through junctions between LYVE1+ SCS-lining cells and SCS macrophages [67]. B cells may acquire the smaller soluble antigens traveled both ways. However, the exact mechanisms of the acquisition of soluble antigens remain unknown.

Antigens greater than 70kDa can be proteolyzed and directly enter the follicles. A recent study showed that cleaved antigens from the surface of beads could encounter follicular B cells without SCS macrophages, DCs or extensive migration of the follicular B cells [68].

1.3.1.3 Acquisition of antigens from dendritic cells and follicular dendritic cells

DCs are specialized in internalizing antigens at peripheral sites. DCs capture antigens, drain them to lymph nodes and display the antigens. The degradation of internalized antigens is slower than that by macrophages, leading to the delivery of intact antigens to B cells. B cells

can encounter DCs and take up the antigens at several places, including high endothelial venules, follicle-T cell zone, SCS and occasionally in the follicles [57].

Follicular dendritic cells (FDCs) are responsible for capturing and displaying opsonized antigens. They are found in germinal centers (GCs) within the B cell follicles and retain the opsonized antigens on the surface for long periods. Complement receptors expressed on germinal center B cells enhance BCR signaling [69]. In contrast, complement receptors expressed on FDCs retain antigen and promote the antigen selection of high-affinity germinal center B cells. [69] FDCs also provide an essential signal for B cell migration. Follicular stromal cells – marginal reticular cells in the subcapsular region of the follicle and FDCs in the center – produce a chemokine, CXCL13, which interacts with a chemokine receptor, CXCR5, expressed by mature B cells [70].

1.3.2 B cell development after antigen acquisition

After antigen encounter, B cells upregulate CCR7 which interacts with chemokines, CCL21 and CCL19. The chemokines are mainly expressed in the T cell zone. However, the CCL21 forms a gradient from the T cell zone to the follicle, guiding the B cells to the B cell – T cell boundary for T cell help [71]. B cells can present the processed peptide on the MHC II to CD4+ T cells and receive CD40L stimulation. CD40L is recognized by CD40 on the B cell surface and triggers the antigen-activated B cells to proliferate and differentiate into plasma cells or memory cells.

Within the first week after immunization, B cells that acquired an antigen and received CD40L-CD40 signaling from co-cognate T cells can proliferate and secrete IgM antibodies without hypermutation. The second development process is the establishment of GCs where B cells undergo somatic hypermutation, affinity maturation and class switch recombination of immunoglobulins. The activated B cells – the GC B cell precursors called centroblasts – rapidly

divide and undergo clonal expansion and somatic hypermutation in the dark zone of GC. The centroblasts produce CXCR4, which interacts with stromal cells in the dark zone [72]. After the process, the centroblasts downregulate CXCR4 and migrate to the light zone, which is rich in FDCs as GC B cells with their expression of CXCR5 [73]. At the light zone of GC, GC B cells are positively selected through interaction with FDCs and T follicular helper cells. Long-lived antibody-secreting plasma cells and memory B cells are eventually produced with high-affinity BCRs for a better defense mechanism.

Hypotheses and approach

The study aimed to elucidate the role of infiltrating B cells in the brain and to investigate the possible mechanisms of B cell pathophysiology in MS. As stated above, monoclonal antibodies contribute to reducing the relapse rate of MS. Particularly, the anti-CD20 and the anti-α4 integrin treatment suggest that brain-infiltrating naïve B cells and/or memory B cells are closely involved with autoimmune inflammation in the CNS. There are a few possible mechanisms, including (i) memory B cells are pathogenic, have acquired the antigen in the lymph tissues and then infiltrate the brain leading to CNS inflammation; or (ii) naïve B cells are pathogenic, infiltrate the brain and acquire the antigen in the CNS which again leads to CNS inflammation. The second mechanism raises a critical question – whether B cells can acquire their cognate antigen outside of the lymph tissues. The antigen acquisition by B cells in the lymph tissues is well understood. However, it is important to investigate the antigen acquisition by B cells outside of the lymph tissues, particularly in the immune-privileged CNS, and its impact on MS pathogenesis.

Our hypothesis was that (1) naïve autoimmune B cells, with other lymphocytes, enter the brain upon CNS infection; (2) the CNS-infiltrating naïve B cells encounter and capture their cognate

antigen; and (3) the antigen capture by autoimmune naïve B cells activates the cells and causes autoantibody secretion which leads to demyelination.

In order to understand the antigen capture by B cells in the brain, we needed to induce B cell entry in the brain as B cells generally do not enter the healthy brain [34]. We used virus infection to induce the infiltration. After establishing the B cell infiltration, we first demonstrated the kinetics of the B cell entry and antigen encounter of anti-viral B cells in the brain. Since we were unable to find a time point at which we could demonstrate antigen capture in fixed tissue, the antigen capture by B cells directly from antigen-expressing cells was assessed *in vitro* and in the lung. Then, we investigated the infiltration of autoimmune B cells and their cognate antigen capture in the virus-infected brain using a transgenic mouse line with myelin-oligodendrocyte glycoprotein (MOG) specific BCR. Finally, we examined the fate of antigen-captured autoimmune B cells using single-cell transcriptomics (scTranscriptomics) analysis.

Chapter 2. Methods and materials

2.1 Mice and viral vectors

2.1.1 WT tomato mice (Ai14 x CD19Cre)

The mouse strain B6.Cg-Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$/J (Ai14) is crossed with B6.129P2(C)-Cd19$^{tm1(cre)Cgn}$/J (CD19Cre)to produce Ai14 x CD19Cre mouse strain which has tdTomato expression in the cells once the CD19 promoter is expressed. Both Ai14 and CD19Cre strains are obtained from The Jackson Laboratory.

2.1.2 BCR transgenic mice

BCR transgenic mice were crossed with Ai14 x CD19Cre to produce tdTomato-expressing B cells with transgenic BCR.

2.1.2.1 IgH MOG

The mouse strain Igh-J^{tm1Aigl} (IgH MOG) is a transgenic strain expressing BCR specific to MOG. IgH MOG was kindly provided by Professor Gurumoorthy Krishnamoorthy at Max Plank Institute of Biochemistry.

2.1.2.2 FluBI

The mouse strain B6(129)-Igk^{FluBI}Igh^{FluBI}/Hpl (FluBI) is a transgenic strain with BCR specific for Influenza hemagglutinin (HA). FluBI strain was kindly provided by Professor Hidde Ploegh at Boston Children's Hospital.

2.1.2.3 V10l

The mouse strain B6-Igh-J$^{tm1(VDJ-VI10)Zbz}$ x B6-Tg(VI10L)C^{tmb} (V10l) is a transgenic strain expressing BCR specific for VSV glycoprotein. V10l strain was kindly provided by Professor Daniel Pinschewer at the University of Basel.

2.1.3 TCR transgenic mice – 2D2

The mouse strain C57BL/6-Tg(Tcra2D2,Tcrb2D2)1Kuch/J is a transgenic strain expressing T cell receptor (TCR) specific for MOG. 2D2 strain was obtained from The Jackson Laboratory.

2.1.4 Viral vectors

2.1.4.1 VSV vectors - VSVΔG and VSVgGFP

VSVΔG and VSVgGFP were kindly provided by PD Dr. Gert Zimmer at Institut für Virologie und Immunologie. The glycoprotein was replaced with GFP for VSVΔG, whereas the glycoprotein was tagged with GFP for VSVgGFP.

2.1.4.2 Adenoviral vectors

AVV-MOG-RFP670 is an adenoviral vector expressing RFP670 tagged MOG. The plasmid is produced and packaged in the lab.

AVV-vPig-CD40L is an adenoviral vector expressing CD40L. The plasmid is produced and packaged in the lab.

2.1.4.3 Lentiviral vectors

LVV-LMP1 is a lentiviral vector expressing LMP1. The plasmid is produced and packaged in the lab.

2.1.5 Influenza virus

Influenza A/WSN/1933 (H1N1) is a neurotropic influenza virus strain purchased from ATCC.

2.2 *In vivo* treatments

Mice were anaesthetized with vaporized isoflurane (2-3% in O_2), and heads were fixed at the stereotaxic device (Stoetling). Virus or viral vectors was administered either in both or in the right striatum. The skull was drilled with a micro drill (Stoelting) to make a small hole (0.2-0.3 mm diameter) at a point 1 mm anterior to and 2 mm left or right from the bregma. 1ul was

injected at a rate of 0.6 ul/min at 2.5 mm depth. Before the surgery, Buprenorphine (0.05 mg/kg) was given as an intraperitoneal (i.p.) injection, and Bupivacaine (10-30 ul of 2.5 mg/ml) was given as a subcutaneous (s.c.) injection at the incision site. Meloxicam (5 mg/kg) s.c. was given right after and within 24 hours of the surgery.

For Interferon-γ ELISpot, histology and scTranscriptomics analysis, a second injection of either VSVgGFP (1:10) or Influenza A/WSN/1933 (H1N1) was performed at the same injection site after 4-7 days of the first injection. For intravital imaging, the second injection of VSVgGFP was performed 1mm posterior to the first injection point. 1ul of 1:10 diluted VSVgGFP was injected with pulled glass capillary sub-dura.

2.3 B cell isolation from tissue

Mice were perfused with phosphate-buffered saline (PBS) followed by MEM-Hepes with Liberase 0.399 units/ml (Roche) and DNase I 0.2 mg/ml (Applichem) (referred to as liberase buffer). Brain, cervical lymph node (CLN), inguinal lymph node (ILN) and spleen (Spl) were harvested. Brain tissue was minced and digested with 3 ml of liberase buffer for 30 minutes in a 37°C water bath. PBS containing 2% fetal calf serum and 2 mM EDTA was used for washing and further processing. Digested tissue was mashed through a 70 um cell strainer (Falcon) then CD45-positive cells were isolated with CD45 microbeads (Miltenyi Biotec). Cells from the CLN, ILN and Spl were isolated by crushing the tissue with a piston and collecting supernatants without centrifugation. The cells in the supernatants were labelled with antibodies after or without fixation with 4% paraformaldehyde (PFA) and permeabilization with 0.1% saponin for detection using a flow cytometer – BD LSRFortessa^{TM} Cell Analyzer or CytoFLEX Flow Cytometer (Beckman Coulter). For scTranscriptomics and ELISpot analysis, tdTomato-positive cells were sorted using BD FACSAria^{TM} III or BD FACSMelody^{TM}.

2.4 ELISpot analysis

Interferon-γ production from T cells in response to antigen presentation by B cells was investigated by Mouse IFN-γ ELSpot set (BD Biosciences) according to the manufacturer's instructions. 1.6 million 2D2 T cells or 1 million L7 T cells and 4,000 – 8,000 B cells isolated from different mouse organs were seeded and incubated in the capture antibody coated ELISpot plate for five days in a humidified carbon dioxide incubator (5% CO_2 at 37°C). The resulting spots were counted using an ELISpot reader and analyzed using FIJI.

2.5 Histology and Immunofluorescence analysis

Mice were perfused with PBS followed by 4% PFA. The brain was harvested and fixed in 4% PFA overnight, then stored in PBS + 0.01% sodium azide. 100-um-thick floating-sections were prepared at the vibratome (Leica VT 1200S). Sections were incubated in PBS with 5% FCS and0.3% triton-X for 15 minutes, then incubated with primary antibody diluted in blocking solution on a shaker at room temperature overnight. The sections were washed with PBS for 5 minutes, followed by washing with PBS + 0.3% triton for 30 minutes, and incubated with secondary antibody diluted in blocking solution overnight at room temperature. The solution was replaced with 1 ug/ml DAPI in PBS for 5 minutes and washed with PBS + 0.3% triton for 10 minutes. The sections were mounted with Fluoromount G (SouthernBiotech. Images were acquired with Nikon A1R confocal microscope or with a Nikon Ti2 fluorescence microscope.

2.6 Intravital imaging

Intravital imaging of the infection site in the superficial cortex was performed after 2 or 3 days after the second injection. Mice were anaesthetized with vaporized isoflurane (2% in O_2). Skulls were fixed with a custom-made metal bar, and the body temperature was kept with a heating pad during the imaging. Imaging was performed with a Nikon A1R MP microscope.

Z-projection of GFP-expressing-infected cells and tdTomato-expressing B cells were recorded for 0.5-3 hours.

2.7 *In vitro* live cell imaging

TE671 rhabdomyosarcoma cells (ATCC) or mouse primary cortical cells were transduced with an adenoviral vector containing MOG-miRFP670 or infected with VSVgGFP on µ-Slide 8 Well (Ibidi 80826).

tdTomato-expressing B cells were isolated from mice. The immune cells were isolated from the spleen and inguinal lymph node by crushing them. Then the B cells were isolated using Pan B cell Isolation Kit II, mouse (Miltenyi Biotec 130-104-443). The tdTomato-expressing B cells were added to the culture. *In vitro* live cell imaging was acquired in a 5-10% carbon dioxide chamber at 37°C with a Nikon Ti2 fluorescence microscope with a 40x objective for 0.5-2 hours.

2.8 *In vitro* antigen capture assay

TE671 rhabdomyosarcoma cells were stably transfected with constructs expressing mCherry-fused MOG (referred to as TE-ratMOG-mCherry), GFP-fused Hemagglutinin (from influenza A/WSN/1933, referred as TE-WSN-GFP) or GFP-fused glycoprotein (from Vesicular stomatitis virus, referred as TE-VSV-GFP). TE-ratMOG-mCherry was cultured in RPMI medium with 10% heat-inactivated fetal calf serum and 100 units/ml of penicillin and 100 ug/ml of streptomycin (referred to as R-10) and with 2 ul/ml of blasticidin added as a selective antibiotic. TE-VSV-GFP was cultured in R-10 with 20 ul/ml of G418 Sulfate (cat no. 10131-035, Gibco). TE-WSN-GFP was cultured in R-10 with 8 ul/ml of Puromycin.

FluBI B cells and wildtype B cells were labelled with 1:100,000, 1:10,000 and 1:1,000 diluted cell trace violet (CTV), respectively. The B cells were then added to TE-MOG-mCherry and

co-cultured in R-10. The B cells were retrieved after 1 hour then the fluorescence of mCherry and CTV was measured with a CytoFLEX Flow Cytometer (Beckman Coulter).

2.9 scTranscriptomics

VSVΔG and Influenza WSN were injected as described above. On day 7 post-injection or day 21 post-injection, the mice were perfused with PBS followed by liberase buffer. The brain was harvested from perfused mice then CD45-positive cells were isolated from the brain as described above. The CD45-positive cells were labelled with TotalSeq-C anti-mouse Hashtag antibody (1:100, Biolegend 155861, 155863, 155865, 155867, 155869 and 155871). The tdTomato-expressing B cells were then sorted using BD FACSMelody^{TM}. scRNA cDNA library was produced using the Chromium Next GEM Single Cell 5' Library kit v2 (Dual Index) (10x Genomics). Sequencing was performed on each sample with NovaSeq 6000 reagent kit (100 cycles) using NovaSeq using NovaSeq 600 System (Illumina)

2.10 Statistics

Quantitative data were analyzed by one-way or two-way analysis of variance (ANOVA) with appropriate post-hoc tests. Data that deviated from normality according to the Kolmogorov-Smirnoff test were instead analyzed with an appropriate non-parametric test. Tests and resulting p-values are specified in each figure legend.

2.11 Antibodies used for labelling

Antibodies used for the labelling are listed in Table 2-1.

Table 2-1. Antibodies used for the thesis.

Antibody	provider	Catalog number
Biotinylated lycopersicon esculentum lectin	Vector	B-1175
Mouse anti-influenza nucleoprotein	Sino Biological	11675
Rabbit anti-GFAP	Cell Signaling Technology	80788S
Rabbit anti-influenza HA	Sino Biological	11692
Rabbit anti-laminin	Abcam	ab11575
Rabbit anti-NEFL	Thermo Fischer	MA5-14981
Rabbit anti-NeuN	Cell Signaling Technology	12943S
Rat anti-CD3	Invitrogen	14-0032-86
Rat anti-MBP	Sigma-Aldrich	MAB386
AF647 anti-B220	Biolegend	103226
FITC anti-mouse IgMa	Biolegend	408606
PE/Cy7 anti-CD40L	Biolegend	157008
AF488 anti-mouse IgG2b	Jackson Immunoresearch	115-545-207
AF647 Donkey anti-rat IgH (H+L)	Jackson Immunoresearch	712-605-153
AF647 goat anti-rabbit IgG (H+L)	Jackson Immunoresearch	111-605-003
AF647 Strepavidin	Jackson Immunoresearch	016-690-084
FITC goat anti-rat IgG (H+L)	Jackson Immunoresearch	112-095-167
Rhodamine Red X goat anti-mouse IgG (H+L)	Jackson Immunoresearch	115-605-003

Chapter 3. Results

3.1 B cells enter the virus-infected brain and interact with infected cells.

Firstly, induction of B cell infiltration was established using a virus infection. VSVΔG was intracerebrally injected into the striatum of a mouse brain. It is a viral vector expressing GFP in the cytoplasm of the infected cells without glycoprotein expression. As its glycoprotein gene was replaced with GFP, the vector is competent for single-round infection. Several BCR transgenic mouse lines were used, including FluBI (BCR specific for Influenza HA) and V10l (BCR specific for VSV glycoprotein).

After the injection, B cell infiltration and the virus infection in the brain were characterized. VSVΔG infected neurons without infecting other cell types in the brain as shown in figure 3-1A. The injected mouse brains were perfusion-fixed and immunofluorescently labelled for several cell markers – NeuN, GFAP, Lectin, and MBP. The nuclei of the infected cells expressed NeuN. Yet, other cell markers did not co-localize with the GFP-positive infected cells.

The B cell infiltration was then analyzed with perfusion-fixed FluBI mouse brains on day 7 post-injection of VSV Δ G. The mouse brain sections were labelled with laminin for visualization of blood vessels to examine the B cell localization. As is shown in figure 3-1B, there were many FluBI B cells accumulated in the perivascular spaces, with some of them infiltrated in the parenchyma.

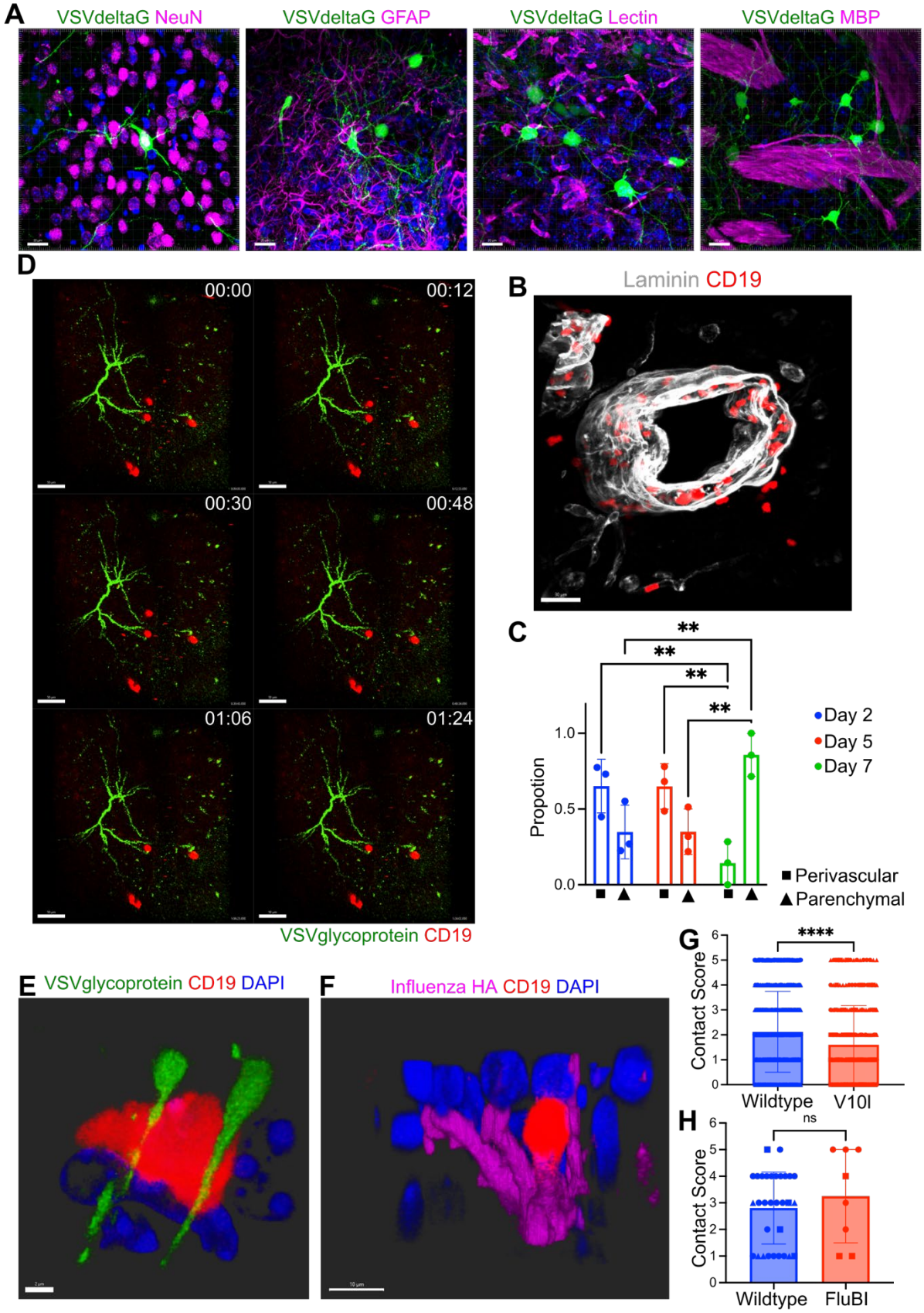

Figure 3-1. Anti-viral B cells infiltrate virus-infected mouse brains independent of antigen

specificity. (A) VSVΔG infected mouse brain sections on day 2 post-injection labelled with NeuN, glial fibrillary acidic protein (GFAP), Lectin, and myelin basic protein (MBP). (B) FluBI tdTomato mouse brain labelled with anti-laminin on day 7 after VSVΔG infection. (C) Quantification of perivascular and parenchymal FluBI tdTomato B cells on day 2, day 5 and day 7 post-injection. (**P<0.01 by 2way ANOVA followed by Sidak's multiple comparisons test) (D) V10l tdTomato B cells interacting with a neuron expressing GFP-tagged VSV glycoprotein. (E) A representative image of interactions between V10l B cells and VSV-glycoprotein-expressing neurons. (F) A representative image of interactions between FluBI B cells and influenza-infected cells labelled with anti-influenza HA. (G) Contact score analyzed for interactions between V10l B cells and VSV-glycoprotein-expressing cells. (****P<0.0001 by T-test) (H) Contact score analyzed for interactions between FluBI B cells and influenza-infected cells.

The kinetics of B cell infiltration was further analyzed with a time course. FluBI B cells started to accumulate in perivascular spaces as early as day 2 post-injection of VSVΔG. On day 5, a lot of the B cells were still localized in perivascular spaces. Most of the B cells were then found in parenchyma on day 7, indicating the majority of FluBI B cells started to infiltrate VSVΔG-injected parenchyma after day 5 (Figure 3-1C). Of note, we demonstrated that the infiltration of FluBI B cells was independent of their antigen specificity since these B cells recognize influenza hemagglutinin and the infection was done with VSV. These data indicate that B cells accumulate in the perivascular spaces quickly after the infection, at least after 2 days, but generally enter the parenchyma after a longer time, appr. 5 days after the injection.

Before analysis of autoimmune B cells, we analyzed the interaction of anti-viral B cells with their cognate viral antigen. V10l transgenic mouse brain was injected first with VSVΔG in the striatum to attract B cells into the CNS, followed by injection of VSVgGFP at the cortex providing the antigen for V10l B cells. VSVgGFP infected neurons were observed with many B cells in an adjacent blood vessel moving with the blood flow. Few of the B cells were extravasated and were found in the parenchyma closed to GFP-tagged VSV glycoprotein-

expressing neurons (Figure 3-1D). We observed a stable and intimate interaction for more than 1.5 hours between the B cells and their antigen with intravital imaging of the infected cortex.

The interaction between the anti-viral B cells and their cognate antigens in the striatum was further investigated using perfusion-fixed mouse brains. Intracerebral injection of VSVΔG was performed in FluBI mice and V10l mice to induce infiltration of the anti-viral B cells. Then, an additional intracerebral injection of either influenza virus or VSVgGFP – viral vector expressing GFP-tagged VSV glycoprotein – was performed to provide the cognate antigen to the corresponding anti-viral B cells. Both of the anti-viral B cells entered the brain parenchyma and interacted with virus-infected cells (Figures 3-1E and F). The interactions were blindly analyzed with the score scale used in figure 3-3. At the analyzed timepoint, the contacts between the B cells and the cognate-antigen expressing cells in the transgenic mouse brains were not significantly different compared to the contacts in wildtype (WT) mouse brains (Figure 3-1G and H).

3.2 B cells capture antigen directly from antigen-expressing cells in vitro

We hypothesized that we had failed to detect antigen capture by histology because we had missed the relevant time point. To better characterize this parameter, we investigated the ability of B cells to capture antigen *in vitro*. Many studies demonstrated the antigen capture by B cells in the lymphoid tissues. Yet, the antigen capture by B cells outside the lymphoid tissues is not well understood. We first investigated whether B cells can capture their antigen directly from antigen-expressing cells, not from antigen-displaying cells.

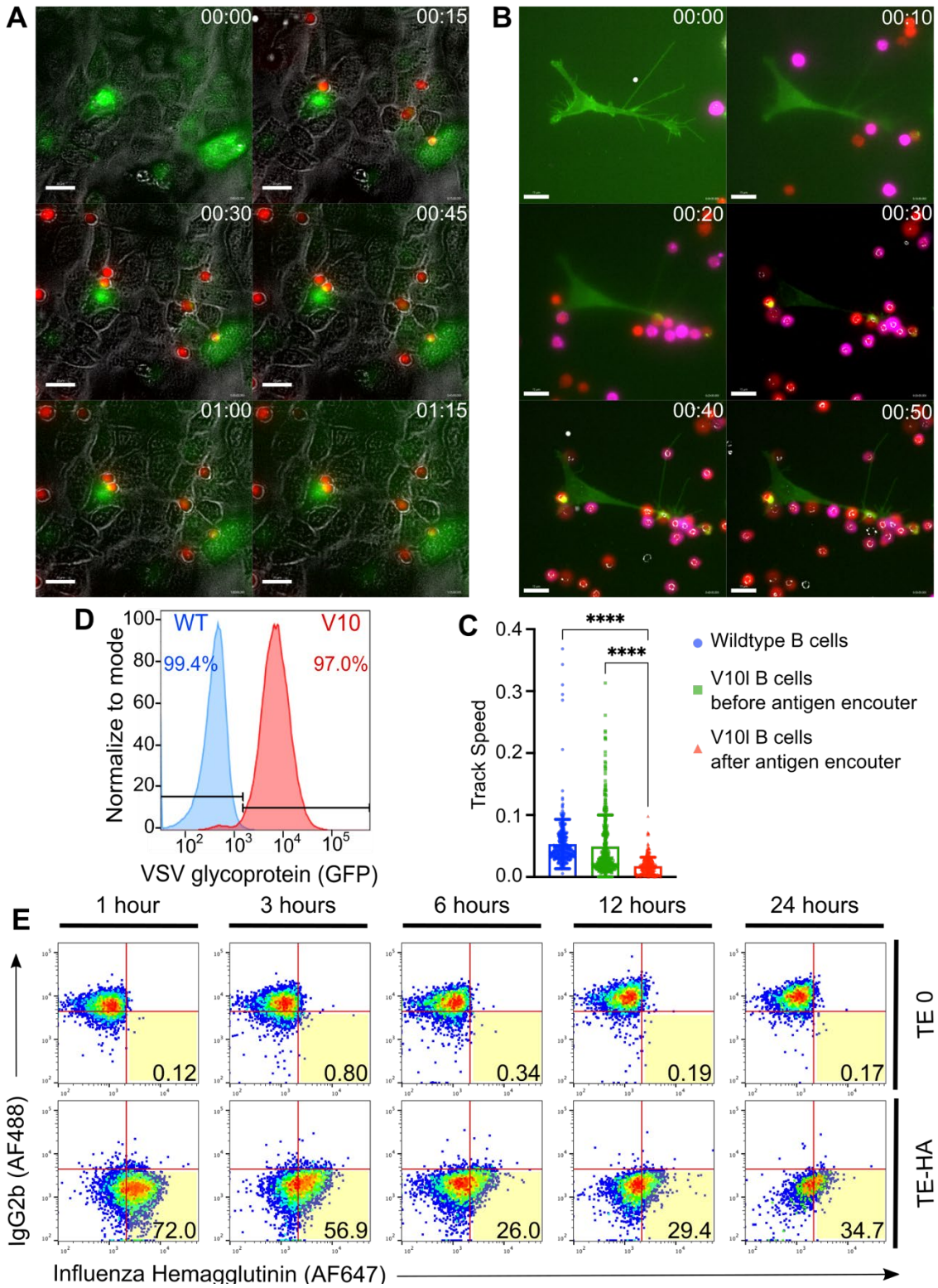

Figure 3-2. B cells capture their cognate antigen directly from antigen-expressing cells *in vitro*. (A) *in vitro* live cell imaging was recorded for 1 hour and 15 minutes while IgH MOG B cells captured MOG proteins expressed on the surface of TE cells. scale bar = 20 um. (B) *in vitro* live cell imaging was recorded for 50 minutes while V10l B cells captured antigens from mouse cortical cultures infected with VSVgGFP. (C) Track speed of V10l B cells and controls before

and after antigen encounter during *in vitro* live cell imaging. Blue represents the speed of wildtype (WT) B cells; Green represents the speed of V10l B cells before the antigen acquisition; Red represents the speed of V10l B cells after antigen encounter. (****$p<0.0001$ by Kruskal-Wallis test followed by Dunn's multiple comparison) (D) Splenic V10l B cells were co-cultured with GFP-tagged VSV glycoprotein-expressing TE cells for 1 hour. Flow cytometry revealed the fraction of GFP-positive B cells, which captured the antigen. (E) FluBI B cells were co-cultured with influenza hemagglutinin HA expressing TE cells or with TE 0 cells as a control for 1, 3, 6, 12, and 24 hours. B cells were then labelled for influenza HA and IgG2b

To examine the antigen capture by B cells, we used B cells isolated from several transgenic mouse lines with BCR specific to viral or self-antigens, including FluBI, V10l and IgH MOG. *In vitro* live cell imaging of IgH MOG B cells co-cultured with RFP670-tagged MOG expressing TE cells (Figure 3-2A) demonstrated that the IgH MOG B cells can capture MOG as soon as they encounter them. In addition, V10l B cells showed antigen capture from virus-infected cortical cells in a similar way (Figure 3-2B).

These data indicated that both autoimmune B cells and anti-viral B cells could capture their antigen directly from antigen-expressing cells as soon as they encounter them. The B cells moved around and briefly interacted with surrounding cells when they were added to the culture. Once they encountered their antigen, B cells strongly and stably interacted with antigen-expressing cells for around an hour and concentrated the fluorescently tagged antigens at the point of contact.

When the track speed of B cells before and after the antigen encounter was quantified, it was significantly decreased after the specific antigen capture (Figure 3-2C), validating the stable interaction by B cells with the antigen-expressing cells. After the co-culture of GFP-tagged VSV glycoprotein expressing TE cells and V10l B cells, GFP expression was observed in V10l

B cells, confirming the capture of their cognate antigen, VSV glycoprotein, from the antigen-expressing cells (Figure 3-2D).

We additionally performed a time course experiment to confirm the antigen capture and further understand the kinetics. FluBI B cells were co-cultured with TE cells with or without influenza HA expression (Figure 3-2E) for 1, 3, 6, 12 and 24 hours. The FluBI B cells started to capture influenza HA at the earliest time point, after 1 hour of co-culturing. The immunolabelling of influenza HA decreases over time since the B cells start to degrade the antigen after the acquisition and then the antigen cannot be recognized by the antibody anymore. Nevertheless, we could still detect influenza HA even at 24 hours of co-culturing. The surface BCR of FluBI B cells was also stably reduced from the earliest time point, which again demonstrates that the B cells captured the antigen as soon as they encountered their antigen.

3.3 B cells enter influenza-infected lung and capture viral antigens.

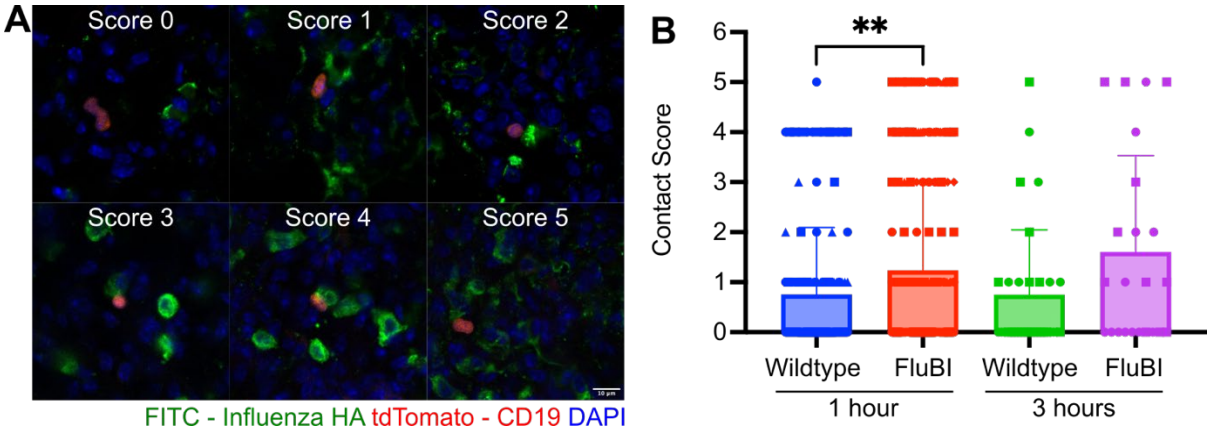

Figure 3-3. Anti-viral B cells infiltrate infected tissue and locally interact with antigen-expressing cells in *vivo*. (A) Representative images of contact scores were used to analyze the intimacy of interactions between B cells and influenza HA-expressing cells. Scale bar = 10 um. (B) Quantification for the intimacy of interactions between B cells and influenza HA expressing cells at 1 hour or 3 hours after the B cell transfer. (**P <0.001 by t-test)

After the *in vitro* analysis of antigen capture by B cells from antigen-expressing cells, Influenza-infected lung was investigated for antigen capture by anti-viral B cells *in vivo*. Infections were performed three days before the transfer of either FluBI B cells or WT B cells. As the duration of antigen capture was shown to be relatively short – around an hour – in the skin of the ear (data not shown), tdTomato-expressing B cells were isolated from FluBI or WT mice and transferred at 1 hour or 3 hours before the sacrifice to detect the antigen capture during the short time window. The intimacy of contact between B cells and influenza HA expressing cells was blindly analyzed and scored from 0 – no contact and far away – to 5 – very intimate contact suggesting the antigen capture (Figure 3-3A). Figure 3-3B demonstrated that the FluBI B cells had stronger interaction with the infected cells compared to the WT B cells. In addition, the FluBI B cells transferred 1 hour before the detection showed significantly more intimate contacts compared to the WT B cells transferred 1 hour before. The results suggested that the anti-viral B cells can enter the infected lung tissue and search for the cognate antigen. Moreover, the interaction between B cells and the antigen usually happens within an hour of B cell infiltration in the tissue.

In figure 3-1, we showed that the anti-viral B cells infiltrated and had intimate interactions with the antigen-expressing cells. The interaction between BCR transgenic anti-viral B cells and antigen-expressing cells was not significantly different compared to the interaction between WT B cells and antigen-expressing cells. The insignificant interaction can be explained as most B cells capture their antigen within the first hour of infiltration. The kinetics of B cell infiltration in the brain cannot be anticipated since the time frame is relatively wide from day 2 – day 5. Distinguishing freshly-entered B cells is also very difficult.

3.4 IgH MOG B cells infiltrate the virus-infected brain and capture MOG.

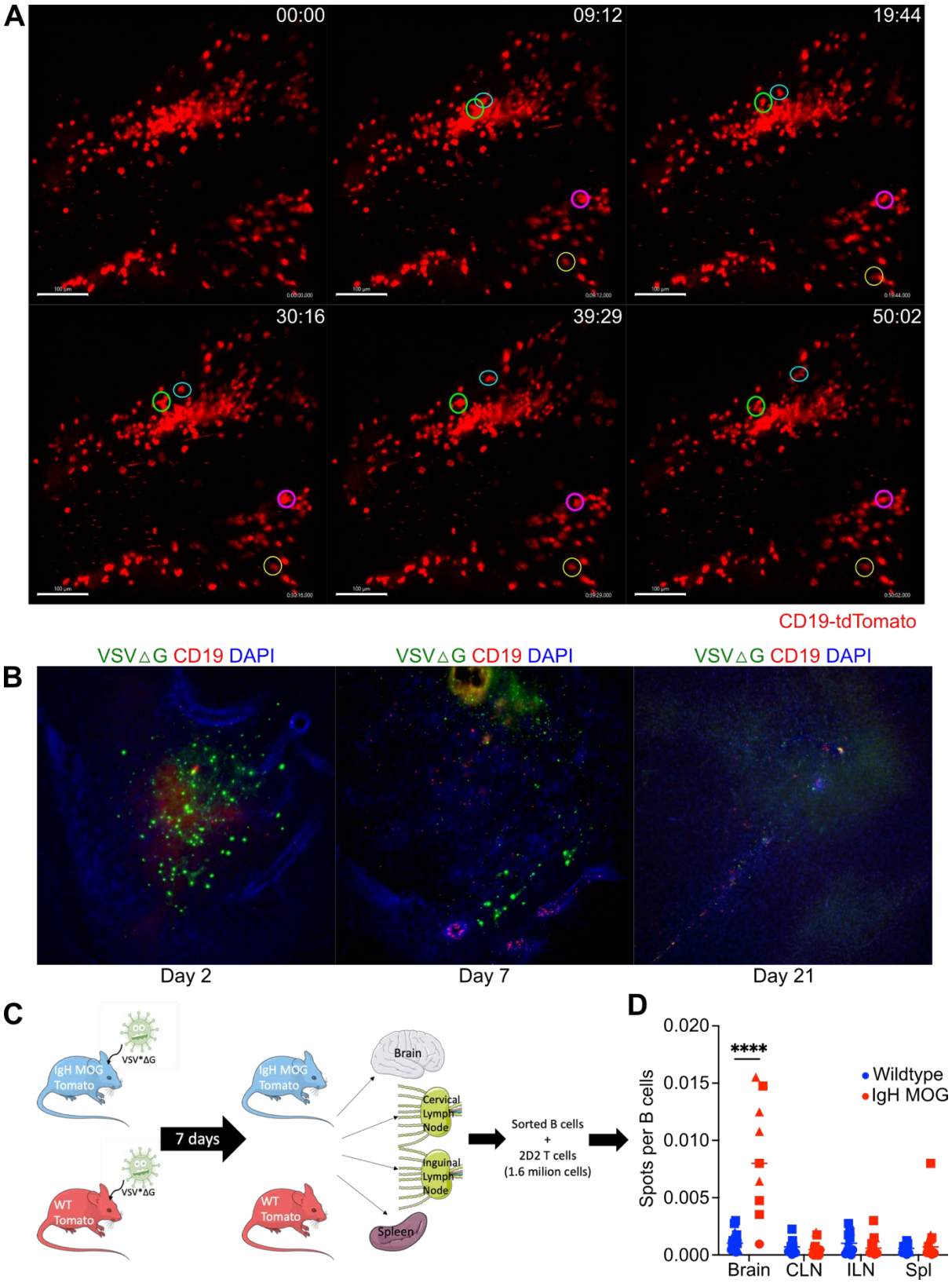

Figure 3-4. IgH MOG B cells infiltrated the brain upon virus infection. (A) Intravital imaging showing B cells in the vessel, perivascular space and parenchyma of VSVΔG infected IgH

MOG mouse brain on day 7 post-injection. (B) Representative image of B cells infiltration in IgH MOG brain on day 2, day 7 and day 21 post-injection. (C) Experiment scheme for ELISpot. IgH MOG and WT mice were injected with VSVΔG. B cells were sorted from different tissues – brain, cervical lymph node (CLN), inguinal lymph node (ILN), spleen (Spl) –on day 7 post-injection and incubated with 2D2 T cells to detect interferon-gamma production. (D) Quantification of Interferon-gamma spots per well (****P<0.0001 by 2way ANOVA followed by Sidak's multiple comparisons test)

With a better understanding of B cell infiltration in the brain and of antigen capture in vitro and in the lung, we finally examined infiltration and antigen capture by autoimmune B cells. Infiltrated IgH MOG B cells were observed at the cortex of VSVΔG injected brain on day 7 post-injection shown in Figure 3-4A. As with the previous intravital imaging of the infected brain, the blood vessel was full of B cells moving with the blood flow. After transmigration, many of the infiltrated B cells were found near the vessel suggesting the accumulation in the perivascular spaces. There, the B cells were still mobile and moved around in the perivascular space (Figure 3-4A). Few of the IgH MOG B cells were observed as early as day 2 post-injection with numerous VSVΔG infected neurons (Figure 3-4B, left). On day 7 post-injection, many more B cells infiltrated the brain parenchyma and also accumulated in the perivascular spaces, similar to the anti-viral B cells (Figure 3-4B, center). GFP signal disappeared at a later time point, day 21 post-injection (Figure 3-4B, right), possibly due to the death of infected cells as the NeuN labelling disappeared from the GFP-positive VSV infected neurons on day 7 post-injection. The number of B cells also decreased possibly due to cell death or egress of the tissue (Figure 3-4B, right).

As it is hard to compare the interaction of transgenic and of non-transgenic B cells with antigen-expressing cells using microscopic analysis, we decided to use a different technique to validate the local antigen capture by B cells in the brain – using MOG recognizing 2D2 T cells as a reporter. To investigate the cognate antigen capture by IgH MOG B cells, the B cells were

isolated from the brain and control tissues – cervical lymph node as draining lymph node, inguinal lymph node as non-draining lymph node, spleen for antigens from blood – 7 days after VSVΔG injection in the striatum of IgH MOG mice and WT mice (Figure 3-4C). The B cells were then incubated with 2D2 T cells, which produce interferon-gamma when the B cells present their cognate antigen in an ELISpot well.

The B cells isolated from IgH MOG mouse brains demonstrated significantly more interferon-gamma production by the 2D2 T cells compared to B cells isolated from WT mouse brains (Figure 3-4D). Interestingly, B cells isolated from any other tissues, including the draining CLN, of IgH MOG mice or WT mice did not induce any significant interferon gamma production. The result indicates that the IgH MOG B cells captured their cognate antigen, MOG, locally in the brain and additionally that the antigen-captured IgH MOG B cells did not migrate to CLN at that timepoint.

3.5 IgH MOG B cells die after antigen capture in the brain

After the investigation of antigen capture by IgH MOG B cells in the brain, we used flow cytometric analysis to characterize the brain-infiltrating B cells at a later time point. The overall number of B cells isolated from IgH MOG brain was decreased compared to the WT brain (data not shown). As IgH MOG transgenic B cells express IgMa as their BCR, we examined the IgMa-positive B cells isolated from the VSVΔG-injected brain on day 7 and on day 21 post-injection. The frequency of IgMa positive B cells isolated from the IgH MOG brain was significantly lower than from other tissues (Figure 3-5A).

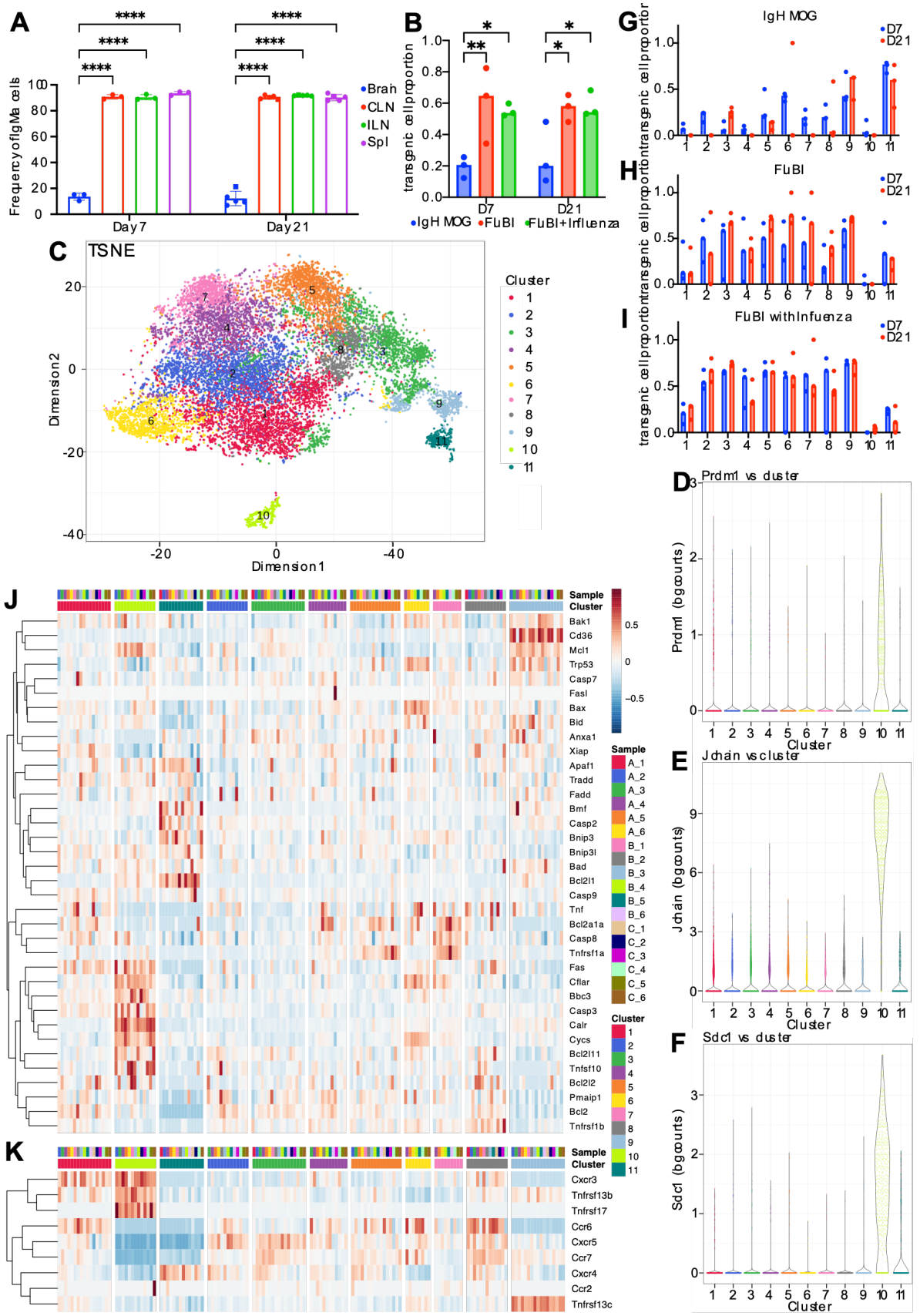

Figure 3-5. B cells disappear in the brain after antigen capture. (A) Flow cytometric analysis showing the frequency of IgMa positive B cells in IgH MOG mouse tissues (****p<0.0001 by

2way ANOVA followed by Sidak's multiple comparisons test). (B) The proportion of transgenic B cells of each sample from the scRNA-seq dataset (**p<0.01 and *p<0.1 by 2way ANOVA followed by Sidak's multiple comparisons test). (C) Two-dimensional TSNE projection of scRNA-seq data from all of the B cells isolated from VSVΔG-injected mouse brains of IgH MOG, FluBI and FluBI, with additional influenza injection, on day 7 and day 21 post-injection. (D, E, F) Expression of *Prdm1*, *Sdc1* and *Jchain* across cell clusters. (G, H, I) Transgenic B cell proportion of each cluster and sample (not significant by 2way ANOVA followed by Sidak's multiple comparisons test). (J, K) Average of the log-transformed and normalized expression levels across cells from each cluster and sample. The rows were centered and scaled to show the relative changes in expression levels. The expression of apoptotic markers is shown in (J), and chemokine receptors and receptors for survival signal expression are shown in (K).

There are several possible explanations for the decreased frequency of IgMa-positive B cells in the IgH MOG brain. It can be that the MOG-recognizing B cells did not enter the brain; antigen-capturing IgH MOG B cells drained to the CLN; IgH MOG B cells internalized their BCRs after the antigen capture or antigen-captured IgH MOG B cells died from AICD. We demonstrated that the first two hypotheses are not valid in the results above (results 3.1 and 3.4). In order to address the remaining hypotheses and further understand the fate of the B cells after the antigen capture, we performed a scTranscriptomics analysis.

One group of the IgH MOG transgenic mice and two groups of the FluBI transgenic mice were intracerebrally injected with VSVΔG in the striatum of the brain. Then one group of the FluBI mice was additionally injected with the Influenza virus at the same location in the brain. On the 7 or 21 days after the VSVΔG injection, B cells were isolated to perform the scTranscriptomics analysis.

Firstly, we could confirm that proportion of transgenic B cells with MOG-specific heavy chain was decreased compared to the FluBI transgenic B cells on both day 7 and day 21 (Figure 3-

5B). The data indicate that the decrease of IgMa positive B cells in the previous flow cytometric analysis was not due to the internalization of BCR but rather the AICD of IgH MOG B cells.

11 clusters of the remaining brain-infiltrating B cells were identified in the two-dimensional TSNE projection (Figure 3-5C). Among those, cluster 10 had a distinct gene expression including several plasma cell markers, like *Prdm1, Jchain* and *Sdc1*(Figures 3-5D, E and F). This cluster 10 was observed in all conditions at shorter and longer time points, including the FluBI mouse group with or without influenza virus injection. Cluster 10 may be either a plasma cell population developed outside of the CNS or developed against a viral antigen before day 7 post-injection.

The remaining brain-infiltrating B cells with MOG-specific heavy chains were found across all clusters but were most abundant in clusters 9 and 11, especially at the later timepoint (Figure 3-5G). In contrast, the FluBI transgenic B cells with or without influenza infection were more evenly distributed (Figures 3-5H and I). We examined the expressions of various apoptotic markers and chemokine receptors to further understand the subsets.

Cells in cluster 9 have higher expression of major apoptotic players, such as *Bak1* and *Trp53* and a high expression of *Tnfrsf13c* which encodes BAFF receptor, the receptor for B-cell survival signal BAFF (Figure 3-5J). We, therefore, concluded that cluster 9 most likely contains dying cells in the brain.

Cluster 11 cells have more expression of BH3-only proteins, such *as Bmf, Bnip3, Bnip3l* and *Bad* (Figure 3-5K). The role of BH3-only proteins is diverse. Some proteins engage with the anti-apoptotic BCL2-family protein [74]. In contrast, some BH3-only proteins regulate the pro-apoptotic process [74]. CXCR4 is highly expressed by the cells in cluster 11. In the lymphoid tissues, antigen-acquired and activated B cells up-regulate CXCR4 expression to migrate to the

dark zone of the GC [72]. Cluster 11 could therefore include cells that acquired their antigen, were activated and were looking for a survival signal.

These results showed that our initial hypothesis – after the local antigen capture in the brain, B cells will become plasma cells and cause demyelination – was not valid. Instead, after capturing the antigen in the brain locally, most B cells die due to AICD in the brain in absence of additional survival signals, including T cell help or CD40-CD40L interaction.

3.6 IgH MOG B cells cause demyelination in the brain upon CD40-CD40L interaction.

AICD of antigen-acquired autoimmune B cells in the brain is an important immune checkpoint to protect the brain from autoimmune responses. To further address the B cell pathogenesis of MS, we tested whether CD40-CD40L interaction is sufficient for IgH MOG B cells to cause an autoimmune response.

We first examined the CD40-CD40L interaction *in vitro*. When none of the survival signals was provided to the antigen-captured B cells, the B cells die due to AICD and did not expand. The antigen-capturing B cells survive and expand very well when 2D2 T cells were provided. In the presence of CD40L signals, B cells could survive and expand much better compared to cells without any survival signal (Figure 3-6A).

After investigating the effect of CD40-CD40L interaction on B cells *in vitro*, scTranscriptomics was performed after recapitulating the CD40L paradigm in an in vivo setting. WT and IgH MOG mice were injected with VSVΔG and AVV-vPig-CD40L. As a negative control, one group of IgH MOG mice was injected with VSVΔG injection only. B cells were isolated from the brains and ILNs of the mice on day 28 post-injection.

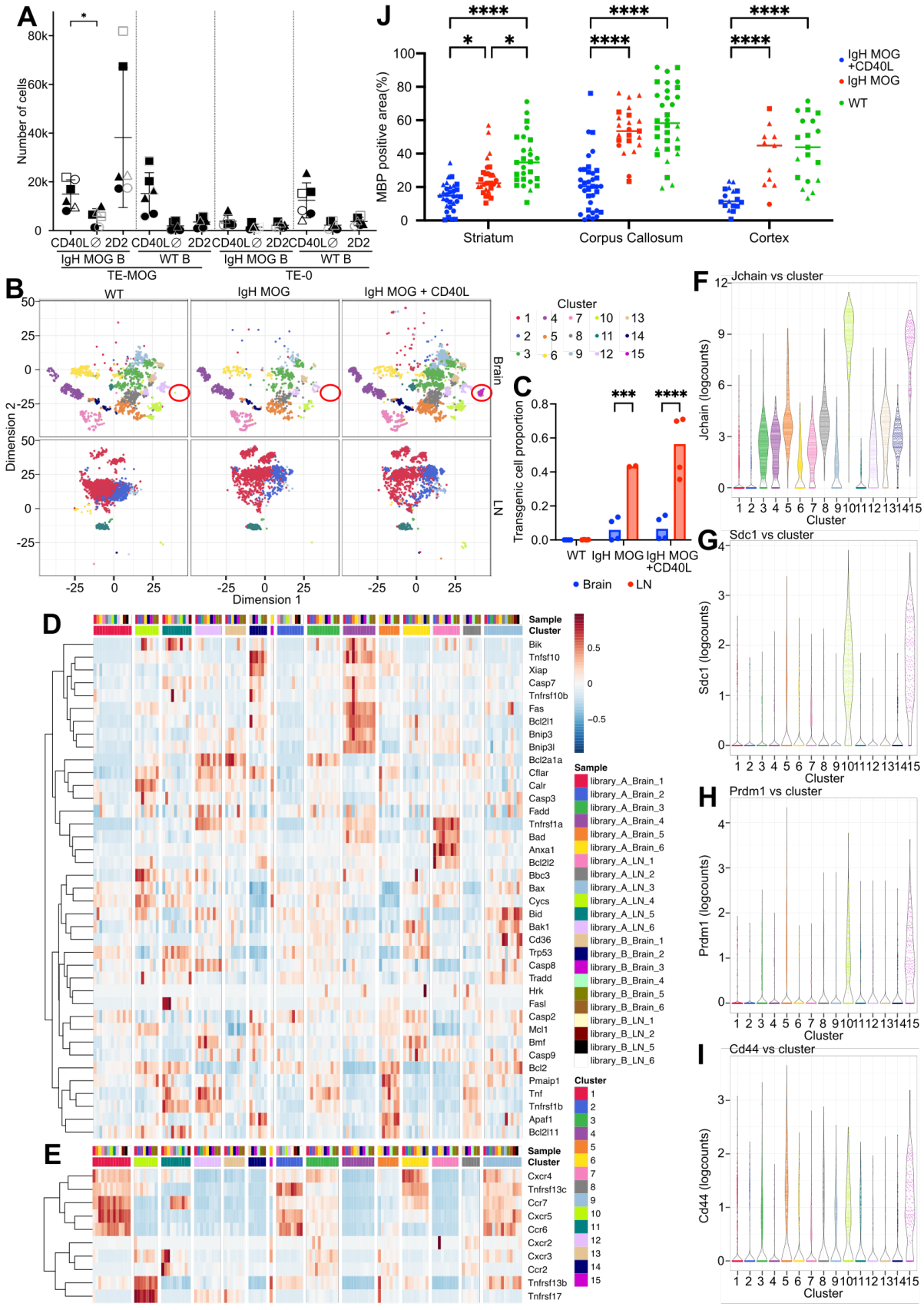

Figure 3-6. CD40-CD40L interaction allows IgH MOG B cells to cause demyelination in the brain. (A) IgH MOG B cells were co-cultured with MOG-expressing TE cells for 72 hours with

CD40L signal or 2D2 T cells or without any additional signal *in vitro* (*P<0.1 by Repeated measure ANOVA followed by Sidak's multiple comparisons test). (B) Two-dimensional TSNE projection of scRNA-seq data from B cells isolated from mouse brains of WT and IgH MOG on day 28 after VSVΔG and AVV-vPig-CD40L injection. A group of IgH MOG mice were only injected with VSVΔG without CD40L. (C) The proportion of transgenic B cells of each sample from the scRNA-seq dataset (****P<0.0001 and ***p<0.001 by 2way ANOVA followed by Sidak's multiple comparisons test). (D, E) Average of the log-transformed and normalized expression levels across cells from each cluster and sample. The rows were centered and scaled to show the relative changes in expression levels. The expression of apoptotic markers is shown in (D), and chemokine receptors and receptors for survival signal expression are shown in (E). (F, G, H, I) Expression of *Jchain*, *Sdc1*, *Prdm1* and *Cd44* across cell clusters. (J) VSVΔG-injected mouse brains of WT, IgH MOG and IgH MOG, with additional CD40L injection, were labelled with MBP and the percentage area above a threshold is compared. The comparison was performed within similar brain areas (*P<0.1 and ****p<0.0001 by 2way ANOVA followed by Tukey's multiple comparisons test).

Two dimensional TSNE projection of scTranscriptomics data shows 15 clusters of B cells isolated from the brains and from the ILNs (Figure 3-6B). By faceting the data, we identified a unique population (cluster 15) developed in the IgH MOG brain when CD40L was provided (Figure 3-6B, marked with a red circle). However, the MOG-specific heavy chain cell proportion in the brain did not differ with or without CD40L signal and is still significantly lower than the proportion in the LN (Figure 3-6C).

Cluster 10 is composed of plasma cells that developed before the manipulation as the cluster; or developed against other viral antigens, such as VSVΔG, which appears in all conditions in the brain and in the ILN (Figure 3-6B). Interestingly, cluster 15 – the unique population in CD40L provided IgH MOG brain – and cluster 10 have a very similar expression pattern of apoptotic markers, chemokine receptors and receptors for survival signal (Figure 3-6D and E). Furthermore, both clusters 10 and 15 highly express several plasma cell markers – *Jchain*, *Sdc1*, and *Prdm1* (Figure 3-6F, G and H). Expression of *Cd44* is differential in both clusters with

higher expression in cluster 15 (Figure 3-6I). CD44 expression varies during the B cell differentiation. The resting IgD+/IgM+ B cells have high levels of CD44 which decreases when they are activated and become GC B cells [75]. The CD38+ centroblasts have also a low expression [75]. Then CD44 is re-upregulated at the post-germinal center stage and plasmablasts express high levels of CD44 [75]. As the cells in cluster 15 have high levels of *Sdc1* and other plasma cell markers, these cells are possibly the newly developed plasmablast-like cells in the brain.

In addition, we examined the histology of the brain with the same manipulation of VSVΔG and AVV-vPig-CD40L injection in the brain of WT and IgH MOG mice on day 28 post-injection. IgH MOG brains provided with CD40L have a significantly lower MBP-positive area in the striatum, corpus callosum and cortex compared to IgH MOG brain without CD40L or WT brain with CD40L (Figure 3-6J).

Chapter 4. Discussion

B cells capture their cognate antigen from antigen-expressing cells in the CNS

Firstly, the induction of B cell infiltration in the brain was established, and both anti-viral and autoimmune B cell entry into virus-infected lungs and brain was demonstrated independent of their antigen specificity. In addition, we validated that B cells can capture antigens directly from antigen-expressing cells *in vitro*. Finally, the B cells have intimate contact with infected cells in non-lymphoid tissues and we demonstrated that the autoimmune B cells captured their cognate antigen locally in the brain.

In the past, the paradigm has been well established that B cells obtain their antigen exclusively in the secondary lymphoid tissue. Our demonstration that antigen capture also occurs in non-lymphoid tissues requires a re-examination of the function and consequences of antigen acquisition by B cells.

Antigen acquisition and presentation by B cells have some unique features in comparison with other antigen-presenting cells. Macrophages and dendritic cells take up antigens in a non-specific way and act as APCs. B cells do not take up antigens non-specifically and only efficiently capture and present their cognate antigens. Although monovalent antigens can also initiate B cells responses, BCR activation is usually triggered by multivalent antigens. DCs digest the internalized antigen slowly compared to the macrophages and present relatively intact antigens to B cells. Yet, the most intact antigen can be acquired by capturing directly from antigen-expressing cells. In addition, antigen density might be higher in antigen-expressing cells compared to presentation by DCs.

It was also shown that the level of T cell activation by B cells differs greatly depending on whether the B cell captured a membrane-bound antigen or a soluble antigen from solution. [76].

B cells can acquire the most intact antigens from antigen-expressing cells directly, which, in the end, results in better T cell response and better antibody production.

The antigen acquisition by the B cells has been mainly studied in the lymphoid tissues. B cells are either presented with processed antigens by other APCs or captured small soluble antigens in the fluid. Our results showed that the B cells can capture their cognate antigen directly from antigen-expressing cells other than antigen-displaying cells *in vitro* and *in vivo*.

An interesting follow-up investigation would be to examine the events following B cell antigen capture in the non-lymphoid tissues, particularly in the CNS.

We have three possible hypotheses regarding the fate of B cells after antigen acquisition. After the antigen capture by B cells in the CNS, (i) lymphatics may drain B cells to lymphoid tissues, (ii) B cells may remain in the tissue and form tertiary lymphoid structures (TLSs), or (iii) B cells may undergo AICD.

B cells may leave the CNS after antigen capture.

How freely B cells can traffic across the tissue barriers of the CNS is controversial. One study shows the possibility of B cell migration from CSF to the draining lymph node. When the B cells were transferred into the CSF of another mouse by intracisternal magna injection, the donor B cells were observed in the dura lymphatic vessels and also accumulated in the CLN of the recipient mouse 24 hours after the transfer [77]. Paired tissues and high-throughput sequencing of B cells isolated from multiple sclerosis patients suggest that the B cells can freely traffic between the CNS and secondary lymphoid tissues, and the clonal expansion may occur in multiple compartments [78]. Another study claims that there is no direct evidence showing the trafficking of immune cells from CNS parenchyma to the periphery [79].

For virus-infected brains, it was shown that GC formation in the CLN is initiated earlier than the appearance of virus-specific antibody-secreting cells in the brain, suggesting that B cells obtain antigen, or at least T cell help in the periphery and then traffic into the CNS. During mouse hepatitis virus (MHV) infection in the CNS, somatic hypermutation and GC reaction was followed by the expression of Aicda promoter – promoter for activation-induced cytidine deaminase – and by GL7 expression. GCs are first formed in the CLN at day 7 post-infection and increased until day 21 [80, 81]. The B cells, which have undergone somatic hypermutation, enter and accumulate in the CNS after the GC formation in the periphery [80, 81].

We did not observe antigen-captured IgH MOG B cells in CLN on day 7 after the virus injection. There was no significant activation of 2D2 T cells from IgH MOG B cells isolated from CLN despite sufficient time for the B cells to capture their antigen and migrate into CLN as the majority of B cells enter the brain at day 5 post-injection. The B cell migration back to lymphoid tissues may also depend on the infected tissue. In other studies by our lab, we could observe that the B cells approach and contact the infected cell and leave within 1 hour at the skin of the ear (data not shown). In the lung, intimate contact and capture happen within the first hour of transfer. However, B cells in the brain stay for 3 hours and longer near the infected neurons. It may be more difficult for B cells to move in and out of the the CNS due to the dense network of cells in the CNS or due to a lack of appropriate cytokine or chemokine signals. In particular, the absence of lymph vessels in the CNS parenchyma limits lymphocyte egress.

B cells may form TLSs in infected CNS.

TLSs were observed in many immune contexts. Inducible bronchus-associated lymphoid tissue (iBALT) is formed in the lung after influenza infections and acts as an immunological hub and promotes rapid and localized immune responses [82]. Infection with *mycobacterium*

tuberculosis also resulted in the formation of the iBALT [83]. Moreover, the presence of TLS within the tumor microenvironment predicts better patient outcomes [84, 85].

However, the TLS in the context MS is speculated to support or even enhance intrathecal inflammation. Recent MRI studies showed the prevalence of cortical lesions at different stages of various MS subtypes [86]. Several studies suggested a correlation between meningeal inflammation and these cortical lesions. Patients with follicle-like structures had a more severe disease course with earlier disease onset and younger age at irreversible disabilities and earlier death [87, 88]. Another study, however, did not find any relationship between meningeal inflammation and cortical lesions in chronic MS [89].

Several EAE models have been described with TLSs. TLSs were described in B cell-dependent chronic EAE resulting from immunization with an MBP-Proteolipid protein fusion protein (MP4) [90]. 2D2 IgH MOG mice – transgenic mice with MOG-specific TCR and MOG-specific BCR – spontaneously develop EAE and were described to fosterTLS-like structures in the CNS [91].

Our results did not support this hypothesis as TLS or TLS-like structures were not found in the brain. We have looked at the infected brains at day 2, 7, 21, 28, 49, and 63 post-injection (data not shown) and never found any similar structures in either IgH MOG or controls brins, which leads to our last hypothesis, AICD.

B cells may undergo AICD

In general, BCR engagement results in the proliferation of B cells. BCR cross-linking activates Src family kinases, which leads to activation of PLCγ, Ras, and phosphatidylinositol-3 kinase (PI3K) [92]. These signals generally initiate DNA synthesis or mRNA translation leading to the proliferation of B cells [92].

However, in the absence of survival signals, such as CD40-CD40L interaction, BCR engagement can lead to AICD. When mature B cells have BCR cross-linking, there is an up-regulation of c-Myc, leading the B cells to enter a cell cycle [93]. However, hypercross-linking of surface IgM or IgD receptors led to apoptosis of the B cells characterized by DNA fragmentation [94]. IL-4 and stimulation of CD40 could reverse the induction of apoptosis in the B cells [94]. The data suggest that the BCR-induced apoptosis of B cells may be due to the overexpression of c-Myc in the absence of T cell help [92].

CD40-CD40L stimulation has profound effects on various cells, including B cells. The stimulation of B cells promotes GC formation, immunoglobulin class-switch, somatic hypermutation and eventually, formation of plasma cells and memory B cells [95]. Moreover, CD40-CD40L stimulation is an essential survival signal for GC B cells, activated B cells and plasmablasts [96, 97]. It was also shown in an EAE model that expansion of the B cell population in the CNS was associated with T follicular helper cells [98].

We demonstrated that the B cells undergo AICD after antigen capture in the brain, which may be due to the lack of survival signals after the BCR engagement. To test this hypothesis, we provided the CD40L signal in the brain. Upon CD40-CD40L stimulation, IgH MOG B cells could survive and expand after the antigen capture as shown with scTranscriptomics analysis. Finally, demyelination was observed in brains of IgH MOG B cell mice that expressed CD40L in the brain.

Conclusion and perspectives

We have demonstrated that when naïve B cells, with BCR recognizing CNS-restricted autoantigen, enter the brain, they can encounter and capture their cognate antigen locally in the CNS. Our results indicate that antigen capture by B cells can happen in non-lymphoid tissues. As autoimmune B cells are part of the human B cell repertoire, autoimmune B cells may contribute to the pathogenesis of MS by capturing their antigen in the brain. Yet, an important mechanism protects the brain from the autoimmune response, which is the lack of CD40-CD40L interaction. Without the CD40-CD40L interaction, activated B cells do not proceed to the next activation step and eventually undergo AICD. By providing CD40L signal in the brain, the IgH MOG B cells could expand and cause demyelination after capturing MOG locally in the brain.

Our results raise a fundamental question about antigen capture by B cells and the following immune responses outside lymphoid tissues. Several hypotheses exist. It is possible that the B cells cannot find the necessary stimulation in the periphery and die, as is shown in the brain. Another possibility is that after the antigen acquisition from cells in the periphery, B cells may immediately traffic to lymphoid tissue to get T cell help as tissues outside of the CNS are well supported by the lymphatic system. It may be that B cells find the T cells in the TLSs or even contribute to the formation of the TLSs after the antigen capture. In our study, we have only investigated the antigen acquisition by naïve B cells in the brain. Memory B cells may behave very differently in the brain and also in the peripheral tissues.

Further understanding of the antigen capture by B cells in the brain and other non-lymphoid tissues will provide new insights into possible mechanisms of B cell-mediated pathogenesis in autoimmune diseases, including MS.

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Education

2018-2022	PhD Candidate in Clinical Neuroimmunology, *University Hospital Basel, Switzerlnad*
2011-2015	Master of Science in Experimental Medicine *McGill University, Canada*
2008-2011	Bachelor of Science in Biochemistry *McGill University, Canada*

Professional experience

2023-present	Project Manager *Innovation Office, University of Basel, Switzerland*
2018	Research Assistant *University of Zurich, Switzerlnad*
2015-2016	Sales application specialist *QIAGEN, Republic of Korea (South)*
2011-2012	Research assistant *McGill University, Canada*

List of skills

- Microscopy: multiphoton microscopy, confocal and fluorescence microscopy.
- Cell Biology: transfection and transduction, primary cell culture, drug toxicity testing.
- Molecular biology: PCR-related techniques and cloning, Designing and production of adenoviral and lentiviral vectors
- Multicolor flow cytometry and FACS
- Biochemistry: western blotting and ELISpot

- Single-cell transcriptomics
- Animal experimentation (LTK 1 Certified): Maintenance of transgenic mouse strains, Injection (IP, SC, IV) and surgical dissection of mice, intracerebral surgery, behaviour testing
- In-house production of formalin-fixed, paraffin-embedded tissue sections, cryo-sections and free-floating
- IT: Graphpad Prism, Inkscape, FlowJo, Imaris, FIJI, R, MS Word, MS PowerPoint, and MS Excel

Publications

Kim et al, Local antigen acquisition of LMP1 expressing B cells in the brain can survive and trigger autoimmune response in multiple sclerosis. (Manuscript in preparation) **[first author]**

Rose et al., (2022) Receptor clustering and pathogenic complement activation in myasthenia gravis depend on synergy between antibodies with multiple subunit specificities. Acta Neuropathologica, 144, 5 **[co-author]**

Zimmermann et al., (2019) Antigen extraction and B cell activation enable identification of rare membrane antigen specific human B cells. Frontiers in immunology, 10, 829 **[co-author]**

Awards

Best Poster Presentation Recipient at ISNI Congress 2021
Graduate Excellence Award in Medicine in McGill University 2014
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Language skills

Korean | Fluent
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