The role of B cells in the pathogenesis and therapy of multiple sclerosis

Bulić, Katarina

SVEUČILIŠTE U RIJECI

ODJEL ZA BIOTEHNOLOGIJU

Preddiplomski sveučilišni studij

"Biotehnologija i istraživanje lijekova"

Katarina Bulić

Uloga B stanica u patogenezi i terapiji multiple skleroze

Završni rad

Rijeka, 2019. godina

SVEUČILIŠTE U RIJECI

ODJEL ZA BIOTEHNOLOGIJU

Preddiplomski sveučilišni studij

"Biotehnologija i istraživanje lijekova"

Katarina Bulić

Uloga B stanica u patogenezi i terapiji multiple skleroze

Završni rad

Rijeka, 2019. godina

UNIVERSITY OF RIJEKA

DEPARTMENT OF BIOTECHNOLOGY

Undergraduate university study

"Biotechnology and medical research"

Katarina Bulić

The role of B cells in the pathogenesis and therapy of MS

Final thesis

Rijeka, 2019

Završni rad obranjen je dana __10. rujna 2019.__ pred povjerenstvom:

1. __Docent dr.sc.Nicholas Bradshaw_____
2. __Izvanredni profesor dr.sc.Hrvoje Jakovac_____
3. __Izvanredni profesor dr.sc.Ivana Munitić_____

Završni rad ima _43_ stranice, _9_ slika, _1_ tablicu i _46_ literaturnih navoda.

Summary

Multiple sclerosis (MS) is an autoimmune disorder that targets myelin sheaths in the central nervous system. It is a chronic disorder and although the axonal damage is initially reversible, at later stages it eventually results in neuroaxonal loss. It has a wide range of symptoms including sensory, autonomic nervous system, motoric and cognitive problems. It affects over 2.5 million people in the world, and starts in most patients at 20 to 40 years of age. MS is a multifactorial disease, with both genetic and environmental factors playing a role in its pathogenesis. Based on its clinical course it is categorized into four types: relapse-remitting, secondary progressive, primary progressive, and progressive relapsing MS. There is still no definite cure for MS, and all current therapies work solely by alleviating symptoms and the severity of the disease. The immune system plays a key role in MS. Autoreactive $CD4^+$ T cells in the periphery are accepted as the most likely cause of the disease, although the triggering mechanisms for their activation are still unclear. Microglia and macrophages have both pro- and anti-inflammatory effects in MS, whereas the natural killer cells have been shown to have a regulatory role and could suppress the autoreactive $CD4^+$ T cells. B cells have been extensively studied in MS in recent years, and were found to play an important role in its pathogenesis and course, thus presenting an attractive therapeutic target. Oligoclonal immunoglobulin bands in the cerebrospinal fluid are produced by B cells and are found in over 95% of MS patients. B cells also act as antigen-presenting cells to T cells, and secrete cytokines which can be either pro-inflammatory or anti-inflammatory. In this thesis we will elaborate on the pathogenesis of MS and review MS therapies, all of which target the immune system. Specifically, we will list and compare the traditional therapies such as interferon-β and glatiramer acetate, which are still the first-line therapy for MS, with second- and third-line therapies that include various B cell-targeting approaches. With a higher efficacy than

older therapies and a smaller risk of serious side effects, B cell-focused therapies are proving to be the future of MS treatment.

Key words: MS, autoimmune disease, immunomodulatory therapies, B cells, antibodies

Sažetak

Multipla skleroza (MS) je kronični autoimuni poremećaj koji pogađa mijelinske ovojnice u središnjem živčanom sustavu. U ranijim stadijima aksonalna šteta je reverzibilna, ali u kasnijima dolazi do neuroaksonalnog gubitka. Ima širok spektar simptoma uključujući probleme u senzornom, autonomnom živčanom i motoričkom sustavu, kao i kognitivne poteškoće. Više od 2,5 milijuna ljudi u svijetu boluje od ove bolesti, a u većine pacijenata započinje u dobi od 20 do 40 godina. MS je multifaktorijalna bolest u čijoj patogenezi ulogu imaju i okolišni i genetski faktori. Na temelju kliničkog tijeka, MS je kategorizirana u četiri tipa: relapsno-remitirajuća, sekundarno progresivna, primarno progresivna i progresivno relapsirajuća. Još uvijek ne postoji definitivni lijek za MS i sve trenutno dostupne terapije samo umanjuju simptome. Imuni sustav igra ključnu ulogu u ovoj bolesti. Autoreaktivne $CD4^+$ T stanice na periferiji su prihvaćene kao najvjerojatniji uzrok bolesti, iako mehanizmi njihove aktivacije još uvijek nisu jasni. Mikroglija i makrofagi imaju i proupalne i protuupalne učinke u MS-u. NK stanice imaju regulatornu ulogu i mogle bi utišati autoreaktivne $CD4^+$ T stanice. Posljednih godina B stanice su opsežno istraživane i pokazalo se da igraju bitnu ulogu u patogenezi i tijeku MS-a te ih to čini dobrom terapijskom metom. B stanice proizvode oligoklonalne vrpce imuoglobulina koje se nalaze u preko 95% pacijenata. B stanice su također antigen-prezentirajuće stanice T stanicama, te proizvode citokine koji imaju ili proupalni ili protuupalni učinak. U ovom završnom radu bit će pojašnjena patogeneza MS-a i opisane terapije za MS. Sve terapije za MS djeluju na imunosni sustav. Tradicionalne terapije poput glatiramer acetata i interferona-β usporedit će se s novijim terapijama koje na neki način djeluju na B stanice. Zbog veće učinkovitosti od starijih terapija i manjeg rizika od ozbiljnih nuspojava, terapije fokusirane na B stanice su se pokazale kao budućnost tretiranja MS-a.

Ključne riječi: multipla skleroza, autoimuna bolest, imunomodulacijske terapije, B stanice, antitijela

Contents

1. Introduction

MS is an autoimmune disorder targeting the myelin sheaths in the central nervous system. Demyelination is seen in the form of lesions within the white matter of the brain and spinal cord. In the early stages of the disease, myelin is destroyed while the axons – nerve fibers of neuron cells – remain largely spared. Ongoing disease, on the other hand, results in neuroaxonal loss, with patient disability correlating to the extent of the loss (1).

MS has a wide range of symptoms, varying from sensory loss or distortions (vision problems, pain, numbness etc.), autonomic nervous system disruptions in bowel and bladder functions, motoric problems, and cognitive defects (Figure 1, Figure 2) (2). Symptoms differ depending on where in the white matter lesions appear (spinal cord, basal ganglia, brain stem). It is most commonly diagnosed between the ages of 20 and 40, but it can affect both younger and older individuals (3).

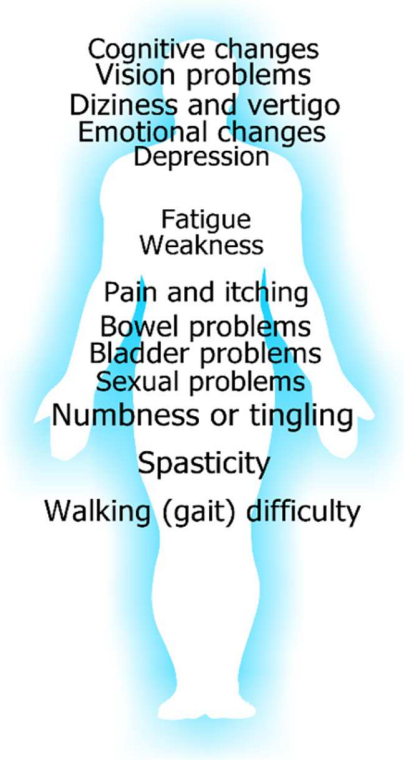

Figure 1. More common symptoms of MS (figure created with Photoscape software).

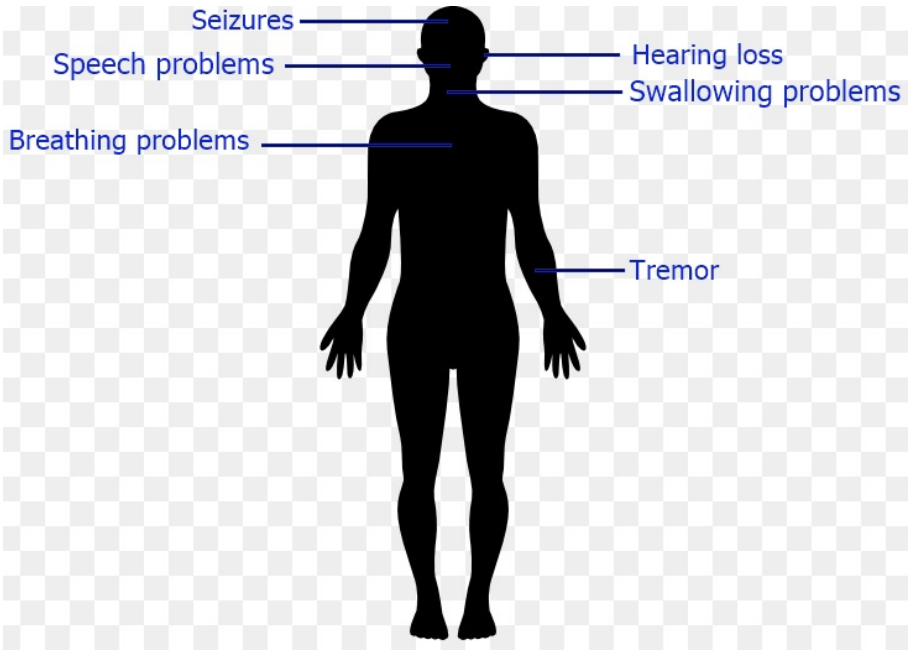

Figure 2. Less common symptoms of MS (figure created with Photoscape software).

Today, over 2,5 million people are affected by MS, with a higher prevalence of the disease the further south or north of the equator (for example, Norway and Canada have the highest prevalence). Prevalence of MS is also growing over time; improved diagnostics most likely plays a role, but it has also been theorized that lifestyle changes, such as increased smoking and lack of vitamin D also contribute to this (3).

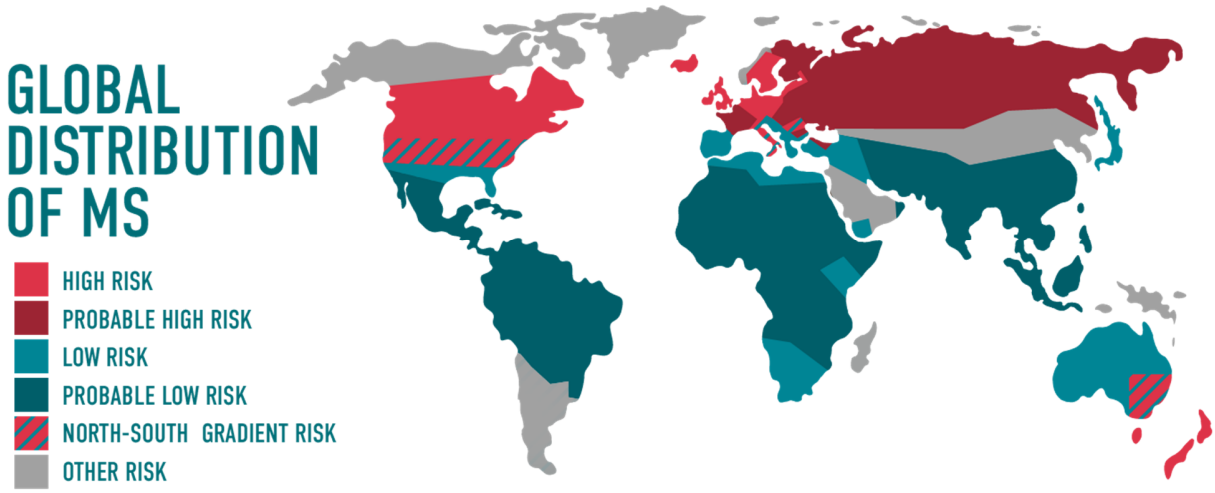

Figure 3. Global distribution of MS (picture taken from https://multiplesclerosis.net/what-is-ms/statistics/).

MS is generally considered to be a multifactorial disease. There is a genetic component, with first relatives and siblings of the people with the disease being more susceptible (4). A region on the sixth chromosome which codes for human histocompatibility system (human leukocyte antigens – HLA) exerts the strongest genetic effect in MS. Besides that region, several genome-wide association studies (GWAS) have shown that other genes have modest effect in MS, including the genes encoding interleukin 7 (IL-7) receptor alpha and IL-2 (5). There has been evidence to suggest that MS may be maternal in origin (maternal half-siblings had greater risk than paternal half-siblings (2.35% as opposed to 1.31%, respectively) (6). Environmental factors (e.g. occupation, smoking, obesity, and birth control)

which most likely act at the population level also possibly have an impact (7).

Based on the disease's clinical course, MS is categorized into four distinct types characterized by increasing severity:
(1) The relapsing/remitting MS (RRMS) is the most common (85% of patients) and the least severe type of MS. It is characterized by relapses (attacks), the periods of active symptomatic disease, followed by periods of remission (3). The attacks evolve over days to weeks, and remission periods last months. During the remission period there is some degree of recovery, and no worsening of neurological functions. It often escalates to a progressive type of disease in later stages (2).
(2) Secondary progressive MS (SPMS) is characterized by initial relapses, which stop with time and are followed by gradual neurological deterioration (2). It is mostly diagnosed retrospectively based on a history of worsening symptoms and irreversible progression of disability regardless of relapses (8).
(3) Primary progressive MS (PPMS) has no remissions, but a steady functional decline since the onset of the disease (2). A prominent pathological feature in both primary and secondary progressive MS is brain atrophy (9).
(4) Progressive relapsing MS (PRMS) is characterized by steady functional decline from onset of disease, with superimposed acute attacks in later stages. It cannot be differentiated from PPMS until the attacks occur (2).

Out of all MS cases, 80% present as clinically isolated syndrome (CIS), an isolated acute attack targeting one or more CNS sites. It can convert to chronic RRMS. The risk of that depends on the presence of white matter lesion on MRI scans; the risk of conversion is considerably higher if there are white matter lesions found on MRI scans (10).

2. Aims of the thesis

MS is one of the most prevalent neurological disorders among young adults. It severely impacts an individual's quality of life and their physical capability. Its prevalence has also been slowly growing.

In the past, most of the focus was put on T cells and their role in MS pathogenesis and therapy. Although B cells have long been suspected to have a role in MS, it was not known what effect they had in the disease. That role has been researched extensively in recent years, and it has been found that they do more in MS than just produce myelin-targeting antibodies. Knowledge of those roles has helped with the development of new B cell-targeting therapies have shown most promising results.

This thesis will explain the pathogenesis of MS, and the role of different immune cells in it, focusing primarily on B cells. Therapies targeting and not targeting B cells will be listed, elaborated, and compared to each other.

3. MS pathogenesis

MS most likely starts with the activation of autoreactive T cells in the periphery. As mentioned earlier, it is not clear why and how they are activated, probably through bystander activation or molecular mimicry. The autoreactive T cells then travel to the CNS through a disrupted blood-brain barrier (BBB) (11). Damage to the BBB is done mostly by a subset of T helper cells called Th17 (12).

In the CNS, T cells are re-activated by autoantigens presented by different antigen-presenting cells (APCs), which trigger the production of pro-inflammatory mediators. Examples of such mediators are cytokines and chemokines (for example IL-17, IFN-γ) which can be produced either by T cells or other infiltrating cells, such as monocytes. These mediators recruit other pro-inflammatory cells, like macrophages and B cells. B cells cross the BBB, undergo local stimulation, affinity maturation and hypermutation driven by antigens, and clonal expansion. The combined pro-inflammatory effect of autoreactive T cells, B cells and other immune cells leads to demyelinating damage and lesions in the CNS (11).

MS mainly targets myelin sheaths, but any cell of the CNS can be affected by the disease. Myelinating cells of the CNS are called oligodendrocytes. Oligodendrocytes are differentiated from oligodendrocyte progenitor cells. This differentiation occurs during the CNS development, as well as following injury. The demyelination in MS is the result of the dysfunction of myelin-producing oligodendrocytes and the reduced generation of oligodendrocyte progenitor cells. Damage to myelin sheaths is mediated by autoreactive T cells and autoantibodies, as well as activation of microglia and macrophages through pro-inflammatory cytokines (12,13).

Experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model of MS, and the two share key pathological features of demyelination, inflammation, and axonal loss. EAE can be induced in most species of mammals, including humans, through external immunization typically achieved by using myelin antigens delivered with strong adjuvants containing bacterial components highly capable of activating the innate immune system. EAE is not MS because MS is a uniquely human disease without a singular cause (14). However, EAE has shown to be valuable in understanding MS pathogenesis, as well as the development of new therapies. In the following part, the role of different cell types in MS pathogenesis will be stated and explained.

3.1. T cells

T cells have been the focus of MS research for a long time, and during that time, many possible therapies that target T cells have been considered. Genome-wide MS susceptibility studies have identified ~100 genes, nearly all of which belong to the immune system. Most of these genes are in some way associated with T cell function, followed by those associated with B cell function. Some of the genes associated with B cells are indicative of their role as APCs to T cells (12). Further proof of the importance of T cells in MS is that almost all of the treatments target and affect T cells, both directly and indirectly.

It is believed that activated peripheral autoreactive $CD4^+$ T cells cause MS by migrating into the CNS and initiating the disease process. This "outside-in-hypothesis" opposes the "inside-out" hypothesis, which states that MS is caused by an initiating event inside the CNS (Figure 4).

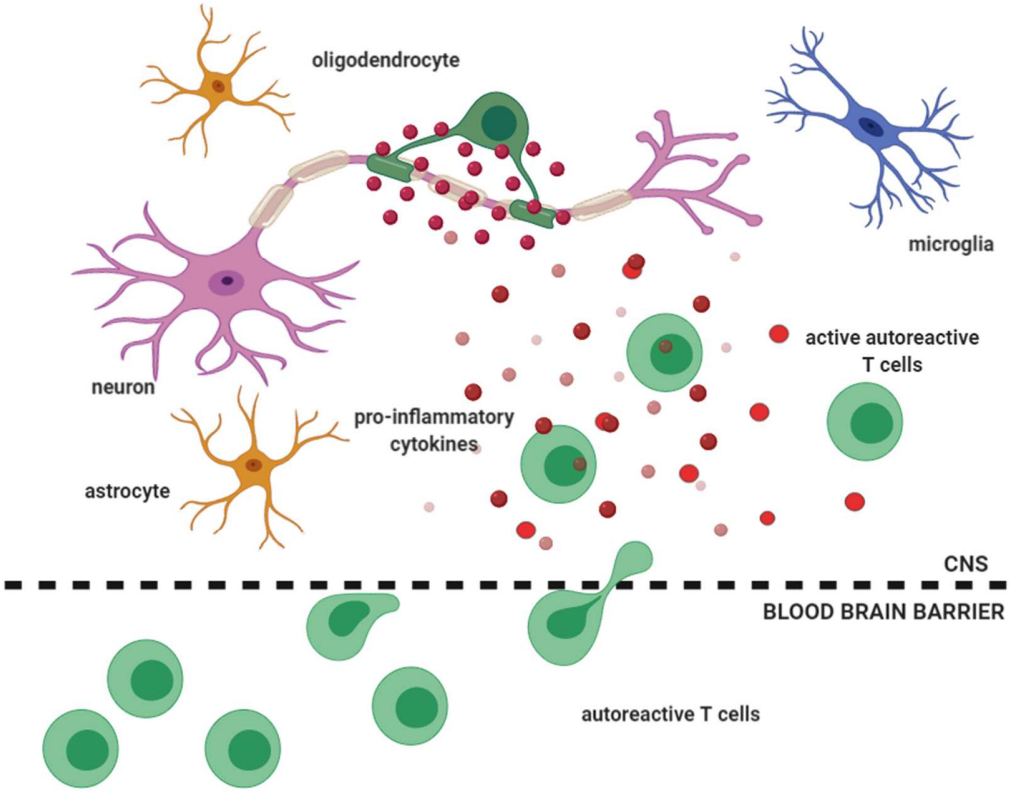

Figure 4. Possible course of MS pathogenesis. According to the "outside-in" hypothesis, once they are activated by autoantigens, autoreactive T cells cross the BBB and reach the CNS where they are reactivated and start causing damage to myelin sheaths by secreting the pro-inflammatory cytokines. Microglia are a subset of macrophages that are found in the CNS parenchyma. Microglia in MS can have both neuroinflammatory and neuroprotective effect. They internalize myelin, produce neurotoxic mediators, and present autoantigens that reactivate the T cells once they've crossed the BBB. Astrocytes have the ability of communicating with neurons, as well as other glial cells (oligodendrocytes and microglia, for example). In MS, astrocytes are abundantly present within the demyelinated plaques. They can also present myelin epitopes to T cells. Figure was created with BioRender software.

Inflammatory lesions are formed after the reactivation of $CD4^+$ T cells by autoantigen-presenting cells and the recruitment of additional macrophages and T cells. These lesions contain cytotoxic $CD8^+$ cells on the edges, and $CD4^+$ T cells deep inside the lesions. T cells cause the loss of myelin, the destruction of oligodendrocytes, and axonal damage through secretion of pro-inflammatory cytokines (Figure 4). All of this leads to neurological dysfunction (15).

3.1.1. T helper cells

A subset of $CD4^+$ T helper cells known as Th1, which produce interferon gamma (IFN-γ), were long considered to be the main pathogenic T cells in both MS and EAE. The conclusion was based on the observation that mice lacking the cytokine interleukin (IL)-12 were resistant to EAE, and IL-12 was required for the differentiation and development of Th1 cells. Also, treating MS patients with IFN-γ worsened the disease. However, there was a paradox because mice that lacked Th1 cells would develop more severe EAE. The paradox was partially resolved when a new cytokine was discovered, IL-23, which shared a part of its structure with IL-12. This has led to a discovery of an entirely new subset of T helper cells for whose development IL-23 is necessary, named Th17 because of their secretion of IL-17. Th17 cells produce a number of other cytokines, have a pro-inflammatory effect and, are pathogenic in many autoimmune disorders (12,16).

3.1.2. T regulatory cells

T regulatory cells are self-reactive cells whose role is the regulation and suppression of the immune responses. In normal circumstances, they play a pivotal role in preventing or suppressing autoimmunity and are deficient in many autoimmune diseases, including MS (12). In the context of MS, two subsets of these $CD4^+$ T cells have been identified and studied, and both are thought to be important in MS.

Tregs are a subset of T regulatory cells that express FoxP3 transcription factor and many inhibitory immune checkpoint molecules. These help their ability to suppress the proliferation of effector T cells. They are activated by self-antigens and have been shown to suppress the ability of pathogenic T cells to induce EAE in healthy mice when co-transferred with them. Their function is disrupted and reduced in MS (15).

Tr1 regulatory $CD4^+$ cells are a second type of regulatory T cells. They prevent T cell proliferation mainly by secreting the anti-inflammatory cytokine IL-10. They have not been as extensively studied as $FoxP3^+$ cells, but research has shown that their function is also disrupted in MS and that $CD4^+$ T cells of MS patients express less IL-10 than those of healthy individuals (15).

3.1.3. Cytotoxic $CD8^+$ T cells

$CD8^+$ cytotoxic T cells recognize short peptide epitopes, which are presented by APCs as part of MHC class I molecules. In MS, $CD8^+$ T cells have been suggested to act against myelin oligodendrocyte protein (MOG), myelin basic protein (MBP), and proteolipid protein (PLP), but their pathogenic role is still not clear. A much higher number of $CD8^+$ cells has been found in the lesions than $CD4^+$ cells, and MHC class I molecules are expressed in large numbers in MS lesions. Cytotoxic $CD8^+$ T cells within those lesions secrete IL-17 and TNF-α (3).

3.2. Natural killer cells

Natural killer (NK) cells are a part of the innate immune system. They are lymphocytes that act as sentinels by recognizing and destroying transformed, infected or autoreactive cells. They do this in part by exocytosis of cytotoxic granules containing granzymes and perforin. Their function is controlled via a balance of positive and negative signals, which are transmitted through germ line-encoded activating and inhibitory receptors (17).

There has been evidence showing that the cells of the innate immune system have a regulatory role in MS (18,19). NK cells, specifically those that have a high surface expression of a molecule known as CD56, or the neural cell adhesion molecule (NCAM), produce cytokines in larger amounts and have a lower cytotoxic effect compared to NK cells without CD56 expression. Natural killer $CD56^{bright}$ cells express NKG2A, an inhibitory receptor, as well as receptors for cytokines like IL-18, IL-15, and IL-12. These cytokines trigger proliferation of NK cells and also their production of granulocyte-macrophage colony stimulating factor (GM-CSF), tumor necrosis factor beta (TNF-beta), IFN-γ, and interleukins 13 and 10 (18). Using the regulatory features of NK cells, it has been shown that it is possible to eliminate the activated autoreactive T cells (20).

3.3. Microglia and macrophages

Macrophages are a type of phagocytes, and their functions vary from host defense, immune regulation, wound healing, as well as organ- and tissue-specific homeostatic functions. Microglia and three types of classical macrophage subsets (macrophages in the perivascular space, meninges, and choroid plexus) are present in the CNS. All of these subsets have the task of maintaining the CNS homeostasis. They accomplish this through clearing the cellular debris, and preventing pathogens and systemic mediators from entering the CNS (21). Monocyte-derived macrophages can develop in the CNS during MS attacks and have an anti-inflammatory and neuroprotective role in injured and healthy CNS. They also have the ability to promote secondary neurodegenerative events and neuroinflammation (21).

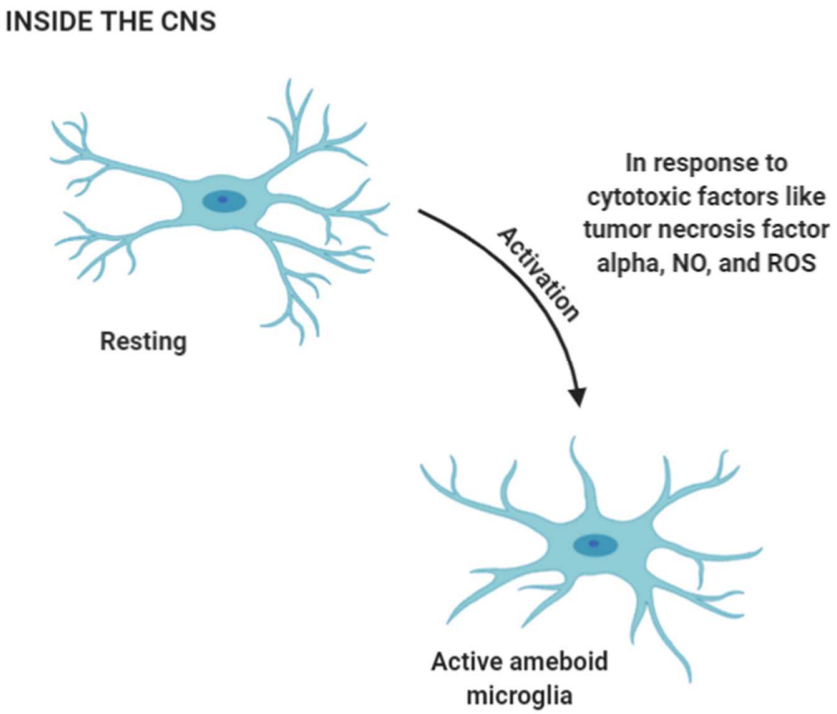

Figure 5. Microglia inside the CNS are activated by injury and pro-inflammatory cytokines. In the course of MS pathogenesis, microglia are activated by pro-inflammatory cytokines released by reactivated autoreactive T cells. Microglia remove debris and present processed antigens to T cells, contributing to their expansion. Microglia itself can cause damage to myelin by releasing free radicals (ROS, NO), glutamate, or pro-inflammatory cytokines (IL-1 and TNF-α, for example). Figure was created with BioRender software.

Microglia are the most numerous of all brain macrophage subsets and are the only ones found in the CNS parenchyma. Microglia proliferate when activated, gaining increased phagocytic ability and motility (Figure 5). They also release a number of inflammatory mediators, and their antigen presentation is heightened. Microglia are implicated in both neuroinflammatory and neuroprotective processes.

Activated microglia are found in all stages of MS and EAE, and correlate with oligodendrocyte and axon pathology. Experiments on bone marrow chimeras showed that activated and proliferating microglia precede the onset of EAE, and that their inhibited activation slows the maintenance and development of lesions in CNS (21). On the other hand, microglia also have an active role in neuroprotective processes. As mentioned above, inhibited activation of microglia delays EAE symptoms, but it also causes a more severe case of the disease, and the recovery of the neurological functions in mice is delayed. Microglia can promote and accelerate CNS recovery in animal models by producing growth factors and removing the damaged myelin (21).

Therefore, although MS is likely mediated in large part by T cells, innate immune cell types also contribute to its complexity. NK cells have a role in regulating T cells and the disruption of that regulation could be an important factor in MS pathogenesis, as well as therapeutic target. Microglia and macrophages have both neuroprotective and neuroinflammatory role in MS, and it is not yet clear which factors determine which of those two activities will prevail. Finding a way of changing the effects of microglia and macrophage in MS could also be a potential therapy in the future.

4. B cells in the pathogenesis of MS

B cells in MS were a long time ago considered only to produce antibodies (also known as immunoglobulins, or Ig) that do not contribute to MS pathogenesis, and CNS damage was primarily attributed to pro-inflammatory T cells. Now we know that B cells play a much larger role in the autoimmune pathogenesis of the disease. Experiments done on B cell deficient mice gave a good insight into their pathological role. These mice were shown to be resistant to EAE when injected with human recombinant MOG, and transfer of the antigen-specific antibodies from wild-type mice rescues susceptibility to EAE. This indicates that antigen recognition by antibodies may be crucial for the development of EAE (22), and that these findings can be used in further research of the antibody role in MS. The benefits of anti-CD20-mediated depletion of B in MS showed that B cells likely have a contribution to its development and progression. The activation of peripheral and CNS B cells, their chronic inflammatory effect, and the shift towards memory B cells indicates an activation mediated by antigens (23).

Our understanding of the roles of B cells in MS pathogenesis has grown, and will certainly continue to do so as new technologies are developed. It has been shown that B cells are important in MS, and their roles should be researched further. B cell-targeting therapies with promising results will be discussed later in the thesis, and therapies should improve as B cell roles in MS become clearer.

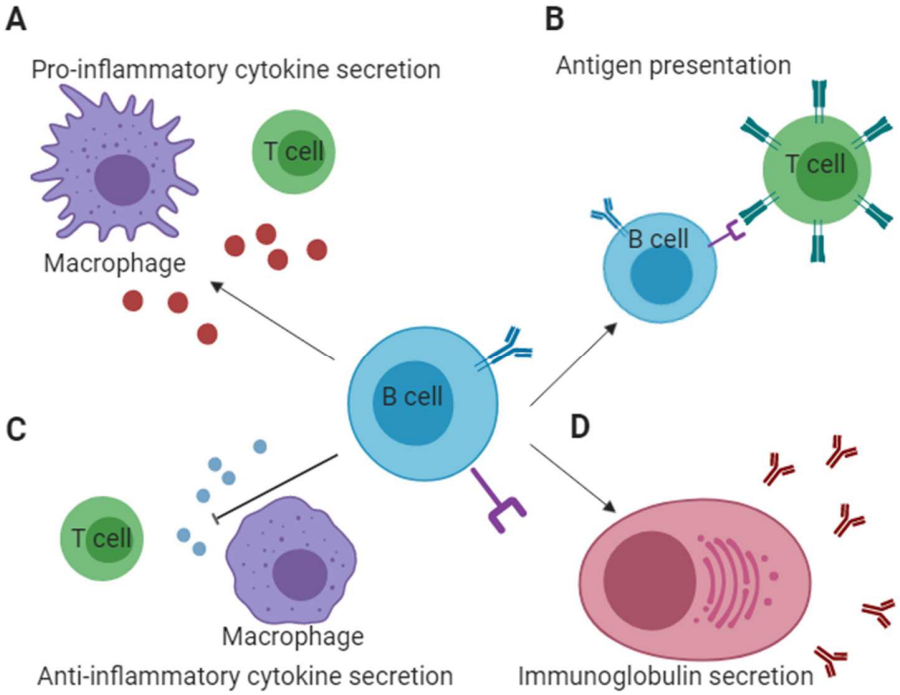

Figure 6. B cells in MS secrete cytokines, produce antibodies, and act as APCs. A) Secretion of pro-inflammatory cytokines, such as IL-35, and transforming growth factor-β1, which have a negative effect in MS. B) They act as APCs to autoreactive T cells. C) Secretion of anti-inflammatory cytokines (IL-6, TNF-α, TNF-β, GM-CSF) which have a neuroprotective effect in MS. D) Secretion of Ig, which can act as facilitators of disease and CNS damage (picture created with BioRender software)

4.1. Oligoclonal Ig bands are a biomarker present in MS lesions

Kabat et al. showed in 1942 that MS patients have oligoclonal immunoglobulin bands (OCBs) present in their cerebrospinal fluid, and these bands are presently the only immunological biomarker that has been confirmed to have both prognostic and diagnostic relevance (24,25). IgG OCBs are present in up to 95% of MS patients, and around 40% also have IgM OCBs (26). Oligoclonal bands themselves are Ig species produced by clonally expanded B cells (Figure 6) and are highly mutated, something which is unusual for IgM. Possible reason for this is high exposure to antigens, and subsequent affinity maturation (23). They can be visualized on isoelectric focusing gels by analytical immunoblotting (Figure 7) (24).

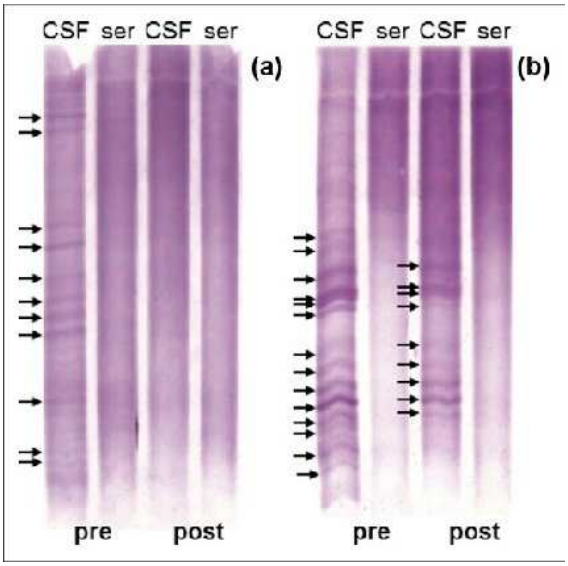

Figure 7. Visualization of OCBs on agarose gel. Figure shows complete (a) and partial (b) disappearance of OCBs from CSF and serum samples of MS patients after a 24-month treatment with natalizumab (figure taken with permission from (27).

The role of OCBs in the progression and evolution of MS is not yet clear. An additional open question is which antigens the antibodies in OCBs recognize. It was noted that different antibodies produced in humoral response to MS target a range of antigens, from rubella to measles virus (23). One theory suggests that OCBs target myelin autoantigens and viruses which have the potential to harm the CNS. Another theory suggests that OCB antibodies target cell debris, based on a study done by Brändle et al. which found that those antibodies were directed against various intracellular proteins (24). The success of B-cell depleting MS therapies, in spite of their failure to target plasma cells and reduce OCB levels, suggests that despite their prevalence, OCB are not essential in MS pathogenesis. Their long-term survival also implies that CNS is a favorable environment for B cells and Igs. In MS and other autoimmune diseases, there are high levels of B cell activating factor (BAFF) and a proliferation inducing ligand (APRIL), two molecules that are involved in B cell development, survival, and differentiation. BAFF and APRIL are produced by macrophages, astrocytes, monocytes, and activated T cells (28,29). Treatments that target BAFF and APRIL could present an area of research in MS therapy.

4.2. The pathological role of B cell-derived plasma cells and antibodies

After recognizing antigens, B cells mature to plasma cells which then produce large amounts of antibodies (Figure 6). MS lesions usually show several histopathological patterns. Specifically, the most frequent pattern is characterized by large amounts of antibody and complement deposits, indicating that locally produced antibodies have an effect on overall demyelination (22).

There have been a number of potential targets for the autoreactive antibodies in MS. Some of them include the mentioned myelin antigens such as MOG and MBP. MOG is the most well researched out of all of these due to its location on outermost myelin layer (23). For example, in animal models (nonhuman primates and rodents), immunization with an intact MOG protein leads to fulminant EAE, characterized by an antibody response against MOG. These antibodies most likely are not sufficient to cause the disease themselves, but studies have indicated that they can lead to a progression of already ongoing EAE and facilitate CNS damage (22), especially after the blood-brain barrier is weakened by T cell-mediated inflammation which acts as a 'first hit'. Following that logic, antibodies represent the 'second hit', and this model has been confirmed by *in vivo* experiments in primates and rodents (30).

4.3. B cells as antigen-presenting cells in MS

Anti-CD20 treatment induces long-lasting depletion of B cells expressing CD20, which includes all B cell subsets except antibody-forming plasma cells and early B cell differentiation stages. The response to such treatment occurs much earlier than it would be expected if reduced antibody production played a significant role in treatment outcome. Therefore, it is assumed that this treatment targets $CD20^+$ B cells as APCs to T cells and as the source of different pro-inflammatory cytokines (31).

B cells are professional APCs (Figure 6). They express major histocompatibility complex (MHC) class II molecules. MHC II expression is elevated on B cells in MS. Along with MHC II, B cells in MS express a higher level of different co-stimulatory molecules. These molecules have the ability to promote differentiation of pro-inflammatory effector $CD4^+$ T cells. B cells recognize antigens via their highly specific B cell receptor (BCR), which is a membrane-bound Ig. A captured antigen is internalized and processed before being presented on the surface of the B cells to T cells. This process is sufficient for mice to develop EAE, and studies have shown that myelin-reactive B cells and T cells can spontaneously induce the disease (23). In contrast, the selective ablation of MHC II molecules made the mice resistant to EAE. All of these findings point to the APC role of antigen-specific B cells in MS pathogenesis.

4.4. B cells secrete different cytokines which affect the course of MS

Another antibody-independent function of B cells in MS is that both naïve and activated B cells secrete different cytokines with either protective of pathogenic effect (Figure 6). This allows them control over the activity of some of the other immune cells. For example, B cells secrete IL-6, which induces differentiation of Th17 cells, but also prevents the differentiation of regulatory T cells. As mentioned earlier, Th17 have a pathogenic effect in most autoimmune disorders. In line with that, IL-6 deficiency restricted to B cells leads to diminished Th17 responses and less severe EAE disease course (23). Besides IL-6, B cells secrete other cytokines with pro-inflammatory functions, such as tumor necrosis factor alpha (TNF-α) and tumor necrosis factor beta (TNF-β). Another important pro-inflammatory cytokine produced by a specific subset of B cells is granulocyte-macrophage colony stimulating factor (GM-CSF), and this same subset co-expresses TNF and IL-6. The deletion of GM-CSF-producing B cell subset led to a reduced pathogenic response from immune myeloid cells (23).

B cells also secrete anti-inflammatory cytokines (IL-35, IL-10, transforming growth factor-β1) (11). IL-10 suppresses myeloid APCs like dendritic cells and monocytes, and is an inhibitor of pathogenic Th17 and Th1 cell differentiation. It is produced by the naïve B cells, but also antigen-experienced and fully differentiated plasma cells. IL-35 is a recently discovered regulatory cytokine produced by B cells, especially fully differentiated and developed plasma cells (23).

This dichotomy of the contribution of B cells to the pathogenesis of MS is explained by the classification of B cells into two subsets: B1 and B2. These subsets differ in their lineage, location, genes expression, antibody

repertoire, proliferation, and secretion of Igs. B1 cells are predominantly found in the peritoneal cavity and are rare in secondary lymphoid organs such as the spleen. As APCs, B1 cells activate generation of Th17 and Th1 cells, and are considered to have a role in autoimmunity. Conventional follicular B2 cells, on the other hand, promote the development of induced Tregs that have a suppressive capacity. MS patients have a higher frequency of B1 cells in the peripheral blood and CSF, with patients with progressive MS having a higher frequency than those with RRMS. This frequency has been shown to positively correlate with the activity of the disease (22).

5. MS therapies

MS therapies are either immunosuppressive, immunomodulatory or both. Glucocorticoids and plasma exchange can be used to stop the acute attacks, but are usually poorly efficient in the long-term and necessitate the use of disease modifying treatments (DMT) such as treatments that affect the disease course. The latter can be sorted into first-, second-, and third-line. Glatiramer acetate (GA) and interferon-beta (IFN-β) are considered first-line therapies, having moderate efficacy and high safety, so MS treatment usually starts with them. Natalizumab and fingolimod target receptors on T cells and prevent them from entering the CNS. They are included in second-line treatments, with greater efficacy but a higher risk of adverse effects. Several B-cell or combined B/T-cell targeting antibody treatments, comprise second- and third-line therapies, such as alemtuzumab, rituximab, ocrelizumab, ofatumumab, and antibodies that target APRIL or BAFF. Another important factor in choosing the appropriate the DMT is cost, with newer DMTs being more expensive (11,32).

5.1. Glucocorticoids

Glucocorticoids are a class of steroids with anti-inflammatory properties often used in the treatment of different inflammatory diseases. They are used in the management of acute MS attacks. Despite the limited research data, it seems to be that a limited glucocorticoid treatment for a duration of about two weeks helps with quicker recovery from the MS attack and is tolerable. However, glucocorticoids do not help with the degree of recovery and is only a short term treatment (33).

5.2. Glatiramer acetate

Glatiramer acetate (GA, Copolymer-1, Cop-1) was first discovered in the late 1960s during research on immunological properties of amino acids' synthetic polymers and copolymers with the goal of producing a synthetic antigen which would be capable of inducing EAE. In contrast to the original hypothesis, they did not induce EAE, but were protective against it. Cop-1 - a heterogeneous mixture of L-lysine, L-alanine, L-tyrosine, and L-glutamic acid - proved to be especially active against EAE by reducing its incidence, its prevalence, and the severity of lesions. Research of other EAE models showed that the activity of GA was not restricted to a single species or a disease type or the component used to induce EAE (34).

Clinical research was started on GA in the late 1970s with the goal of testing its safety and efficacy as a possible DMT for MS. In 1996, it was approved for RRMS treatment and for the treatment of CIS, and to this date it is the only peptide approved for therapy of MS. The exact mechanism of action of GA in MS is not yet known (34,35).

5.3. Interferon beta

Interferon-beta (IFN-β) is a cytokine naturally produced in the immune system, and its recombinant forms are used as a DMT for MS (36,37). IFN-β therapy results in a reduced number of brain lesions, attacks, and relapses in RRMS by about 30%. It also slows down the progression of the disease. Its mechanism of action is not fully understood yet, but it has many immunomodulatory functions. IFN-β affects the immune system by increasing the levels of anti-inflammatory cytokines such as IL-10, inhibiting the production of pro-inflammatory cytokines like IL-17, and preventing the

migration of leukocytes across the BBB (37). There are dilemmas about whether the therapeutic effect of IFN-β could be helped by IFN-β acting as an inhibitor to viral replication (Epstein-Barr virus is often associated with MS). IFN-β therapy may cause some side-effects like symptoms similar to flu, shortness of breath, anxiety, and spasms (36).

5.4. Fingolimod

Fingolimod is an immunomodulatory drug which is used in the therapy of RRMS. It is an oral drug, the first of its kind that FDA licensed for use in RRMS. Fingolimod prevents lymphocytes from leaving the lymphoid tissue to enter the circulation by blocking their sphingosine-1-phosphate (S1P) receptors. As such it blocks the entrance of auto-reactive lymphocytes to the CNS. In EAE, fingolimod reduced and even reversed symptoms like paralysis, while in clinical trials it reduced the number of lesions and the annual relapse rate to a higher degree than IFN-β (38). Considering that fingolimod targets receptors present in a wide range of tissues and organs (CNS, smooth muscle cells, cardiac myocytes), there is also a number of side effects connected with the therapy. Some of these side effects are heart block, hypertension (heightened blood pressure), and bradycardia (heart rate slower than 60 beats per minute) (39).

5.4. Natalizumab

Natalizumab is a humanized monoclonal antibody, which binds to the α4 subunit of two different integrins, α4β1 and α4β7 integrins. These integrins are found on T cells, among others. When the antibody binds to the integrins, it prevents their binding to endothelial receptors and infiltration to the CNS, thus reducing inflammation. A rare but serious side effect to therapy with natalizumab is progressive multifocal leukoencephalopathy (PML), an infective and demyelinating disease of the central nervous system which occurs due to the reactivation of John Cunningham virus (JCV) (40). This suggests that T cell-mediated patrol of CNS is crucial for suppression of chronic infections and that increased scrutiny is warranted when applying such immunosuppressive therapies.

6. MS therapies that directly target B cells

6.1. Plasmapheresis

One of the possible ways MS symptoms could be lessened is through the removal of pro-inflammatory cytokines and Ig from circulation (23). The process of removing these and other soluble products from the plasma is called plasmapheresis. It was the first therapy for MS that targeted B cell components specifically, with the first report of its efficacy in MS therapy dating back in the 1980s (22). Despite intrathecal OCBs being a biomarker in more of 95% of MS patients, not all patients respond well to plasmapheresis, only those with prominent deposition of immunoglobulin and activation of complement (41). Therapeutic success and clinical usage of plasmapheresis and similar procedures shows that plasma can contribute to relapses, and its

removal improves the state of the patients, although it has no long-term effects (23).

6.2. Anti-CD20 monoclonal antibodies

Using therapies that deplete B cells in MS treatment began with the assumption that important players in MS pathogenesis are autoreactive Igs. The initial phase II clinical trial of rituximab, a monoclonal antibody targeting CD20, a molecule which is expressed on all B cells from the late pro-B stage to the memory cell stage, showed promising results in RRMS. Rituximab success opened the door to the development of various DMTs that target the B cells.

As mentioned, CD20 is a surface protein expressed on almost all B cells (only plasma cells, pro-B cells, and stem cells do not express it). It is also expressed by a limited number of mature T cells in circulation (5-7%). This makes it a great target for depletion of B cells. Anti-CD20 therapies deplete nearly all $CD20^+$ B cells in circulation. Anti-CD20 monoclonal antibodies (anti-CD20 mAbs) do not pass the BBB, but they do eliminate the B cells in CSF, though they do not have an effect on OCBs (42). After depletion, immature and naïve B cells are mostly reconstituted within months, but memory B cells levels do not go up for at least one to two years. Secretion of pro-inflammatory cytokines (TNF-α, GM-CSF) is also decreased. T cell function is also altered by this therapy because of the lack of B cells acting as APCs: $CD4^+$ and $CD8^+$ proliferate and secrete pro-inflammatory cytokines at a lower rate, and T regulatory cells are increased (42). This next part will describe various anti-CD20 antibodies that are used in MS treatment.

6.2.1. Rituximab

Rituximab is a chimeric IgG antibody. Chimeric antibodies are monoclonal antibodies produced by mice in laboratory conditions. Because of their origin, they have large stretches of non-human together with human sequences. Rituximab depletes B cells through complement-dependent cytotoxicity (CDC), and main adverse effects connected with it are mild to moderate infusion-associated reactions (IARs) (42). It was the first anti-CD20 monoclonal antibody that was tested as a possible treatment of MS. The promising results in phase II clinical trial provided reason to further research anti-CD20 mAbs as effective MS therapy, especially in RRMS (41). In PPMS there was no noticeable effect, possibly as low rituximab levels in the CSF after intravenous administration were not enough to affect the CNS inflammation. Three phase II studies have been done on the efficacy of rituximab, but the antibody has not entered any phase III trials. The development for usage in MS was never completed due to adverse reactions to the chimeric antibody, and research has shifted to different kinds of anti-CD20 mAbs. Despite that, rituximab is still used off-label in MS treatment (42).

6.2.2. Ocrelizumab

Ocrelizumab is a humanized version of the anti-CD20 mAb and for this reason it is less immunogenic than rituximab. In comparison, rituximab therapy led to creation of antibodies as a response in 24.6% of the patients. Another advantage is that the Fc region of a humanized mAb showed a greater affinity for FcγRIIIa receptors on NK cells. Ocrelizumab acts as a B-cell depleting mAb through antibody-dependent cellular cytotoxicity (ADCC) (41). Only a small part of adverse effects connected with ocrelizumab are serious infections and neoplasms.

There have been three phase III clinical trials done on Ocrelizumab. All three confirmed its efficacy, and Ocrelizumab was the first Anti-CD20 monoclonal antibody approved by the FDA for RRMS and PPMS treatment (41,42). Low rates of serious immunogenic reactions and heightened affinity for its target receptors make ocrelizumab a great alternative to rituximab.

6.2.3. Ofatumumab

Ofatumumab is a fully human IgG1 monoclonal antibody. Because it is fully human, it is even less immunogenic than ocrelizumab. It binds to a distinct epitope at a slower rate of dissociation from CD20 in comparison with rituximab. This leads to enhanced CDC activity, decreased ADCC, and a low risk profile for immunogenic reaction. A phase-II trial led to a reduction in lesions, and partial depletion of B cells dependent on the dose. Two phase-III trials are currently in progress (41,42). If the trials show its efficacy and safety, ofatumumab could potentially be a great therapy if immunogenic reactions want to be avoided.

6.2.4. Ublituximab

Ublituximab is a chimeric monoclonal antibody that binds a unique CD20 epitope. It has an increased ADCC activity due to a glycoengineered Fc region that binds to already mentioned FcγRIIIa receptors. Recent phase-II study showed promising results in B-cell depletion and lesion reduction with minimal adverse effects (headache, fatigue, injection-related reactions), and two phase-III trials are currently ongoing (41,42). The unique epitope that ublituximab binds, its enhanced activity in recent trials, and a lack of serious side effects show that ublituximab is a promising new therapy with a potential to open up a new area of research.

6.3. Anti-CD19 (MEDI-551)

Inebilizumab (MEDI-551) is a humanized monoclonal antibody which targets CD19, a molecule expressed on plasmablasts and some plasma cells. Inebilizumab binds to FcγRIIIa receptors, and acts through ADCC. Anti-CD19 mAb therapies in general could have a longer-lasting depletion effect due to CD19 expressed on cells that lack the expression of CD20. This kind of therapy would also remove short-lived cells which secrete antibodies, while leaving others intact. (23,41).

6.4. Alemtuzumab

Alemtuzumab is a humanized mAb therapy, which binds to CD52, a glycoprotein expressed on monocytes, lymphocytes, and some dendritic cells. The function of CD52 is not known yet. Alemtuzumab has been approved for active RRMS therapy in over 30 countries.

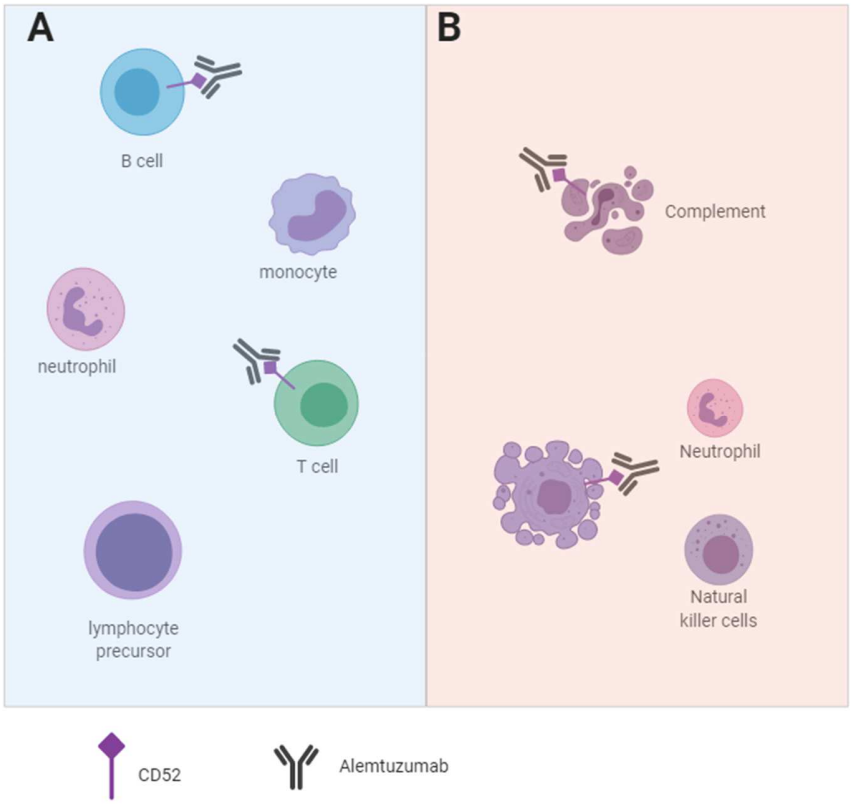

Figure 8. Mechanism of action of alemtuzumab. A) Alemtuzumab binds to CD52, which is expressed on lymphocytes. B) The cells expressing CD52 are depleted through complement activation and ADCC (NK cells, neutrophils). After depletion, a new repertoire of T and B cells is produced from primary lymphoid organs.
(picture created with BioRender software)

Alemtuzumab causes cell depletion through ADCC and CDC (Figure 5). A meta-analysis showed that alemtuzumab had higher efficacy than IFN-β, with better results in lowering annual relapse rate, and lower discontinuation rate due to adverse effects (43). Repopulation after depletion starts with lymphocytes (within weeks). B cell levels return to baseline six months after treatment and T cells reached normal levels 12 months after treatment. $CD4^+$ cell repopulation is delayed in particular, with a long-term study from

the 1990s showing that median time for $CD4^+$ T cell recovery after patients received a single treatment was 12 years. Innate immune cells, plasma cells, serum Ig levels, and some T-cell subsets have not been affected by alemtuzumab in animal studies (44).

Data suggests that alemtuzumab is not effective in patients with SPMS and may not be the most appropriate therapy for patients with mild MS or CIS. According to its EMA label, it should be used on patients with active RRMS (44). Considering it is a newer therapy, the benefits and risks of alemtuzumab should be assessed, especially long-term in order to make the treatment as effective as safe as possible, as well as ensure that the right patients receive this treatment.

Table 1. MS monoclonal antibody therapies, their target antigens, and mechanisms of action.

NAME	KIND	TARGET	MECHANISM OF ACTION
Natalizumab	Humanized antibody	α4 subunit of α4β1 and α4β7 integrins	Prevention of CNS infiltration
Rituximab	Chimeric antibody	CD20	CDC
Ocrelizumab	Humanized antibody	CD20	ADCC
Ofatumumab	Human antibody	CD20	Enhanced CDC Decreased ADCC
Ublituximab	Chimeric antibody	CD20 (unique epitope)	Increased ADCC
Inebilizumab (MEDI-551)	Humanized antibody	CD19	ADCC
Alemtuzumab	Humanized antibody	CD52	ADCC CDC

6.5. Cytokine antagonists

BAFF and APRIL are cytokines crucial for B cell survival and are elevated in patients with MS. There have been attempts to develop DMTs which target these molecules, but none have progressed beyond phase-II trials. Of note is the fusion protein atacicept (TACI-Ig). It is made up of the extracellular domain of transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) - a receptor for BAFF and APRIL - and the Fc domain of human IgG. Atacicept captures BAFF and APRIL, thus stopping their interaction with B cells. However, two phase-II trials done on patients with RRMS and unilateral optic neuritis have been terminated prematurely. The reasons for this were heightened activity of RRMS in one of the trials and the patients in the other having higher conversion rates to clinically definite MS than the placebo group. This has shown that roles of B cells and humoral immunity in general is not yet fully understood in autoimmune disorders, and that testing new possible therapies should be approached with caution (42).

6.6. Inhibitors of Bruton's tyrosine kinase

Bruton's tyrosine kinase (BTK) is a cytoplasmic enzyme expressed not only on B cells, but also on neutrophils, monocytes, osteoclasts, and mast cells. It affects B cell signaling through different surface molecules, including BCR (Milo, 2019). BTK is required for B cell maturation, antigen presentation, proliferation, and differentiation into antibody-producing plasma cells (45). BTK inhibition results in B cell inhibition, which is quickly reversed once the treatment stops (Figure 9).

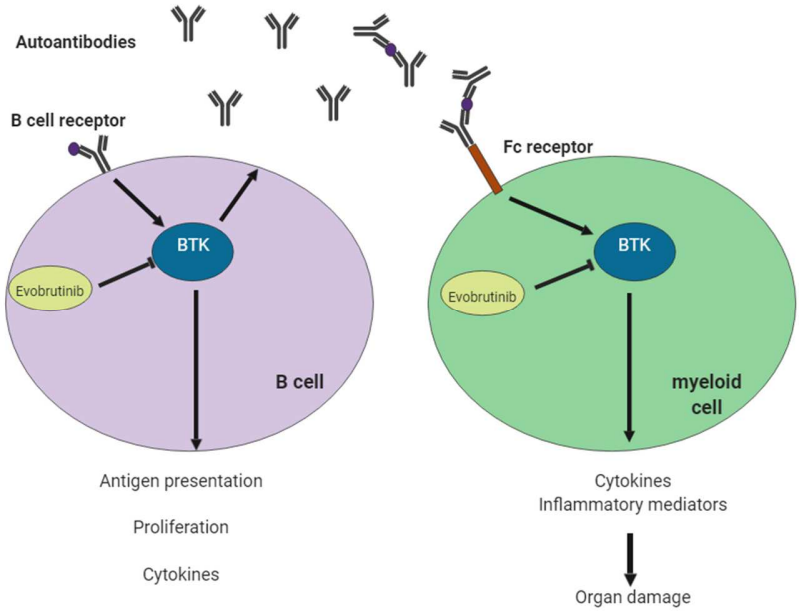

Figure 9. Mechanism of action of evobrutinib. By inhibiting BTK, evobrutinib stops B cells from proliferating, maturing, and differentiating into antibody-secreting plasma cells. Without antibody-antigen complex to bind to Fc receptors of myeloid cells, no pro-inflammatory cytokines or inflammatory mediators can be secreted.
(picture created with BioRender software)

Evobrutinib is an oral BTK inhibitor that promotes polarization of anti-inflammatory human monocytes and inhibits pro-inflammatory cytokine and macrophage release. A phase-II trial with RRMS patients was recently completed and there has been a decrease in the number of lesions, with main adverse effects being elevations in the levels of lipase and reversible transaminase. The effect of evobrutinib on both the innate and adaptive immunity certainly supports further development and research for clinical use (42).

7. Discussion

MS was first discovered in the second half of the 19th century. Despite considerable progress in understanding its pathogenesis, it still has no definite cure. All MS treatments modulate the course of the disease by targeting the immune system. Efficacy of MS DMTs has improved as our understanding of MS pathogenesis and available technologies have advanced.

An all-encompassing comparative study of all available MS therapies has not been done yet, but meta-analyses comparing different placebo-controlled, comparative, and randomized trials have been conducted. In one such study, Fogarty et al. found that annual relapse rate was reduced following all DMTs when compared to placebo, though the efficacy of the reduction varied, as did side-effects of different treatments. First-line therapies, such as IFN-β and GA had lower ranking, with annual relapse rate reduction being 15-36% and the risk of the disease progressing being 19-28% lower than the placebo. Natalizumab and fingolimod, the two second-line therapies in this study, were shown to reduce annual relapse rate by 50-69%, together with alemtuzumab, the only third-line therapy analyzed there. Second- and third-line therapies differed in the risk of disability progression inside a 3-month period. While fingolimod reduced the risk about the same as IFN-β and GA, natalizumab reduced it by 38-45%. Alemtuzumab had a risk reduction of 68% (38). Unfortunately, no other therapy targeting B cells except alemtuzumab was mentioned in this study.

Another study has been done by Granqvist and colleagues in Sweden on 494 patients with RRMS. It compared rituximab and its effectiveness to different first- and second-line therapies. It is been found that patients receiving rituximab treatment had the lowest annual discontinuation rate, lower annual

relapse rate, and lower neuroradiologic activity of the disease (46). This shows the advantages of rituximab as opposed to older treatments, and the improvement made in anti-CD20 mAb treatments suggests the direction in which MS therapy needs to move once those treatments prove to be not as effective.

There have been no comprehensive comparative studies done on other third-line therapies. These kinds of studies would help in identifying the most effective therapy, or their combination. Phase of the disease and clinical profile of individual patients would have to be taken into account, as would the risks and benefits connected with each therapy. Despite the lack of exhaustive comparative studies, early research and clinical trials show promise, with a lot of new mAb therapies having good efficacy with minimal adverse effects. They could provide an opportunity for a personalized therapy approach, something which could help in a disease as diverse in its symptoms and clinical image as MS. Overall, B cell-focused therapies offer a new approach to MS treatment, as is shown with newer DMTs that are going through clinical trials or are already approved for clinical use.

The atacicept clinical trial has led to worsening of annual relapse rates and the disease course, but that does not mean that BAFF and APRIL should be discounted as potential DMT targets. Lowering their concentration or preventing their interaction with B cells could lead to B cell depletion, which in turn leads to an improved MS course. Before such a therapy could be developed, however, BAFF and APRIL should be studied more carefully in connection to MS pathogenesis.

Not all B cell functions are equally important as therapeutic targets. For example, despite the fact that OCBs are an important biomarker in diagnosing MS, the success of DMTs which have had no effect on OCB levels has shown that they probably do not have a big role in neuroinflammatory

processes that occur in MS. New biomarkers, ones not necessarily connected to B cells, could prove useful in MS diagnostics and potential patient screenings to determine risk factors. This ties in with the personalized treatment approach mentioned above, and knowing from the start which therapy would be the most effective would save a lot of time and money. The best way to save money would of course be a definitive cure for MS, and hopefully one day, technology and research will advance enough to find such a cure.

8. Conclusion and future directions

B cells undoubtedly play a big role in MS. They produce antibodies, and OCBs are the only immunological diagnostic marker for MS. They also have a role as APCs for T cells, which are considered to be key players in the MS pathogenesis. B cells can affect the course of MS through production of different cytokines, both anti- and pro-inflammatory. All of this makes better understanding of B cells a crucial step in understanding MS itself.

Antibodies and blood plasma affect the course of the disease, indicated by the success of trials for different DMTs targeting them. However, trials done on atacicept have shown that we don't understand everything about MS and that treatments can work in unexpected ways. What needs to be researched more is determining which patients respond favorably to a certain therapy, and keeping the effects of those therapies under control, especially possible consequences of long-term B-cell depletion. There has been significant process in understanding B cells in MS therapies, and future research needs to focus on developing safer therapies that cater to the individual patients.

To conclude, B cells are not only important in the pathogenesis of MS, but the therapy as well. With MS being a chronic disease whose prevalence is slowly growing, personalized therapy done through B cell-targeting mAbs will likely become more important than ever before.

9. References

1. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nature Reviews Immunology. 2015 Sep;15(9):545–58.

2. Loma I, Heyman R. Multiple Sclerosis: Pathogenesis and Treatment. Curr Neuropharmacol. 2011 Sep;9(3):409–16.

3. Dargahi N, Katsara M, Tselios T, Androutsou M-E, de Courten M, Matsoukas J, et al. Multiple Sclerosis: Immunopathology and Treatment Update. Brain Sci. 2017 Jul 7;7(7).

4. Files DK, Jausurawong T, Katrajian R, Danoff R. Multiple sclerosis. Prim Care. 2015 Jun;42(2):159–75.

5. Leray E, Moreau T, Fromont A, Edan G. Epidemiology of multiple sclerosis. Revue Neurologique. 2016 Jan 1;172(1):3–13.

6. Ebers GC, Sadovnick AD, Dyment DA, Yee IML, Willer CJ, Risch N. Parent-of-origin effect in multiple sclerosis: observations in half-siblings. Lancet. 2004 May 29;363(9423):1773–4.

7. Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. The Lancet Neurology. 2010 May 1;9(5):520–32.

8. Lorscheider J, Buzzard K, Jokubaitis V, Spelman T, Havrdova E, Horakova D, et al. Defining secondary progressive multiple sclerosis. Brain. 2016;139(Pt 9):2395–405.

9. Mahad DH, Trapp BD, Lassmann H. Pathological mechanisms in progressive multiple sclerosis. Lancet Neurol. 2015 Feb;14(2):183–93.

10. Doshi A, Chataway J. Multiple sclerosis, a treatable disease. Clin Med (Lond). 2017 Dec;17(6):530–6.

11. Claes N, Fraussen J, Stinissen P, Hupperts R, Somers V. B Cells Are Multifunctional Players in Multiple Sclerosis Pathogenesis: Insights from Therapeutic Interventions. Front Immunol [Internet]. 2015 Dec 21 [cited 2019 Jun 21];6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4685142/

12. Constantinescu CS, Gran B. The essential role of T cells in multiple sclerosis: a reappraisal. Biomed J. 2014 Apr;37(2):34–40.

13. Dulamea AO. Role of Oligodendrocyte Dysfunction in Demyelination, Remyelination and Neurodegeneration in Multiple Sclerosis. Adv Exp Med Biol. 2017;958:91–127.

14. Constantinescu CS, Farooqi N, O'Brien K, Gran B. Experimental autoimmune encephalomyelitis (EAE) as a model for multiple sclerosis (MS). Br J Pharmacol. 2011 Oct;164(4):1079–106.

15. Baecher-Allan C, Kaskow BJ, Weiner HL. Multiple Sclerosis: Mechanisms and Immunotherapy. Neuron. 2018 Feb 21;97(4):742–68.

16. Fletcher JM, Lalor SJ, Sweeney CM, Tubridy N, Mills KHG. T cells in multiple sclerosis and experimental autoimmune encephalomyelitis. Clinical & Experimental Immunology. 2010;162(1):1–11.

17. Zhang C, Tian Z. NK cell subsets in autoimmune diseases. Journal of Autoimmunity. 2017 Sep 1;83:22–30.

18. Gross CC, Schulte-Mecklenbeck A, Wiendl H, Marcenaro E, Kerlero de Rosbo N, Uccelli A, et al. Regulatory Functions of Natural Killer Cells in Multiple Sclerosis. Front Immunol [Internet]. 2016 Dec 19 [cited 2019 Jun 17];7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5165263/

19. Mauri C, Bosma A. Immune Regulatory Function of B Cells. Annual Review of Immunology. 2012;30(1):221–41.

20. Leavenworth JW, Schellack C, Kim H-J, Lu L, Spee P, Cantor H. Analysis of the cellular mechanism underlying inhibition of EAE after treatment with anti-NKG2A F(ab')2. PNAS. 2010 Feb 9;107(6):2562–7.

21. Bogie JFJ, Stinissen P, Hendriks JJA. Macrophage subsets and microglia in multiple sclerosis. Acta Neuropathol. 2014 Aug 1;128(2):191–213.

22. Lehmann-Horn K, Kronsbein HC, Weber MS. Targeting B cells in the treatment of multiple sclerosis: recent advances and remaining challenges. Ther Adv Neurol Disord. 2013 May;6(3):161–73.

23. Lehmann-Horn K, Häusser-Kinzel S, S. Weber M. Deciphering the Role of B Cells in Multiple Sclerosis—Towards Specific Targeting of Pathogenic Function. International Journal of Molecular Sciences. 2017 Sep 23;18:2048.

24. Brändle SM, Obermeier B, Senel M, Bruder J, Mentele R, Khademi M, et al. Distinct oligoclonal band antibodies in multiple sclerosis recognize

ubiquitous self-proteins. Proc Natl Acad Sci USA. 2016 12;113(28):7864–9.

25. Kabat EA, Moore DH, Landow H. AN ELECTROPHORETIC STUDY OF THE PROTEIN COMPONENTS IN CEREBROSPINAL FLUID AND THEIR RELATIONSHIP TO THE SERUM PROTEINS 1. J Clin Invest. 1942 Sep;21(5):571–7.

26. Delgado-García M, Matesanz F, Alcina A, Fedetz M, García-Sánchez MI, Ruiz-Peña JL, et al. A new risk variant for multiple sclerosis at the immunoglobulin heavy chain locus associates with intrathecal IgG, IgM index and oligoclonal bands. Mult Scler. 2015 Aug;21(9):1104–11.

27. Mancuso R, Franciotta D, Rovaris M, Caputo D, Sala A, Hernis A, et al. Effects of natalizumab on oligoclonal bands in the cerebrospinal fluid of multiple sclerosis patients: a longitudinal study. Mult Scler. 2014 Dec;20(14):1900–3.

28. Nakayamada S, Tanaka Y. BAFF- and APRIL-targeted therapy in systemic autoimmune diseases. Inflammation and Regeneration. 2016 Jul 21;36(1):6.

29. Wekerle H. B cells in multiple sclerosis. Autoimmunity. 2017 Feb;50(1):57–60.

30. Krumbholz M, Derfuss T, Hohlfeld R, Meinl E. B cells and antibodies in multiple sclerosis pathogenesis and therapy. Vol. 8. 2012.

31. Sospedra M, Martin R. Immunology of Multiple Sclerosis. Semin Neurol. 2016 Apr;36(2):115–27.

32. Hartung DM, Bourdette DN, Ahmed SM, Whitham RH. The cost of multiple sclerosis drugs in the US and the pharmaceutical industry. Neurology. 2015 May 26;84(21):2185–92.

33. Goodin DS. Glucocorticoid treatment of multiple sclerosis. Handb Clin Neurol. 2014;122:455–64.

34. Weinstock-Guttman B, Nair KV, Glajch JL, Ganguly TC, Kantor D. Two decades of glatiramer acetate: From initial discovery to the current development of generics. Journal of the Neurological Sciences. 2017 May 15;376:255–9.

35. Bell C, Anderson J, Ganguly T, Prescott J, Capila I, Lansing JC, et al. Development of Glatopa® (Glatiramer Acetate): The First FDA-Approved

Generic Disease-Modifying Therapy for Relapsing Forms of Multiple Sclerosis. J Pharm Pract. 2018 Oct;31(5):481–8.

36. Haji Abdolvahab M, Mofrad MRK, Schellekens H. Interferon Beta: From Molecular Level to Therapeutic Effects. Int Rev Cell Mol Biol. 2016;326:343–72.

37. Kieseier BC. The mechanism of action of interferon-β in relapsing multiple sclerosis. CNS Drugs. 2011 Jun 1;25(6):491–502.

38. Fogarty E, Schmitz S, Tubridy N, Walsh C, Barry M. Comparative efficacy of disease-modifying therapies for patients with relapsing remitting multiple sclerosis: Systematic review and network meta-analysis. Multiple Sclerosis and Related Disorders. 2016 Sep 1;9:23–30.

39. Mandal P, Gupta A, Fusi-Rubiano W, Keane PA, Yang Y. Fingolimod: therapeutic mechanisms and ocular adverse effects. Eye (Lond). 2017 Feb;31(2):232–40.

40. Clerico M, Artusi CA, Liberto AD, Rolla S, Bardina V, Barbero P, et al. Natalizumab in Multiple Sclerosis: Long-Term Management. Int J Mol Sci. 2017 Apr 29;18(5).

41. Negron A, Robinson RR, Stüve O, Forsthuber TG. The role of B cells in multiple sclerosis: Current and future therapies. Cell Immunol. 2019 May;339:10–23.

42. Milo R. Therapies for multiple sclerosis targeting B cells. Croat Med J. 2019 Apr;60(2):87–98.

43. Li H, Hu F, Zhang Y, Li K. Comparative efficacy and acceptability of disease-modifying therapies in patients with relapsing-remitting multiple sclerosis: a systematic review and network meta-analysis. J Neurol. 2019 May 25;

44. Havrdova E, Horakova D, Kovarova I. Alemtuzumab in the treatment of multiple sclerosis: key clinical trial results and considerations for use. Ther Adv Neurol Disord. 2015 Jan 1;8(1):31–45.

45. Haselmayer P, Camps M, Liu-Bujalski L, Nguyen N, Morandi F, Head J, et al. Efficacy and Pharmacodynamic Modeling of the BTK Inhibitor Evobrutinib in Autoimmune Disease Models. The Journal of Immunology. 2019 May 15;202(10):2888–906.

46. Granqvist M, Boremalm M, Poorghobad A, Svenningsson A, Salzer J, Frisell T, et al. Comparative Effectiveness of Rituximab and Other Initial

Treatment Choices for Multiple Sclerosis. JAMA Neurol. 2018 Mar 1;75(3):320–7.