Chapter

Cellular Cytotoxicity and Multiple Sclerosis

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Abstract

Multiple sclerosis (MS) is an autoimmune disease in which discrete central nervous system lesions result from perivascular immune cell infiltration associated with damage to myelin (demyelination), oligodendrocytes and neurons. This culminates in debilitating neurological symptoms, primarily affecting women in their child-bearing years. Both the innate and adaptive branches of the immune system have been implicated in disease initiation and progression, and although the underlying cause remains elusive, there is compelling evidence for a complex interaction between genetic and environmental factors, leading to inflammation and neurodegeneration. Both direct cellular toxicity and antibody-dependent cellular cytotoxicity (ADCC) involving several cell types have been identified in playing major roles. These cells and their interactions in the pathogenesis of MS will be discussed.

Keywords: multiple sclerosis, cellular cytotoxicity, cell subsets, human, MS mouse models

1. Introduction

Multiple sclerosis (MS) is the most prevalent neurological disease among young adults in developed countries, with approximately 2.8 million people being affected worldwide [1]. It principally affects women in their prime, with diagnosis typically occurring between the ages of 20 and 40. The disease is debilitating due to central nervous system (CNS) damage resulting from activated lymphocytes migrating across the blood brain barrier (BBB) and engaging in a proinflammatory response. This causes cells to attack and destroy the myelin sheaths that coat the axons of neurons of the brain, spinal cord and optic nerve, as well as the myelinating cells or oligodendrocytes, and the axons themselves [2]. As neurons receive sensory input from external sources and send motor commands to the muscles by relaying interneuron electrical impulses, breakdown causes interruption to the signals being sent around the body, and dependent on where the damage occurs, results in different signs and symptoms. These can include vision impairment, muscle spasms and numbness, bladder and bowel issues, fatigue and difficulty walking [3]. Most people with MS have progressive neurological disability which, though not usual, can culminate in death [4]. The area of damage or scarring caused by the immune system attack is

called a lesion or plaque, and can be visualised by magnetic resonance imaging (MRI). A definitive diagnosis of MS is made when these plaques are shown to be reoccurring and when there is the clear presence of clinical symptoms [3].

Two major types of MS have been recognised, primary progressive multiple sclerosis (PPMS), diagnosed in approximately 15% of patients and which results in steady progression of disease from onset, and relapsing remitting multiple sclerosis (RRMS), which affects approximately 80% of patients and is characterised by periods of relapse separated by periods of remit without worsening of symptoms [5–7]. Most patients with an initial diagnosis of RRMS will, within 20 years of diagnosis, progress to secondary progressive multiple sclerosis (SPMS) where the stages between relapse and remit shorten and there is a steady decline with an increase in symptoms and disease progression [8]. Up to approximately 5% of MS patients have progressive relapsing multiple sclerosis (PRMS) and this characterised by steady disease progression with occasional relapses [9].

The exact cause of MS is still unknown, however research has determined that it is an autoimmune disease, arising from complex interactions between environmental and genetic influences. There is a latitude incidence variance, with prevalence of MS increased the further one is from the equator; sunlight and vitamin D are therefore being investigated as disease triggers [1, 10–12]. Childhood exposure to bacteria and viruses have also been investigated, due to a person's disease risk being set as the incidence of the region they moved to prior to puberty [13, 14]. Of note, every patient with MS have previously been exposed to Epstein–Barr virus (EBV) [15, 16]. Smoking also increases a person's risk and worsens symptoms following diagnosis [17].

Although the disease is not inherited, it has a genetic component, with those having an affected first degree relative exhibiting an increased incidence of disease [18], and twin studies indicate that there is a 30% chance of developing disease in the second twin if the first has been diagnosed with MS [19]. Genome wide association studies (GWAS) have identified more than 230 genes associated with a person's MS risk, several being immune genes, particularly those of T cells, B cells, natural killer (NK) cells, monocytes and microglia, implicating involvement of both major branches of the immune system, the innate and adaptive immune responses, in initiation and progression of disease [20–23]. These studies are supported by several human and animal model functional studies [24–26].

Cellular toxicity, or the ability to kill other cells, is an important effector mechanisms of the immune system to protect us from infections, cancer or autoimmune diseases. There is a close association between inflammation and neurodegeneration, and cellular toxicity has been implicated as a having a major role in MS [27]. The main players are CD8, or cytotoxic, T cells and NK cells. Cellular toxicity can operate by many mechanisms including NK cell release of lytic granules containing perforin or granzymes to kill directly, or by inducing death receptor-mediated apoptosis via tumour necrosis factor (ligand) superfamily member 10 (TRAIL) or Fas Ligand (FasL) expression on CD8 T cells [28]. There are also antibody-dependent cell-mediated cytotoxic mechanisms (ADCC), where B cells produce antigen specific antibodies or immunoglobulins, that will coat a pathogen or foreign body, marking them for killing or destruction through cell to cell cytoloysis by effector immune cells expressing FcγRIIIA (CD16A), including classical NK cells, monocytes/macrophages, neutrophils, eosinophils, NKT cells, or γδT cells (reviewed in [29]).

2. Innate and adaptive immunity

The immune system of vertebrates is commonly divided into two main complementary parts, innate and adaptive immunity, the bridge between which is critical for an efficient and effective immune response.

The innate immune system is evolutionary the most primitive, where there is non-specific response to a broad class of antigens. The haematopoietic cells involved include macrophages, dendritic cells, mast cells, neutrophils, eosinophils, NK cells and NKT cells. Although 1908 Nobel Prize winner, Elie Metchnikoff, first described an important role for the innate immune system [30], it is only now being recognised as a critical regulator of human inflammatory disease. Innate immunity involves the recognition of infected cells through surface recognition receptors. These are termed pattern recognition receptors (PRRs) which recognise pathogen associated molecular patterns (PAMPs) unique to non-vertebrate cells, including bacteria and fungi. They are also on internal vesicle membranes for recognition of viral ssRNA and dsRNA and for distinguishing lysed bacterial components [31]. Cytotoxic innate lymphocytes can lyse abnormal or infected cells through the release of cytotoxic granules containing perforin or granzymes, and antigen presenting cells (APCs) can be activated by the innate immune system to present pathogen antigens on their surface. Once activated they will migrate to secondary lymph organs to present their antigen to T cells, and in so doing also activate the adaptive immune system response [32, 33]. The innate immune system therefore functions through a combination of cellular defences and humoral components to defend against nonspecific antigens before activating B and T cells, triggering an adaptive immune response. Speed is the main advantage of innate immunity, with a protective inflammatory response being generated within minutes of pathogen exposure.

Another part of innate immunity is the complement system, which is made up of several small proteins that have been synthesised in the liver and circulate in the blood as active precursors that when stimulated are proteolytically cleaved to release cytokines, leading to a cascade of reactions, ultimately resulting in complement activation or fixation [34]. As the name suggests, they complement or enhance the ability of antibodies and phagocytic cells to clear damaged or diseased cells by promoting inflammation and attack of the cell membrane of the pathogen. Antibodies, generated by the adaptive immune system, can activate the complement system.

Adaptive immunity, sometimes referred to as acquired immunity, is highly specialised and helps to protect the body by recognising antigens, whether they are foreign to the host's immune system (exogenous), produced by intracellular bacteria or viruses (intracellular) or produced by the host (autoantigen). The adaptive immune system also remembers previously encountered antigens, leading to quicker response times [35]. T and B lymphocytes are the main cells mediating adaptive immunity, with T cells being further divided into the cytotoxic CD8 T cells and CD4 T cells that constitute several classes of what are commonly referred to as "helper T cells". These cell have produced highly specific receptors for recognition of hundreds or even thousands of antigens through genetic recombination, and this facilitates pathogen specific immunologic effectors pathways, the generation of immunological memory and the regulation of host immune homoeostasis [36].

CD8 T cells recognise infected cells through interaction of T cell receptors with antigens presented by major histocompatibility complex (MHC) class I on the infected cell. The target cell is then killed by the release of cytotoxins, such as perforin and

granzymes, from the CD8 T cell [28]. CD4 T cells, on the other hand, recognise antigens presented in the context of MHC II on an APC. Binding to MHC II molecules activates CD4 T cells to release cytokines, which can stimulate CD8 T cells, macrophages and B cells to form an immune response (reviewed in [37]). They can, for example, release cytokines as instructors to CD8 T cells to release cytotoxins, or to B cells to produce pathogen specific antibodies. They therefore instigate and shape adaptive immune responses dependent on the cytokines they release. These can be mainly Th1, or inflammatory, in nature, such as IFN-γ and IL-12, responsible for the control of intracellular pathogens, or polarised to a more anti-inflammatory Th2 response, where cytokines such as IL-4, IL-5 or IL-13 are produced [38, 39]. A disturbance in this Th1/Th2 response can have severe consequences, be they more Th2 in nature, driving asthma and allergy, or Th1 driven, resulting in autoimmune diseases, including MS (reviewed in [40]). A couple of the more recently identified CD4 T cells subsets include Th17 cells that are characterised by production of IL-17 and IL-23, and have been linked to inflammatory diseases, and T regulatory (Treg) cells, which are important in maintaining homeostasis and tolerance of the immune system [41–43]. Tregs express the transcription factor FoxP3 which is essential for their development and function [44–46]. In humans, mutations in FOXP3 have been found to result in immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, providing evidence that anomalies of Tregs can cause autoimmune disease and allergy [47].

During production of the T cell receptor (TCR) on T cells and B cell receptor (BCR) on B cells, random genetic recombination events can lead to receptors being produced that are specific to autoantigens [48, 49]. To prevent reaction to self, cells undergo central and peripheral tolerance events through which autoreactive cells are apoptotically removed, first in the primary lymphoid organs of the thymus (T cell) and bone marrow (B cell), and if this fails, in the secondary lymphoid organs after cells migrate to the periphery [49]. Self-reactive antibodies account for 55–75% of all antibodies expressed by early immature B cells, including polyreactive and anti-nuclear specificities [49]. However, it is estimated that the majority of newly produced B cells do not reach maturity, and during central and peripheral tolerance most of the self-reactive B cells are removed. If both selection processes fail in T or B cells, this will result in T and B cells able to react with the body's own cells and tissues. These events lead to autoimmune disease.

3. Autoimmune disease

Inflammation as a response of the body to infection or cell injury is a well-known concept that dates back to the beginning of medicine. However, Metchnikoff pointed out that although normally a method of protection, inflammation that exceeds normal bounds can cause disease [27]. Even with this knowledge, it was not until the 1950s that inflammation was recognised as inducing an autoimmune reaction responsible for disease. Autoimmune disease is characterised by an excessive immune response against self, often resulting in inflammation and tissue destruction, in the absence of a threat to the organism [50, 51]. Aberrant immune responses have been associated with over 80 disorders, including multiple sclerosis, and affects 5–7% of the population [52]. Clinical observations over the past decade have suggested that the prevalence of all autoimmune disease, not just MS, is increasing, bringing the issue to the forefront of scientific interest [53, 54]. Successful treatment of autoimmune disease is also of great societal interest, as they are commonly characterised by

chronic natures, ongoing health care costs, and debilitating issues resulting in loss of productivity.

Immunological self-tolerance is maintained in part by Tregs. Tregs are CD4 T cells that actively and dominantly supress lymphocytes, particularly self-reactive T cells in the normal periphery that exist despite the deletion mechanisms in the thymus [43]. Natural CD25+ CD4 Tregs utilise several modes of suppression, including cell contact dependent mechanisms, such as the killing of APCs or responder T cells by granzyme and perforin, and by mediation of soluble factors, such as the secretion of immunosuppressive cytokines like IL-10, TGF-β or IL-35, or deprivation of cytokines necessary for expansion and survival of responder T cells (reviewed in [55, 56]).

Optimal T cell function relies on a carefully maintained state of equilibrium. When one subpopulation of T helper cells is activated, others are modulated or inhibited to promote the most specific effector response to the threat [57]. The cellular development of Tregs shares a common cytokine with Th17 cells, TGF-β [41, 42]. Th17 cells are the opposing force to Tregs, serving as an effector lymphocyte population that plays a key role in autoimmunity [41, 42]. At homeostasis, Th17 cells promote gut barrier defence, granulopoiesis, granulocyte chemotaxis and immunity against extracellular pathogen [58]. IL-17 induces granulopoesis indirectly through the stimulation of fibroblasts, epithelial and endothelial cells to secrete GM-CSF, IL-6, IL-8 and MIP-2, with IL-8 and MIP-2 enhancing chemotaxis of neutrophils [59, 60]. While Th17 cell mediated immunity is crucial for maintaining mucosal and haematopoietic homeostasis, too strong a response can induce autoimmunity. The relationship between Tregs and effector Th17 must remain balanced to provide the optimal functional immunity and health of an organism.

Another theory of immune regulation is the hypothesis of homeostasis between Th1 and Th2 cells. The subpopulations can be distinguished by the cytokines they produce and the expression of difference cell surface molecules. Th1 cells are responsible for cell mediated immunity, phagocyte dependent protective responses, B cell activation and production of opsonising antibodies such as IgG1, whereas Th2 cells produce cytokines that are responsible for strong antibody production, eosinophil activation and inhibition of several macrophage functions, thus providing phagocyte independent protective responses [61]. Th2 cells are also responsible for the general activation of B cells. When the Th1/Th2 paradigm is thrown out of balance by failure of central or peripheral tolerance, immunological disorders can occur due to uncontrolled responses [61].

4. MS and the immune system

MS arises when there is an imbalance in the body's immune response, shifting it from a beneficial immune process that fights infection and disease towards a self-aggressive immune attack on the cells within the CNS (**Figure 1**). Genetic and environmental factor interaction may facilitate movement of autoreactive T cells, macrophages and NK cells and demyelinating antibodies from the periphery to the CNS. In the periphery self-antigens can be presented on MHC II molecules by APCs to TCRs on T cells, thereby activating proinflammatory T cells [48]. The activated T cells can then migrate through the blood brain barrier to the brain and spinal cord [2]. Once in the CNS the T cells can be reactivated by CNS antigens presented on MHC II by other APCs, primarily microglial cells [62]. Secretion of proinflammatory Th1 cytokines by the reactivated T cells can induce CNS inflammation by activating

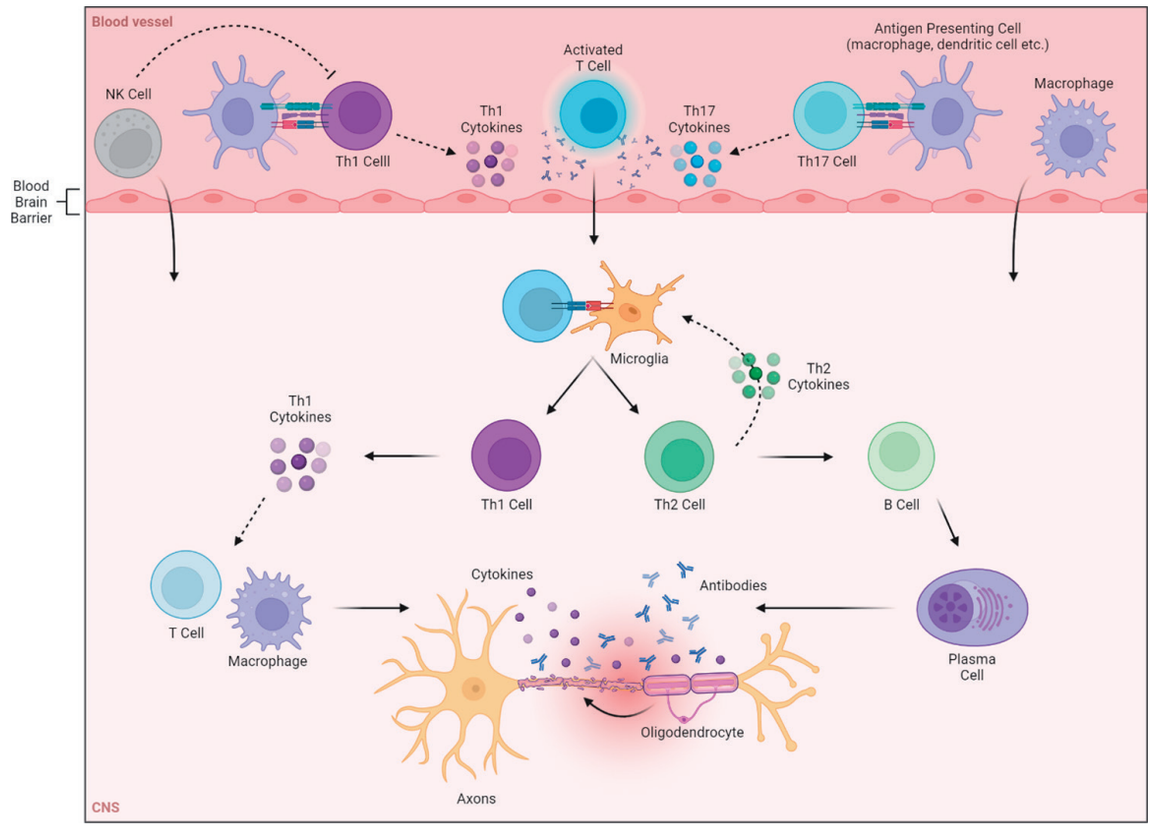

Figure 1.
Multiple sclerosis pathogenesis. Autoreactive T cells, macrophages and NK cells, and demyelinating antibodies, may migrate across a compromised blood brain barrier. T cells are reactivated in the central nervous system by antigen presenting cells (APC). Anti-inflammatory cytokines released by Th2 cells can stimulate B cells to differentiate into plasma cells that secrete demyelinating antibodies. Alternatively, the release of proinflammatory cytokines by Th1 cells can enhance immune response, via activation of other immune cells such as CD8 T cells and macrophages to attack the myelin sheath and oligodendrocytes causing demyelination and the development of clinical symptoms of MS (created with BioRender.com by A Willson).

macrophages, B cells and other T cells [63]. The antibodies can also initiate a complement cascade resulting in assembly of the membrane attack complex, forming pores in the myelin membranes.

5. Cell types

While inflammation and neurodegeneration are correlated in active lesions, research suggests that neurodegeneration may become independent from inflammation in progressive disease [64]. There are many MS therapeutics that suppress proinflammatory cytokines or their effector functions, but not all treatments show equal efficacy and can cause unintended effects. Currently, there is no cure. It is thus becoming clear that there is a need to elucidate the different populations important in initiating and progressing disease, and by studying their interactive networks, identify possible areas for targeted intervention.

5.1 T cells

While there is overwhelming evidence of a role for T cells in the pathogenesis of MS, further studies in humans and in the mouse model of disease, experimental autoimmune encephalomyelitis (EAE), provides compelling evidence that other cell types

play major roles. Linkage to the Human Leukocyte antigen (HLA) locus, including MHC I and II genes, was the first genetic locus identified, and still provides today the strongest linkage to MS. Further studies have identified an extended HLA haplotype, HLA-DRB1*15:01, DQA1*0102, DQB1*0602, within the MHC class II region [65]. As MHC II molecules specifically present peptide antigens to activate CD4 T cells, this suggests that CD4 T cells are important in initiation and progression of MS.

Th1 cells are a lineage of CD4 effector T cells that promote cell mediated immune responses and are necessary for defence against intracellular viral and bacterial pathogens. They were originally believed to be the main pathogenic T cells in MS, not only because susceptibility genes were linked to MHC II molecules, but also because immune surveillance of a healthy brain to scan for infection, showed favouring towards infiltration by Th1 cells, and therapeutic strategies designed to induce a shift from Th1 to Th2 immune response resulted in beneficial outcomes in MS patients [66–68].

The development of Th1 cells is coupled to the involvement of cell-extrinsic and cell-intrinsic factors, including signal transducer activator 1 (STAT1), the transcription factor Tbx21, IL-21 and STAT4 [69]. The CD4-Th1 model for MS was further supported by a trial performed in 1987, which found that administering IFN-γ to RRMS patients exacerbated disease. An accompanying increase in circulating monocytes bearing class II (HLA-DR) surface antigens suggested that the attacks induced by the treatment were immunologically mediated [70].

Th1 cells are also known to drive EAE. However, it was found that transgenic mice that lacked Th1 cells developed more severe EAE, thereby contradicting the Th1 cell theory for MS [71]. This conundrum was partially resolved following further investigation involving IL-23, a heterodimer cytokine composed of a unique p19 subunit and a common p40 subunit shared with IL-12. IL-23 promotes development of Th17 cells as opposed to Th1 cells [72]. Early studies on Th17 cells therefore dismissed a role for the previously favoured Th1 cells, but more recent research suggests that both cell types may play distinct roles in pathology [73]. It was suggested that Th1 cells accessed the CNS initially and subsequently facilitated the recruitment of Th17 cells [73].

Analysis of CNS tissue revealed distinct histopathological features and immune profiles depending on cytokine modulated T cells. IL-12p70 driven disease was characterised by macrophage-rich infiltrates, however in IL-23 driven lesions it was found that neutrophils and the growth factor, granulocyte colony stimulating factor (CSF), were the most prominent [74]. Research has shown that while IL-23 is commonly associated with the expansion of Th17 cells or the stabilisation of the Th17 phenotype, a similar course of EAE has been reported following the transfer of MOG-specific T cells into either wild type or IL-23 knockout mice [75]. This suggests that once encephalitogenic cells have been generated, EAE can develop in the absence of IL-23. IL-23 may therefore only be necessary for disease induction and not the effector phase of disease.

While MHC II molecules were found to be the strongest associated with MS in genetic studies, the MHC I HLA-A*0301 allele, independent of the HLA II haplotype DRB1*15,DQB1*06, was found to be increased in MS patients [65]. There was also a negative association with the MHC I HLA-A*0201 and disease [76]. As MHC I molecules are recognised by CD8 T cells, this suggests that CD8 T cells play a role in MS.

In one of the first studies that shifted from a CD4 T cell focus, CD8 T cells outnumbered the CD4 T cell subset in all parenchyma samples from MS patients, regardless of the MS type, duration or speed of disease progression [77]. Research has also

shown that APCs, including dendritic cells (DCs), interact with T cells and proliferating lymphocytes, predominately CD8 T cells, at the margins of chronic active MS lesions [78]. CD8 T cells have also been found within active lesions of RRMS patients [77]. These T cells, and to a lesser extent, compartmentally differentially distributed B cells, have been shown to correlate with disease progression and damage.

CD8 T cells are an important subpopulation of MHC I restricted T cells, and are mediators of adaptive immunity. Cytotoxic T cells specialise in direct killing of cells that are infected, particularly with viruses, or are cancerous or damaged in other ways. Cytotoxic cells rely on two mechanisms for lytic activity: granule-dependent cytotoxicity (reviewed in [79]) and death receptor dependent cytotoxicity (reviewed in [80]). The principle mechanism used is granule-dependent cytotoxicity. In lesion prone areas of the CNS, T lymphocytes, including CD8 cytotoxic T lymphocytes (CTLs), are recruited to the affected tissue and brain cells are stimulated to present antigens to the T lymphocytes via de novo expression of MHC molecules. Although levels of MHC I and MHC II are very low in normal CNS parenchyma, neural injury leads to a massive increase in activated and phagocytotic microglial, which can serve as competent APCs [81]. To develop into functioning CD8 T cells, the TCR must recognise the MHC-peptide combination along with the costimulatory signal from APCs. While classical MHC I molecules necessary for CD8 T cell activation are not usually expressed on neural cells, they are induced in most inflammatory and degenerative CNS diseases [82].

Oligodendrocytes lack expression of costimulatory molecules and are thus unable to trigger the full effector of T cells, however they have been known to express MHC I in vitro [83]. Therefore, despite the lack of complete activation of the T cells, oligodendrocytes may still be targets of primed CTLs. MHC I expressing oligodendrocytes are susceptible to lysis by blood donor derived CD8 CTLs [83]. IFN-γ treated human oligodendrocytes also express Fas/CD95, and are therefore susceptible to death receptor dependent cytotoxicity [84]. Another component of the CNS, the neurons, were found to be capable of expressing MHC I when treated with IFN-γ [85, 86]. Medana and colleagues in 2000 discovered that hippocampal neurons were highly susceptible to direct application of cytotoxic granules, but showed no signs of perforin mediated lysis or membrane damage following attack by CTLs [87]. This effect was not observed in any other cell type.

Research to date indicates that all cellular elements of the CNS may act as targets to CTLs but that susceptibility and cytotoxic pathways involved vary dependent on the cell type and the immune activations during the course of the inflammatory process.

5.2 B cells

Historically, B cells have not been recognised as major players in regulatory function in the development of autoimmune diseases, although the identification of autoantibodies produced by autoreactive plasma cells and their pathogenic consequences are widely accepted [88]. B cells are considered effector cells as well as cells with immunoregulatory potential. B cells in MS patients express increased levels of costimulatory molecules, increasing the stimulation of antigen-reactive T cells [89]. It has been reported that MS patients have increased levels of IL-6 and GM-CSF, correlating with increased Th17 cells [90, 91]. B cell targeted therapies utilise B cell depleting monoclonal antibodies against the B cell marker CD20. These antibodies trigger B cell lysis through antibody dependent cellular cytotoxicity, complement dependent cytotoxicity or apoptosis induction [92].

5.3 NK cells

Administration of daclizumab, an alpha subunit of IL-2 receptor blocking monoclonal antibody, to MS patients was found to strongly reduce brain inflammation. This therapy, while being associated with a decline in circulating CD4 and CD8 T cells, also correlated with a significant expansion of $CD56^{bright}$ NK cells in vivo. This provided supporting evidence of NK cell-mediated negative immunoregulation of T cells during daclizumab treatment [93], and the identification of NK cells in association with MS, where positive outcome was possibly due to the treatment's effect of increasing the NK cell numbers [94, 95].

For decades, NK cells have been classified as a component of the innate immune system. However, evidence suggests that, like B and T cells, NK cells are educated during development, possess antigen-specific receptors, undergo clonal expansion and generate memory cells (reviewed in [96]). Research originally suggested that NK cells developed and underwent differentiation within the bone marrow, however more recent extensive ex vivo characterisation of haematopoietic precursor cells (HPCs) and downstream NK cell development intermediates (NKDIs) reveals that they are enriched in secondary lymphoid tissues (STLs), including the tonsils, spleen and lymph nodes [97–100]. This suggests that NK cells in humans can differentiate in the SLTs, and may do so preferentially.

Human NK cells are phenotypically defined by expression of CD56 and the lack of CD3 expression [101]. CD56 is the 140-kDa isoform of neural cell adhesion molecule (NCAM) found on NK cells and a minority of T cells [102]. NK cells are categorised into two distinct populations depending on the cell surface density of CD56. The majority of human NK cells, approximately 90%, express low levels of CD56 ($CD56^{dim}$) and high levels of FCyRIII (CD16), while the minority express higher levels of CD56 ($CD56^{bright}$) [103]. $CD56^{bright}$ NK cells have long being associated with an immunoregulatory role, due to increased production of NK-derived immunoregulatory cytokines, including IFN-γ, TNF-β, IL-10, IL-13 and GM-CSF, and reduced cytotoxicity compared to $CD56^{dim}$ NK cells [104]. $CD56^{bright}$ NK cells express receptors for cytokines such as IL-12, IL-15 and IL-18, produced by APCs, which can trigger proliferation of $CD56^{bright}$ NK cells and their production of molecules, including IFN-γ, IL-10 and IL-13 [104]. It has been demonstrated that DCs are a key source of cytokines for the activation of $CD56^{bright}$ NK cells [105]. Modulation and proliferation of $CD56^{bright}$ NK cells can also occur due to DC-derived IL-27 [105]. Activated NK cells can modulate the function of APCs by stimulating monocytes to produce TNF-α and kill immature DCs by a perforin-dependent process referred to as DC editing [106, 107]. However, more recent research has challenged this commonly accepted concept of $CD56^{bright}$ as the primary source of immunoregulatory cytokines. Studies have shown that $CD56^{dim}$ NK cells are also a major source of proinflammatory cytokines and chemokines that are induced rapidly after target cell recognition [108, 109].

The absence of MHC class I molecules, as indicated by virally infected cells or cancerous cells with MHC I downregulated, is not always sufficient to induce NK cell mediated death, suggesting that there must be activating receptors on NK cells whose affinity for target cell ligands dominates over the inhibitory signals of the NK cell. Some activating receptors identified include NKG2D, the NCR, and NKp80 [110–112]. NKG2D is the best characterised of these activating NK cell receptors. It is a C-type lectin-like receptor expressed on the surface of all human NK cells and recognises at least six ligands, each with a MHC class I homology [113]. Following

receptor-ligand interaction, NKG2D phosphorylates an adaptor protein that recruits and activates phosphatidylinositol-3 (PI-3) kinase, which results in perforin-dependent cytotoxicity [114, 115]. Gunesh et al. found that the deletion of CD56 on the NK92 cell line lead to impaired cytotoxic function. The knockout CD56 cells failed to polarise during immunological synapse formation and had severely impaired exocytosis of lytic granules [116].

Treatment of MS patients with IFN-β caused an expansion of $CD56^{bright}$ NK cells, and resulted in the population of $CD56^{dim}$ cells being diminished [117]. The study also found that the proportion of $CD56^{bright}$ NK cells was significantly higher in the secondary lymphoid tissues compared to the peripheral blood for the control group [117]. This suggested that $CD56^{bright}$ NK cells may preferably locate within secondary lymphoid tissues, where they are able to interact with T cells and contribute to control of disease activity in MS [117].

There is an ongoing debate as to whether NK cells have a predominately beneficial or detrimental role in EAE, made even more complex by the lack of CD56 expression on murine NK cells. Studies have shown that enhancing the regulatory features of NK cells ameliorates the disease course of EAE. When the interaction between NKG2A and its ligand Qa-1 (the murine equivalent to the human HLA-E) expressed on target cells were blocked by antibodies specific for either antigen, it was found that NKG2A-expressing NK cells in particular decreased CNS inflammation by killing microglial and T cells [118, 119].

Enrichment of NK cells through treatment with IL-2 coupled with a monoclonal antibody specific for IL-2 (IL-2 mAb) was also found to ameliorate EAE [120]. The IL-2 mAb supplements the proliferation of NK and CD8 T cells in mice by increasing the biological activity of the pre-existing IL-2 by formation of immune complexes [121]. Increased levels of IL-2 was also found to expand Tregs while preventing the induction of Th17 during EAE development [122]. However, NK cells have different effects during the early stages of EAE, and possibly MS, compared to the late stages. In the early stages NK cells were found to protect the CNS whereas NK cells were found to kill neural stem cells (NSCs) during the late stages of EAE, as a result of reduced expression of Qa-1 on NSCs [120, 123].

5.4 NKT cells

NKT cells are unique T lymphocytes that express NK cell lineage markers, and act as a bridge between the innate and adaptive immune system. NKT cells account for a small percentage of lymphocytes, but have profound immunomodulatory roles in a variety of diseases [124]. There are two categories of NKT cells, type I and type II. Type I NKT cells, also known as invariant NKT cells (iNKT cells), express a semi-invariant Vα24-Jα18 (Vα14-Jα18 in mice), paired with a restricted range of β chains, that recognises α-galactosylceramide (α-GalCer) presented by CD1d [125, 126]. Type II NKT cells use TCRα and β chains that are reactive to a broad range of antigens, but do not recognise α-Galcer [127].

Nonobese diabetic (NOD) mice are susceptible to MOG-induced EAE. However, if NKT cells are increased either by transgenesis or adoptive transfer, the mice show protection from disease [128]. EAE protection has been correlated with inhibition of Ag-specific IFN-γ production in the spleen, modulating the encephalitogenic Th1 response [128]. There is conflicting evidence as to the effects of deletion of NKT cells on EAE. Some studies resulted in no effect on disease[129], with other studies

showing disease exacerbation in CD1d-deficient and Jα18-deficient mice [130, 131]. Activation of type I NKT cells by α-GalCer has been shown to improve EAE outcome. These improvements arise by indirectly enhancing Th2 response and reducing the Th1 response, or potentiating the differential of immunosuppressive myeloid cells [131–134]. However conflicting studies showed that high doses of α-GalCer could worsen EAE by directly enhancing Th17 and Th1 differentiation through phosphorylation of STAT3 and activation of NK-κB [135].

NKT cells from MS patients have been reported to have an increased production of cytokines. IL-4 production was increased by CD4 NKT cell clones in RRMS compared to other MS progression types, causing significant Th2 bias [136]. However, NKT cells in progressive MS patients displayed proinflammatory profiles [137]. It has also been suggested that the current available drugs for MS treatment may function through NKT cell targeting. A large reduction of type I NKT cells in peripheral blood was associated with remission of MS [136]. Type 1 interferon-β (T1IFN-β), a popular disease modifying therapy (DMT) for RRMS treatment, has been noted to promote expansion and functionality of type I NKT cells in vitro and to prevent disease in in vivo models of MS [138]. Research indicates a diverse role for NKT cells in MS pathology due to cytokine production.

5.5 Monocytes and macrophages

Besides imbalances in cytokine levels in the CNS and cerebrospinal fluid (CSF), immune imbalances also occur in the blood of MS patients, as reflected by altered levels of cytokines and cytokine producing cells (reviewed in [139]). The cause of these imbalances are thought to be due to circulating monocytes, with monocytes and macrophages influencing early MS, mediating both pro and anti-inflammatory responses [140, 141].

Surface expression of CD14 and CD16 are used to distinguish three distinct monocyte subsets: classical ($CD14^{++}CD16^{-}$), intermediate ($CD14^{++}CD16^{+}$) and nonclassical ($CD14^{+}$ $CD16^{++}$) [142]. Monocytes and macrophages perform the key functions of antigen presentation and co-stimulation vital to the body's immune response, with important roles in T and B cell activation and differentiation via the CD40-CD154 interaction (reviewed in [143]). Macrophages are primarily derived from blood borne monocytes, are present at sites of active demyelination in MS, and are assumed to be a part of the demyelinating process [144]. These inflammatory cells produce a range of toxic oxygen metabolites which mediate host tissue destruction. During MS progression, there is a significant expansion of the $CD16^{+}$ monocyte population, which can primarily be attributed to nonclassical monocytes [145]. Depletion of these nonclassical monocytes may be an alternative to T and B cell depletion with the advantage of leaving the major classical monocyte population untouched. Selective subset depletion of monocytes may also supplement existing therapies to increase efficacy [145].

6. Conclusions

Multiple sclerosis is a complex autoimmune disease. Due to the many cell types involved in pathogenesis of the disease, therapeutics and treatments are often broad ranged and relatively inefficient. Further studies are necessary to uncover the genetic and environmental triggers leading to aberrant cellular toxicity and its role in MS

pathogenesis. Discovering these and the related pathways will potentially lead to more targeted therapeutics and the elimination of not only MS but other autoimmune and neurological diseases in the future.

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Conflict of interest

The authors declare no conflict of interest.

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