The melanoma cell adhesion molecule: a potential therapeutic target for multiple sclerosis

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Introduction

Currently, the vast majority of research indicates that multiple sclerosis (MS) is an inflammatory autoimmune disease of the central nervous system (CNS), in which the infiltration of lymphocytes eventually leads to irreversible damage to neuronal axons and the surrounding myelin[1]. This results in a variety of symptoms, including those affecting movement and sensation [2]. Although several types of MS exist, and the symptoms vary greatly between patients, the majority of individuals initially experience symptoms in the form of relapses, followed by periods of remission[2]. During these relapses, white matter of the CNS becomes inflamed, which is followed by the destruction of myelin by the invading immune cells[3]. Gradually, as the destruction of myelin increases, the damage overwhelms the re-myelination capacity of the resident oligodendrocytes, and the patient regains less and less of their original abilities, resulting in the progressive accumulation of disability [3].

It is proposed that the initial development of this disease begins with an increased migration of lymphocytes across the blood-brain barrier (BBB), which progresses from general surveillance to a pathological function when a regulatory defect allows these cells to mount an immune response within the CNS [4]. In a healthy individual, the infiltration of a small subset of lymphocytes into the CNS is a normal component of immune surveillance. In order for these cells to gain access to the CNS, they must undergo alterations to the molecules expressed on their surface [5]. This may include the expression of entirely new molecules, an increase in the density of certain molecules, or an alteration of the functional state of molecules already being expressed [5]. As both T and B-lymphocytes accumulate in the CNS, along with macrophages, the corresponding increase in the production of pro-inflammatory cytokines amplifies the immune response [6]. Re-myelination by surviving oligodendrocyte precursors can mediate the progression of MS to some extent [7,8], but in most cases demyelination eventually exhausts the capacity for tissue repair [9].

There have been several mechanisms identified that may potentially lead to tissue injury in multiples sclerosis, which vary from T-cell infiltration to hypoxia-like injury [10], or genetic deficiencies in oligodendrocyte activity [11]. There have also been numerous proposals for the cause of the initial onset of MS, which vary from viral infections to environmental conditions or genetic susceptibility, although a combination of these factors is likely involved [2]. Some researchers have even found evidence suggesting that MS may not be an autoimmune disorder at all, but that it may actually be a primary oligodendrogliopathy disease [12]. However, the vast majority of research does point to an autoimmune pathology [1]. Corresponding to the complicated nature of MS, there are currently a wide variety of research projects aimed at furthering our understanding of this unpredictable disease, as well as towards developing potential treatments. Producing alternative treatment options is of particular importance because many of the drugs that are currently in use are associated with a number of negative side effects. For example, corticosteroids are often prescribed during MS relapses, but are known to cause a number of potential severe side effects, including hypertension and osteoporosis.

At the moment some MS patients are being prescribed fingolimod, a drug that has been shown to reduce the frequency and severity of relapses by modulating receptors for sphingosine 1-phosphate [14]. This prevents lymphocytes from leaving tissues such as the thymus and lymph nodes, and migrating to the CNS [14]. While this treatment may be effective at reducing the progressive accumulation of disability, there are also serious implications associated with its prolonged use, which largely revolve around the widespread immunosuppression caused by preventing the egress of lymphocytes from their respective tissues[15]. Due to these adverse

effects, and the fact that fingolimod has some of the highest adherence and patient satisfaction ratings of the most commonly prescribed MS treatments [16], it is apparent that an alternative treatment option is necessary.

Recently, research has indicated the possibility of mobilizing endogenous neural stem cells to replace damaged oligodendrocytes in MS patients[17], which would lead to an increased potential for re-myelination. However, this treatment would not target the source of the demyelination, and would likely need to be used in combination with another treatment that acts to prevent the axonal damage from occurring in the first place. In an attempt to develop a treatment that could potentially prevent damage to the CNS, Larochelle et al.[18] identified a surface marker of pathogenic lymphocytes, which could serve as a potential therapeutic target. Specifically, they identified the melanoma cell adhesion molecule (MCAM), which they believe is used by these pathogenic lymphocytes to enter the CNS. The expression of MCAM by these lymphocytes was shown to be significantly up-regulated during MS relapses[18]. In addition, blocking the expression of MCAM restricted the migration of these lymphocytes across the blood-brain barrier (BBB), and reduced neurological deficits in experimental autoimmune encephalomyelitis (EAE) models (a common animal model used for MS research). If, through additional studies, MCAM is proven to be a crucial component of the adherence and transmigration processes, further research will be required in order to fully elucidate the role of MCAM with respect to these activities. This will enable the development of a specific and effective treatment that will prevent the damage caused by the migration of these lymphocytes into the CNS of MS patients.

Fingolimod: Mechanism of Action and Consequences

Understanding the mechanism of action of fingolimod is necessary in order to fully understand the source and nature of the consequences associated with the prolonged use of this drug. As fingolimod is highly preferred by patients[16], but also causes such serious side effects, it is apparent that alternative treatment options are required. It has been previously demonstrated that sphingosine-1 phosphate (S1P) is involved in the exiting of lymphocytes from the thymus, as well as other peripheral lymphoid organs[19]. Specifically, knockout mice lacking one of the S1P receptors (S1PRs) typically expressed on T and B lymphocytes (S1PR1) have shown that this receptor is required for emigration from the thymus, spleen, lymph nodes, and Peyer's patches [20]. T-cells lacking this receptor develop normally, but fail to exit the thymus, and T-cells that are heterozygous for the deficiency exit slower, compared to the wild-type [21].

Using the information about the role of S1PR1 in the circulation of lymphocytes, the drug fingolimod was developed, which binds to the S1PRs with very high affinity, and subsequently causes the internalization and degradation of these receptors. This inhibits lymphocyte egress from various lymphoid organs, including the thymus and lymph nodes[22]. Fingolimod is a structural analog of S1P, which binds with comparable affinity to each of the five identified S1P receptors [23]. Numerous immune cells, including T and B-lymphocytes, express several of these S1P receptors [15]. A crucial initial step in the development and progression of MS is the activation of these lymphocytes in the lymph nodes, and their subsequent migration to the CNS [24]. As described above, lymphocyte egress from the lymph nodes is based on a S1P gradient [19]. By binding to S1P receptors and causing their internalization and degradation, fingolimod effectively sequesters lymphocytes within lymphoid organs, and prevents their migration into peripheral blood[25].

A major concern associated with the use of fingolimod as a treatment for MS is the inevitable widespread immunosuppression caused by this drug; it not only drastically reduces the number of lymphocytes circulating in the peripheral blood, but it also impacts germinal centers and the migration of B cells [26]. Clinically, the impairment of the immune response in patients treated with fingolimod is comparable to that of patients living with HIV infections and those undergoing myelotoxic chemotherapy [15]. As a result, these patients are incredibly susceptible to infection and other potentially harmful illnesses [27]. During the clinical trials for this drug, a patient receiving fingolimod died from complications of a *Varicella zoster* (chicken pox) infection [27]. However, due to a relatively minimal risk for these infections and the associated complications [15], as well as a lack of a viable alternative, Fingolimod continues to be prescribed to patients suffering from MS. Based on these findings, it is apparent that there is a need for the development of an alternative treatment, which would ideally target the pathological, disease causing lymphocytes of MS patients, without causing widespread immunosuppression by sequestering lymphocytes into their respective organs.

Current Research on Novel Treatments

Stem Cell Recruitment

Researchers have recently presented the possibility of mobilizing endogenous neural stem cells to replace damaged oligodendrocytes in MS patients [17]. It has been reported that some endogenous re-myelination occurs in MS, but this process is insufficient to completely repair the damage and restore function [28]. These studies also identified parenchymal oligodendrocyte progenitor cells and endogenous adult neural stem cells as the sources of the re-myelinating cells[28]. Researchers investigated the possibility of genetically or pharmacologically blocking the action of Gli1, a protein involved in the sonic hedgehog-signaling pathway [29]. They had initially designed this process as a treatment for brain and basal cell cancers [30], but it showed promising potential in mouse models of MS. Blocking the expression of Gli1 was suggested as a possible treatment for MS after elevated levels of this protein, as well as other intermediates of the sonic hedgehog signaling pathway, were found in tissue samples from active MS lesions [31]. Researchers hypothesized that the up-regulation of the Gli1 protein of the sonic hedgehog signaling pathway was interfering with the differentiation of precursor oligodendrocytes [32], as this signaling pathway is involved in neural stem cell differentiation [33]. If this proved to be the case, they suggested that blocking the expression of this protein might increase the endogenous re-myelination capacity of the resident and precursor oligodendrocytes.

In mice induced with EAE researchers blocked Gli1 expression and reported a 50% increase myelin, compared to the control group[17]. They also showed a significant increase in the migration of stem cells to the myelin-damaged areas of the brain, which eventually developed into myelin-producing oligodendrocytes. The mice that experienced these outcomes also recovered from symptoms such as leg paralysis and bladder weakness, which was not seen in the untreated group[13]. This represents an opportunity to develop a treatment that repairs the damage caused by the disease, rather than slowing the progression, which is what many of the currently marketed treatments are designed to do. However, this treatment may be most beneficial if used in conjunction with another that is capable of preventing the progression of the disease. It is not enough to simply repair existing damage, without targeting the cause of the damage; however, a treatment that can prevent damage from occurring, combined with one that can repair damage, would be ideal for patients currently suffering from MS.

Melanoma Cell Adhesion Molecule

The melanoma cell adhesion molecule (MCAM) has recently been identified as a potential therapeutic target for MS, which could be used to slow the progression of this disease. MCAM is an integral membrane glycoprotein consisting of five disulfide bonded extracellular immunoglobulin superfamily domains [5]. This particular cell adhesion molecule was initially identified as a marker for the development and metastasis of basal cell cancers [34]. In healthy individuals, MCAM is normally expressed by various components of the vascular cell wall, including endothelial and smooth muscles cells [35]. The expression of MCAM by these cells can enhance tumor progression in human melanoma and prostate cancers[36], and this may be the result of an increased interaction between the invading cancer cells and the surrounding endothelial cells [37]. Recently, a subset of T lymphocytes expressing MCAM have been identified that are able to bind to the endothelial cells of blood vessels, secrete cytokines, and possess other pro-inflammatory properties[38]. MCAM also plays an important role in maintaining the integrity of endothelial tight junctions [39]. Further studies are necessary in order to determine the exact mechanism(s) by which the ligands of MCAM are involved in the migration and signaling processes characteristic of MCAM+ lymphocytes [38].

The expression of MCAM by circulating lymphocytes was first described by Pickl et al.[5], who also reported that these MCAM+ lymphocytes were not significantly expressed in healthy individuals. It was not until several years later that MCAM+ T cells were found in both the CD4+ and CD8+ subpopulations [40]. The expression of MCAM by these cells was up-regulated by stimulation with mitogens, along with a combination of several other agents [40]. Further studies have indicated that these MCAM+ T cells possess an increased ability to bind to the endothelial cells of blood vessels, compared to MCAM- cells[41]. In addition, antibodies for MCAM have been shown to reverse these effects[42]. More recent studies gone further, by demonstrating that MCAM+ lymphocytes migrate across the BBB more efficiently than MCAM- cells, and that this migration is blocked by MCAM antibodies[43] These findings were supported by an additional study that found MCAM- mice displayed a significantly reduced rate of lymphocyte migration across the BBB [44]. In addition to these migratory properties, lymphocytes expressing MCAM also secrete various cytokines; specifically, both CD4+ and CD8+ MCAM+ T cells produce more IL-17 and INF-γ than their MCAM- counterparts, and that both are able to produce TNF-α[41].

Cumulatively, these results suggest that MCAM may play a role in inflammatory autoimmune diseases, including MS [38]. Specifically, researchers have hypothesized that MCAM expression may be involved in the migration of T cells to sites of inflammation [41]. Studies have shown that pathogenic CD4+ T lymphocytes in EAE commonly express MCAM, and that this cell adhesion molecule plays a role in their recruitment from the peripheral blood, across the BBB and into the CNS [43]. These studies also demonstrated that there is a higher number of MCAM+CD4+ T lymphocytes in MS patients, when compared to healthy individuals [43]. Presently, research is aimed towards CD8+ T lymphocytes, some of which also express MCAM[18]. The exact contribution of these cells to the pathogenesis of MS remains unclear, although research indicates that there is a marked increase in their concentration within the peripheral blood and CNS of MS patients [45]. In EAE, CD4+T cells play a significant role in the inflammatory response [46]. However, research indicates that CD8+T cells actually outnumber CD4+ T cells in active MS lesions, and that their numbers correlate with the extent of damage to nerve axons [47], demonstrating the importance of focusing research efforts towards this subset of T cells. If the expression of MCAM by these cells increases their ability enter sites of

inflammation, this could represent a break through in the understanding of the pathogenesis of MS.

In order to further elucidate the role MCAM may play in autoimmune disorders such as MS, Larochelle et al.[18] examined the function and expression of MCAM on CD8+ T cells. They found that there was a significant increase in the presence of CD8+ MCAM+ T cells in the peripheral blood and cerebral spinal fluid of MS patients during relapse periods, which was reduced in patients receiving treatment [18]. Based on the co-expression of a variety of other proteins and surface markers, MCAM+ CD8+ T cells were determined to posses an effector memory profile, and are therefore likely pro-inflammatory in nature, as opposed to regulatory [18]. These researchers, as well as others, previously demonstrated that the endothelial cells of the human BBB also express MCAM, especially during inflammation [43,44]. Studies also show that MCAM interacts with itself [48] and other ligands expressed at the BBB [35]. Using this information, Larochelle et al.[18] demonstrated that there is an increase in the number of CD8+ T cells that adhere to the BBB during inflammation, and that blocking MCAM expression reverses these results. In addition, the cells adhering to the BBB in this experiment had a significantly higher proportion of cells that expressed MCAM, when compared to free-floating cells[18]. They also found that blocking the expression of MCAM significantly reduced the migration of CD8+ T cells across the BBB[18].

In order to determine if these cells expressing MCAM contribute to demyelination and lesion formation, researchers co-cultured MCAM+ CD8+ T cells with human adult oligodendrocytes and found that exposure to oligodendrocytes led to an increase in MCAM expression by the T cells, and that the survival of the oligodendrocytes was significantly reduced in the presence of MCAM+ CD8+ T cells [18]. In order to determine if MCAM is a valid target for potential treatments, mice induced with EAE received either MCAM+ CD8+ T cells, or MCAM- CD8+ T cells, and the mice that received MCAM deficient cells showed a reduction in the neurological deficits caused by EAE [18]. In addition, mice with EAE that received an antibody for MCAM also showed significant reductions in neurological impairment, indicating that blocking expression of MCAM is effective for controlling the severity of EAE [18]. Together, these data suggest that MCAM is a reliable surface marker for pathogenic T-lymphocytes in MS, and represents a potential therapeutic target for this disease.

Issues with using MCAM as a Therapeutic Target for MS

Because many healthy cells normally express MCAM, several potential consequences exist for the use of this protein as a therapeutic target for MS. For example, MCAM may play a role in the maintenance of the structural integrity of endothelial tight junctions[39]. The breakdown of these junctions between the endothelial cells of the BBB, and the subsequent infiltration of lymphocytes into the CNS, is a hallmark of MS [49]; therefore, targeting a protein that maintains these junctions could potentially exacerbate the symptoms and progression of this disease. This clearly emphasizes the need to develop a treatment that is designed to selectively target only lymphocytes expressing MCAM, rather than all cell types that also express MCAM. Thus, further research is required to design a treatment that selectively represses its expression in CD8+ lymphocytes.

Future Directions

According to Gilbert[33], strong causational scientific evidence requires three phases of research, deemed "find it, lose it, and move it". Larochelle et al.[18] completed both the find it and

lose it phases; they have identified MCAM as an up-regulated protein during MS relapses (find it), and they have knocked out MCAM and found that the T-cells are no longer able to cross the endothelial cells of the BBB (lose it). However, they have not completed the third phase, or "move it" phase; in this stage, also known as gain-of-function, the outcome is induced in circumstances where the first event does not usually occur [33].

In attempting to determine whether or not MCAM is involved in the trafficking of lymphocytes across the blood-brain barrier (BBB), it is important to make a distinction between the necessary and sufficient conditions required for an event to occur. The find it-lose it-move it phases, according to Gilbert (2010), correspond to identifying an issue, determining if it is necessary, and discovering if it is both necessary *and* sufficient, respectively. When a condition is necessary for an event, but not sufficient, no claim is made that this condition causes the event in question; rather, there may be many necessary conditions that together cause the event to occur [50]. However, being that the condition is necessary, the event will be unable to occur in its absence [50]. In other words, necessary events are relevant through their absence, which corresponds to the "lose it" phase of research, as described by Gilbert (2010). The key implication here is that disease can be eliminated by removing one of the necessary conditions, regardless of whether or not that condition is also sufficient [50]. In contrast, when a condition is sufficient, it is definitively said to be the cause of the outcome or event [50].

Sufficient conditions, as opposed to necessary conditions, work through their presence; two conditions may be separately insufficient, but when combined (with no other influential external factors), may be sufficient to cause a certain outcome [49]. Since removing MCAM from pathogenic T-cells impedes their migration across the BBB [17], this molecule is evidently necessary for this process, and therefore represents a potential target for treatments. However, MCAM alone may not be sufficient for migration across the BBB- this process likely requires the interaction of various cellular components. Although overly rigorous for using MCAM as a target for treatment, determining if MCAM is sufficient for this process would provide insight into the pathology of MS, the cause of which remains largely unknown. Any contributions to an improved understanding of the development and progression of this disease would be useful for the further production of treatments. It is very unlikely this one protein is both necessary *and* sufficient for migration across the BBB, given the unpredictable and multifactorial nature of this disease. As such, several options exist for further research with the eventual goal of verifying that MCAM is a suitable target for potential treatments for MS.

Conclusion

Based on the current research, MCAM seems to represent a promising potential future therapeutic target for MS. Although Fingolimod, one of the current treatment options, has been shown to reduce the progressive accumulation of disability in MS patients, its prolonged use is associated with several complications, all of which pertain to the inevitable widespread immunosuppression caused by this drug. As demonstrated by Larochelle et al. (2015), MCAM may represent a reliable surface marker for pathogenic T cells in MS patients, that assists with the adhesion of these cells to and transmigration across the endothelial cells of the BBB. The use of MCAM as a therapeutic target for MS has several advantages over Fingolimod, as blocking the expression of MCAM would not sequester lymphocytes within various lymphoid organs, and would therefore not cause widespread immunosuppression. With this treatment, lymphocytes would still be able to circulate and perform their necessary immune function, without being able to penetrate the CNS. However, MCAM has been shown to play a role in maintaining the

integrity of endothelial tight junctions. If a treatment was not able to block MCAM expression specifically in pathogenic lymphocytes, this could exacerbate the symptoms and progression of MS, by damaging the integrity of endothelial tight junctions. This is because the breakdown of these tight junctions between the endothelial cells of the BBB is a hallmark of MS, and allows for the infiltration of lymphocytes with greater ease. Since it is unlikely that a single protein is both necessary *and* sufficient for this transmigration process, it may be necessary to determine which other cellular components MCAM interacts with during adhesion and transmigration, in order to further elucidate the mechanism by which lymphocytes use MCAM to infiltrate the CNS in MS. A broader understanding of this mechanism would improve our ability to develop more effective treatments, as well as broaden our understanding of the cause or trigger for this complex, multifactorial disease.

Endnotes

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