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A novel HLA-DR α 1-MOG-35-55 construct treats experimental stroke

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Abstract

Chemoattraction of leukocytes into the brain after induction of middle cerebral artery occlusion (MCAO) increases the lesion size and worsens disease outcome. Our previous studies demonstrated that partial MHC class II constructs can reverse this process. However, the potential application of pMHC to human stroke is limited by the need to rapidly match recipient MHC class II with the β 1 domain of the pMHC construct. We designed a novel recombinant protein comprised of the HLA-DR α 1 domain linked to MOG-35-55 peptide but lacking the β 1 domain found in pMHC and treated MCAO after 4 h reperfusion in humanized DR2 mice. Infarct volumes were quantified after 96 h reperfusion and immune cells from the periphery and CNS were evaluated for expression of CD74 and other cell surface, cytokine and pathway markers. This study demonstrates that four daily treatments with DR α 1-MOG-35-55 reduced infarct size by 40 % in the cortex, striatum and hemisphere, inhibited the migration of activated CD11b⁺CD45^{high} cells from the periphery to the brain and reversed splenic atrophy. Furthermore, DR α 1-MOG-35-55 bound to CD74 on monocytes and blocked both binding and downstream signaling of macrophage migration inhibition factor (MIF) that may play a key role in infarct development. The novel DR α 1-MOG-35-55 construct is highly therapeutic in experimental stroke and could be given to all patients at least 4 h after stroke onset without the need for tissue typing due to universal expression of DR α 1 in humans.

Keywords

Stroke; Inflammation; Immunotherapy; Recombinant T-cell receptor Ligand; MHC class II invariant chain

Introduction

It has now been well established that experimental stroke triggers inflammation in brain as well as rapid activation of the peripheral immune system, resulting in migration of monocytes, neutrophils and T-cells across the blood–brain barrier into the growing infarct and further activation of microglial cells (Gee et al. 2007; Nilupul Perera et al. 2006; Wang et al. 1993). These infiltrating cells contribute to ischemic damage through localized inflammation. The magnitude of the inflammatory response is strongly associated with stroke outcome in patients (Emsley et al. 2005; Smith et al. 2004). Furthermore, the peripheral immune system is massively activated after cerebral ischemia. This vast activation is followed by immunosuppression that is marked by atrophy of the spleen and thymus (Offner et al. 2006a, b). Thus, immunotherapeutic approaches for treatment of ischemic stroke could reduce the inflammatory milieu, target specific mechanisms of the inflammatory pathway and maintain homeostasis of peripheral immunity.

Recombinant T-cell receptor ligand (RTL) molecules consist of the α 1 and β 1 domains of MHC class II molecules expressed as a single polypeptide with or without antigenic amino terminal extensions (Burrows et al. 1999; Vandenbark et al. 2003). We previously demonstrated that RTL could prevent and/or reverse clinical signs of experimental autoimmune encephalomyelitis (EAE) and subsequently showed that an RTL construct could effectively treat experimental stroke in mice (Akiyoshi et al. 2011; Burrows et al. 1998, 2001; Huan et al. 2004; Subramanian et al. 2009; Vandenbark et al. 2003). We reported previously that treatment with RTL1000 that is comprised of an HLA-DR2 moiety

linked to human MOG-35-55 peptide in humanized DR2 mice reduced infarct size (Akiyoshi et al. 2011). Recently, we found that the RTL and HLA-DR α 1 domain directly binds to and downregulates the cell surface expression of the MHC class II invariant chain (CD74) on CD11b⁺ monocytes, and the RTL inhibit binding of macrophage migration inhibitory factor (MIF) to CD74 and blocks downstream inflammatory effects in the CNS (Benedek et al. 2013; Vandenbark et al. 2013). We have further demonstrated that the potency of the DR α 1 domain could be enhanced by addition of a peptide extension (MOG-35-55 peptide) (Meza-Romero et. al. manuscript in preparation). Moreover, because the DR α 1 domain is present in all humans and thus would not be recognized as foreign, treatment using DR α 1 constructs would not require HLA screening of potential recipients and could be used for treatment of CNS diseases.

We demonstrate herein that DR α 1-MOG-35-55 strongly reduces infarct size and reverses splenic atrophy after stroke when administered at a clinically relevant time-point; 4 h after the onset of stroke, mediated in part by reduced expression of the CD74 MIF cell surface receptor and migration of CD11b⁺ monocytes into the ischemic brain.

Materials and methods

Ethics statement

The study was conducted in accordance with National Institutes of Health guidelines for the use of experimental animals, and the protocols were approved by the Animal Care and Use Committees at Oregon Health & Science University and the Portland Veteran Affairs Medical Center.

Animals

All experiments used age-matched, sexually mature (20 to 25 g) male HLA-DRB1*1502 (DR2-Tg) mice produced by Dr. Chella David (Gonzalez-Gay et al. 1996). The mice were housed and bred at the Veterans Affairs Medical Center, and studies were conducted at Oregon Health & Science University.

HLA-DR α 1-MOG-35-55 cloning, production and purification

DR α 1-MOG-35-55 domain cloning, production and purification have been described elsewhere (Meza-Romero et. al. manuscript in preparation). Briefly, DR α 1-MOG-35-55 was constructed using the DR α 1 construct as a template. The mouse MOG (35–55) peptide DNA encoding sequence was attached to the N-terminus of the DR α 1 domain with a linker-thrombin-linker intervening element.

Treatment with DR α 1-MOG-35-55

Mice were randomized to receive 0.1 ml (500 μ g) DR α 1-MOG-35-55 or 0.1 ml Vehicle (5 % dextrose in Tris–HCl, pH 8.5) by subcutaneous injection 4 h after the onset of reperfusion followed by similar doses at 24, 48, and 72 h of reperfusion for a total of 4 treatments each of DR α 1-MOG-35-55 or Vehicle. Both DR α 1-MOG-35-55 and Vehicle treated MCAO mice were euthanized at the 96 h time-point for evaluation of tissues and cells.

Transient middle cerebral artery occlusion

Transient focal cerebral ischemia was induced in male DR2-Tg mice for 1 h by reversible right MCAO under isoflurane anesthesia followed by 96 h of reperfusion, as described previously with slight modifications (Offner et al. 2006a). Head and body temperature were controlled at 36.5 $\pm$ 0.5 °C throughout MCAO surgery with warm water pads and a heating lamp. Laser-Doppler flowmetry (LDF; Model DRT4, Moor Instruments Ltd., Oxford,

England) was monitored throughout the ischemic period with a LDF probe affixed to the skull to ensure effective occlusion and reperfusion. The common carotid artery was exposed and the external carotid artery was ligated and cauterized. Unilateral MCAO was accomplished by inserting a 6–0 nylon monofilament surgical suture (ETHICON, Inc., Somerville, NJ, USA) with a heat-rounded and silicone-coated (Xantopren comfort light, Heraeus, Germany) tip into the internal carotid artery via the external carotid artery stump. Animals were excluded if mean intra-ischemic LDF was greater than 30 % pre-ischemic baseline. At 1 h of occlusion, the occluding filament was withdrawn to allow for reperfusion. Mice were then allowed to recover from anesthesia and survived for 96 h following initiation of reperfusion.

Determination of infarct size

The brains were harvested after 96 h of reperfusion and sliced into four 2-mm-thick coronal sections for staining with 1.2 % 2,3,5-triphenyltetrazolium chloride (TTC; Sigma, St. Louis, MO, USA) in saline as described previously (Hurn et al. 2007). The 2-mm brain sections were incubated in 1.2 % TTC for 15 min at 37 °C, and then fixed in 10 % formalin for 24 h. Infarct volume was measured using digital image analysis software (Systat, Inc., Point Richmond, CA, USA). To control for edema, infarct volume (cortex, striatum, and hemisphere) was calculated by subtraction of the ipsilateral non-infarcted regional volume from the contralateral regional volume. This value was then divided by the contralateral regional volume and multiplied by 100 to yield regional infarct volume as a percent of the contralateral region.

Leukocyte isolation from brain and spleen

Spleens from MCAO-treated mice were removed and a single-cell suspension was prepared by passing the tissue through a 100 µm nylon mesh (BD Falcon, Bedford, MA). The cells were washed using RPMI 1640 and the red cells lysed using 1× red cell lysis buffer (eBioscience, Inc., San Diego, CA) and incubated for 3 min. The cells were then washed twice with RPMI 1640, counted and resuspended in stimulation medium (RPMI, containing 2 % FBS, 1 % sodium pyruvate, 1 % L-glutamine, 0.4 % βME). The brain was divided into the ischemic (right) and nonischemic (left) hemispheres, digested for 60 min with 1 mg/ml Type IV collagenase (Sigma Aldrich, St. Louis, MO) and DNase I (50 mg/ml, Roche Diagnostics, Indianapolis, IN) at 37 °C with shaking at 200 rpm. Samples were mixed every 15 min. The suspension was washed 1× in RPMI, resuspended in 80 % Percoll overlaid with 40 % Percoll and centrifuged for 30 min at 1600 RPM. The cells were then washed twice with RPMI 1640, counted and resuspended in staining medium.

Flow cytometry

All antibodies were purchased (BD Biosciences, San Jose, CA or eBioscience, Inc., San Diego, CA) as published. Four-color (FITC, PE, APC and PerCP) fluorescence flow cytometry analyses were performed to determine the phenotypes of cells following standard antibody staining procedures. One million cells were washed with staining medium (PBS containing 0.1 % NaN₃ and 1 % bovine serum albumin (Sigma, Illinois) and incubated with combinations of the following monoclonal antibodies: CD80 (16-10A1), HLA-DR (TU39), CD11b (MAC-1), CD74 (In-1), CD45 (Ly-5), CD62L (MEL14), ICAM-1 (3B2), and CD44 (IM7), CCR2 (475301), CD4 (GK1.5) for 20 min at 4 °C. Propidium iodide was added to identify dead cells. Data were collected with CELLQUEST (BD Biosciences, San Jose, CA) and FCS EXPRESS (De Novo Software, Los Angeles, CA) software on a FACSCalibur (BD Biosciences).

Intracellular staining for TNF- α

Splenocytes were resuspended (2×10^6 cells/ml) in stimulation media (RPMI 1640 media containing 2 % FCS, 1 mM pyruvate, 200 μ g/ml penicillin, 200 U/ml streptomycin, 4 mM L-Glutamine, and 5×10^{-5} M 2- β -ME with PMA [50 ng/ml], ionomycin [500 ng/ml], and Brefeldin A [10 μ g/ml] [all reagents from BD Bioscience]) for 4 h. Fc receptors were blocked with mouse Fc receptor-specific mAb (2.3 G2; BD PharMingen) before cell-surface staining and then fixed and permeabilized using a Cytotfix/Cytoperm kit (BD Biosciences) according to the manufacturer's instructions. Permeabilized cells were washed with 1 $\times$ Permeabilization Buffer (BD Bioscience) and stained with either PE-conjugated TNF- α (MP6-XT22) or Isotype matched mAb that served as a negative control. Data were collected with CELLQUEST (BD Biosciences, San Jose, CA) and FCS EXPRESS (De Novo Software, Los Angeles, CA) software on a FACSCalibur (BD Biosciences).

Real time PCR

Splenocytes or brain cells were isolated from DR*1502-Tg mice. Total RNA was isolated from cells using an RNeasy cultured cell kit according to the manufacturer's instructions. (Qiagen, Valencia, CA, USA). Quantitative real time PCR was performed using the ABI7000 sequence detection system with gene-on-demand assay products (Applied Biosystems) for TaqMan array mouse immune response or for IL-4 (Assay ID: Mm00445259_m1), ACE (Assay ID: Mm00802048_m1) and CCL3 (Assay ID: Mm00441249_g1). GAPDH housekeeping gene was amplified as an endogenous control. Primers were used according to manufacturer's instructions.

Statistical analysis

Data are presented as mean $\pm$ SEM. Statistical differences in cortical, striatal, and total (hemispheric) infarct volume, as well as spleen and brain cell counts and percentages of cellular subtypes from FACS analyses were determined by Student's *t*-test. Statistical significance was $p < 0.05$.

Results

DR α 1-MOG-35-55 treatment significantly reduces infarct size after MCAO in DR2-Tg mice

Evaluation of brain infarcts 96 h after MCAO demonstrated that DR α 1-MOG-35-55-treated male DR2-Tg mice had significantly reduced infarct volumes compared with the Vehicle-treated group as shown in Fig. 1a. Cortical infarct volume was 25.9 ± 4.67 % in DR α 1-MOG-35-55-treated compared to 47.0 ± 2.5 % in vehicle-treated mice ($p < 0.01$). Similarly, striatal infarct volume was 40.8 ± 5.4 % vs. 64.5 ± 2.0 % respectively in DR α 1-MOG-35-55-treated vs. Vehicle-treated mice ($p < 0.01$), whereas the total hemispheric infarct volume was 19.4 ± 3.6 % vs. 31.1 ± 1.71 %, respectively ($p < 0.01$). Quantitative assay of TTC stained cerebral sections after 96 h of reperfusion illustrated the smaller infarct area in DR α 1-MOG-35-55-treated mice compared with Vehicle-treated mice (Fig. 1b). There were no significant differences in laser-Doppler perfusion before, during or immediately after MCAO between DR α 1-MOG-35-55-treated and Vehicle-treated groups (data not shown).

DR α 1-MOG-35-55 reduces the number of activated microglia and infiltrating monocytes and their CD74 cell surface expression in the ischemic brain

We sought to determine if the reduced infarct size after DR α 1-MOG-35-55 treatment was due to diminished number of infiltrating inflammatory cells in the brain after MCAO. DR α 1-MOG-35-55 treatment significantly reduced the absolute number of mononuclear cells in the right ischemic brain compared with Vehicle-treated mice (13.14×10^4 vs. 21.33×10^4 respectively, $p < 0.05$) (Fig. 2a). This difference is attributed mainly to the

reduction in the number of activated CD11b⁺CD45^{high} monocytic cells (4.7×10^4 vs. 11.85×10^4 respectively, $p < 0.01$). There were no significant differences in the absolute numbers of the CD4⁺ T cells or the resting microglia (CD11b⁺CD45^{low}) (Fig. 2b). In the non-ischemic left brain there were no differences in the total absolute number of mononuclear cells or in any cell type (Supplemental Fig. 1).

Previously, it was demonstrated that DR α 1 treatment of EAE also leads to reduction of activated CD11b⁺ cells in the Central Nervous System (CNS) and that DR α 1 reduces the cell surface expression of the MIF receptor, CD74, on the activated CD11b⁺ cells. In order to determine if DR α 1-MOG-35-55 treatment has the same effect in MCAO, expression of CD74 cell surface levels was evaluated on CD11b⁺CD45^{high} cells. As shown in Fig. 2c, there was a significant reduction in the level of CD74 expression as measured by the Mean Fluorescent Intensity (MFI) in the DR α 1-MOG-35-55-treated mice compared with the Vehicle-treated mice ($p < 0.01$). Taken together, these results demonstrate that DR α 1-MOG-35-55 can reduce the number of activated microglia and infiltrating monocytes in the ischemic brain and affect the expression of CD74 on the surface of these cells.

DR α 1-MOG-35-55 treatment affects the immune gene expression profile in the ischemic brain after MCAO

In order to assess the DR α 1-MOG-35-55 treatment effect on the expression of immune related genes in brain, mRNA was isolated from the ischemic brains of 3 Vehicle-treated mice and 3 DR α 1-MOG-35-55-treated mice. A real-time PCR assay was performed on pooled cDNA samples and expression levels of the DR α 1-MOG-35-55-treated sample was analyzed relative to the Vehicle-treated sample (Fig. 3). Validation of 3 genes: IL-4, CCL3 and ACE, using individual samples, demonstrated that the expression trends were the same as in the immune array, although not all of the genes showed a significant difference (Supplemental Fig. 2). The immune array data demonstrate that there was a decrease in the expression of monocyte-related genes such as CCL3, CCL2 and increases in Th1 and Th2 related genes such as IL-12, Tbx21, IL-4 and IL-13. It is important to note that several genes that were associated previously with cerebrovascular function and ischemic brain injury, including ACE and EDN1, were down regulated after DR α 1-MOG-35-55 treatment relative to Vehicle treatment.

DR α 1-MOG-35-55 treatment reverses MCAO-Induced splenic atrophy

To evaluate the effects of DR α 1-MOG-35-55 treatment on stroke-induced splenic atrophy, cell numbers in the spleen were counted in post-ischemic Vehicle- and DR α 1-MOG-35-55-treated mice. As expected, MCAO induced a significant decrease in spleen numbers in the Vehicle-treated group (Fig. 4a). Interestingly, viable cell counts were significantly increased in spleens of DR α 1-MOG-35-55-treated versus Vehicle-treated mice (64.67×10^6 vs. 32.11×10^6 respectively, $p < 0.01$) 96 h after reperfusion. The increase in spleen cell numbers was reflected in the absolute numbers of CD11b⁺ cells ($p < 0.01$) and CD4⁺ cells ($p < 0.05$) (Fig. 4b). Although DR α 1-MOG-35-55 treatment increased the cell numbers in the spleen, it reduced the frequency of CD4⁺ cells and did not change the frequency of the CD11b⁺ cells in the spleen (Fig. 4c).

DR α 1-MOG-35-55 treatment increases the frequency of activated CD4⁺ cells but does not change the activation state of CD11b⁺ cells in the spleen after MCAO

We further sought to assess the activation state of CD11b⁺ and CD4⁺ cells in the spleen 96 h after reperfusion. As shown in Fig. 5a there were no differences in the level of expression of CD80, ICAM-1, HLA-DR and CCR2 on CD11b⁺ cells between DR α 1-MOG-35-55- versus Vehicle-treated mice. Evaluation of CD4⁺ activation by the expression of CD44 and CD62L revealed that the frequency of both CD62L low and high activated CD4⁺ cells in the

spleen were increased after treatment with DR α 1-MOG-35-55 compared with Vehicle ($p < 0.05$ for both). In addition, spleen cells from DR α 1-MOG-35-55- or Vehicle-treated mice were stimulated with PMA/Ionomycin for 4 h and evaluated by flow cytometry for intracellular staining of TNF- α . There was no difference in production of TNF- α by CD4 $^{+}$ or CD11b $^{+}$ cells in spleens from DR α 1-MOG-35-55- or Vehicle-treated mice (Supplemental Fig. 3).

DR α 1-MOG-35-55 treatment affects the immune gene expression profile in the spleen after MCAO

In order to assess the DR α 1-MOG-35-55 treatment effect on the expression of immune related genes in the spleen, mRNA was isolated from spleens of 6 Vehicle-treated mice and 6 DR α 1-MOG-35-55-treated mice. A real-time PCR assay was performed on pooled cDNA samples and expression levels from the DR α 1-MOG-35-55-treated mice were analyzed relative to the Vehicle-treated mice (Fig. 6). The expression levels of 3 genes: IL-4, CCL3 and ACE were validated using individual samples (Supplemental Fig. 4). Interestingly, the expression of several of the genes in the spleen, such as CCL3, IL-4, Stat6, ACE, FN1 and C3 had an opposite trend compared with their expression in the brain after DR α 1-MOG-35-55 treatment. In addition, the expression of monocyte related genes, such as CD68, were increased in the spleen of DR α 1-MOG-35-55-treated mice relative to Vehicle-treated mice. These expression results are in correlation to the absolute numbers of cells in the spleen.

Discussion

Our study demonstrates for the first time that treatment with DR α 1-MOG-35-55 after onset of MCAO reduces infarct size, inhibits infiltration of activated monocytes into the ischemic brain and reverses splenic atrophy, which is typically induced after MCAO. We have previously demonstrated that partial MHC class II constructs which are comprised of the linked β 1 α 1 domains tethered to antigenic peptides, could treat CNS diseases such as experimental stroke and EAE (Akiyoshi et al. 2011; Sinha et al. 2009, 2007; Subramanian et al. 2009). However, the novel DR α 1-MOG-35-55 construct represents an improved immunotherapy which would not require HLA screening prior to use. In contrast to RTL1000, in which the MOG peptide is linked to the DR2 β 1 domain and thus can be given only to HLA-DR2 matched subjects, the MOG peptide in the new construct is linked to the DR α 1 domain. Hence, the lack of the polymorphic β 1 domain and the fact that the DR α 1 domain is invariable and thus universally tolerogenic enables its use without pre-screening for HLA genotype.

We have recently shown that DR α 1-MOG-35-55 binds to and down-regulates CD74 on CD11b $^{+}$ cells. This binding has a bi-functional inhibitory effect on MIF/CD74 interactions and blocks downstream MIF effects in the CNS during EAE (Meza-Romero et al. manuscript in preparation). In addition, we have demonstrated the RTL can inhibit MIF binding to CD74 (Benedek et al. 2013). However, it is not yet clear whether the treatment effect of DR α 1-MOG-35-55 in MCAO is MIF dependent, MIF independent or both. In the literature, the contribution of MIF to the outcome of MCAO is controversial and one report even indicates that deletion of MIF worsens MCAO in females (Inacio et al. 2011a, b, c; Turtzo et al. 2013), suggesting a protective effect. It is thus important to note that the parent RTL1000 construct can treat MCAO in both male and female mice ((Akiyoshi et al. 2011) and HO unpublished data). Assuming similar mechanisms with RTL1000, the DR α 1-MOG-35-55 treatment might thus have a MIF independent effect, at least in female mice, either via the tethered MOG-35-55 peptide or by reduction of CD74 surface expression on activated CD11b $^{+}$ cells. Consistent with this line of reasoning, Fan et. al. demonstrated that

macrophages from CD74 KO mice showed increased random migration compared with WT macrophages, while the random migration levels of MIF KO and WT macrophages did not differ (Fan et al. 2011). This result would suggest a MIF-independent role of CD74 on macrophage migration that might also be affected by DR α 1-MOG-35-55.

The infiltration of inflammatory cells into the brain after MCAO was reported in detail previously and it was shown that of the activated cells in the brain the predominant population is macrophages/activated microglial cells (both cell types are CD11b⁺CD45^{high} in inflamed CNS) (Stevens et al. 2002; Subramanian et al. 2009). Our results clearly demonstrate that DR α 1-MOG-35-55 treatment reduces the infarct volume and the number and frequency of CD11b⁺CD45^{high} cells in the ischemic brain. This result is very similar to the previously described effect of RTLs in blocking activation of resident microglia and cellular infiltration into the CNS during EAE and MCAO (Benedek et al. 2013; Subramanian et al. 2009).

The gene expression profile of immune related genes in the brain of DR α 1-MOG-35-55-treated relative to Vehicle-treated mice was concurrent with the reduction of activated CD11b⁺ cells in the ischemic brain 96 h after MCAO. Interestingly, several genes that were shown to be involved in the induction and outcome of stroke, such as Endothelin 1 (EDN1) (Ziv et al. 1992) and Angiotensin-converting enzyme (ACE) were down regulated (Yusuf et al. 2000) at this time point. It is also important to note that up-regulated genes such as CXCL10, CXCR3 and CCR7 in DR α 1-MOG-35-55-treated vs. Vehicle MCAO mice could reflect a lesser degree of MCAO-induced immunosuppression (similar to sham-treated vs. untreated MCAO mice) at 96 h (Offner et al. 2006b). Thus, treatment with DR α 1-MOG-35-55 is directly affecting the number of infiltrating cells, mainly monocytes, into the brain and in addition is restoring immune homeostasis to the ischemic brain.

The effects of cerebral damage from stroke on the peripheral immune system are profound (Offner et al. 2006a, b; Seifert et al. 2012). This drastic loss of cell numbers in the spleen 96 h after MCAO induction was largely reversed after DR α 1-MOG-35-55 treatment. The absolute numbers of CD11b⁺ and CD4⁺ cells as well as total splenocytes were significantly increased. We also observed that DR α 1-MOG-35-55 treatment did not alter the frequency of CD11b⁺ cells or their activation state. As for the CD4⁺ cells, although the frequency of these cells was reduced in the spleen after DR α 1-MOG-35-55 treatment, the frequency of the activated CD4⁺ cells was significantly higher suggesting that the activated cells are retained in the spleen.

The gene expression profile of the spleen from DR α 1-MOG-35-55-treated mice relative to Vehicle-treated mice was almost a mirror image to the expression in the ischemic brain. Taken together, these data suggest that the DR α 1-MOG-35-55 mechanism of action in stroke involves alteration of the migration patterns of activated cells in the periphery. Down regulation of CD74 on activated CD11b⁺ cells by DR α 1-MOG-35-55 could induce random migration or inhibit chemotactic migration of these cells, as was shown in CD74 deficient macrophages or by down regulation of CD74 cell surface expression by RTLs.

In summary, our study demonstrates that four daily treatments with DR α 1-MOG-35-555 beginning 4 h after MCAO reduces the migration of activated cells from spleen to brain, thus contributing to a reduction in infarct size. This immunomodulatory agent might thus represent the next generation of partial MHC class II constructs with therapeutic activity for CNS diseases. Further studies are underway to determine how long after MCAO DR α 1-MOG-35-55 is effective and what is the minimal effective dose that can be given.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

MCAO	Middle cerebral artery occlusion
RTL	Recombinant T-cell receptor ligand
MHC	Major histocompatibility complex

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Fig. 1.

DRα1-MOG-35-55 treatment decreased infarct size compared to vehicle 96 h after MCAO.

a Infarct volumes were significantly decreased in DRα1-MOG-35-55 ($n=10$) versus Vehicle-treated ($n=10$) mice after 60 min MCAO and 96 h of reperfusion in cortex (CTX) and caudateputamen (CP), as well as in total hemisphere (HMSPHR) ($*p < 0.05$). **B**). Representative 2,3,5-triphenyltetrazolium chloride stained cerebral sections after 96 h of reperfusion following 1 h of MCAO

Fig. 2.

DRα1-MOG-35-55 treatment reduces number of activated CD11b⁺ cells and their CD74 surface expression in the ischemic brain 96 h after MCAO. **a** Absolute lymphocyte numbers in the non-ischemic left and ischemic right brain from DRα1-MOG-35-55-treated ($n=7$) versus Vehicle-treated mice ($n=9$). **b** Absolute numbers of CD11b⁺CD45^{low}, CD11b⁺CD45^{high} and CD4⁺ cells in the right brain. **c** CD74 cell surface expression on CD11b⁺CD45^{high} cells in the right brain. Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ Student's t -test

Fig. 3.

Gene expression profile of right ischemic brain from DR α 1-MOG-35-55 treated relative to Vehicle treated mice 96 h after MCAO. Relative expression of mRNA of inflammatory genes was analyzed by real-time PCR from pooled ischemic brain samples of DR α 1-MOG-35-55-treated ($n=3$) relative to Vehicle-treated ($n=3$) mice. Values <1, 1, >1 represent down-regulated, unchanged or up-regulated genes, respectively, of samples from DR α 1-MOG-35-55-treated mice relative to Vehicle-treated mice. ND, not detected

Fig. 4. DRα1-MOG-35-55 treatment reverses MCAO-induced splenic atrophy. **a** Absolute cell numbers in the spleen from DRα1-MOG-35-55-treated ($n = 10$) versus Vehicle-treated mice ($n = 12$). **b** Absolute numbers of CD11b⁺ and CD4⁺ cells in spleen **c**. Frequency of CD11b⁺ and CD4⁺ cells in spleen. Data are presented as mean $\pm$ SEM. * $p < 0.05$, ** $p < 0.01$ Student's t -test

Fig. 5.

DRα1-MOG-35-55 treatment increases the frequency of activated CD4⁺ cells but does not change the activation state of CD11b⁺ cells in the spleen 96 h after MCAO. **a** CD11b⁺ cells in the spleen of DRα1-MOG-35-55-treated ($n=10$) versus Vehicle-treated mice ($n=12$) were analyzed for the expression of CD80, HLA MHC class II, ICAM-1 and CCR2. **b** CD4⁺ cells in spleen were analyzed for the expression of CD44 and CD62L. Data are presented as mean $\pm$ SEM. * $p < 0.05$, Student's t -test

Fig. 6.

Gene expression profile of spleens from DR α 1-MOG-35-55 treated relative to Vehicle treated mice 96 h after MCAO. Relative expression of mRNA of inflammatory genes was analyzed by real-time PCR from pooled spleen samples of DR α 1-MOG-35-55-treated ($n=6$) relative to Vehicle-treated ($n=6$) mice. Values <1, 1, >1 represent down-regulated, unchanged or up-regulated genes, respectively, of samples from DR α 1-MOG-35-55-treated mice relative to Vehicle-treated mice. ND, not detected