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H₁R Expression by CD11b⁺ Cells is not Required for Susceptibility to Experimental Allergic Encephalomyelitis

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Abstract

The histamine H₁ receptor (*Hrh1*/H₁R) was identified as an autoimmune disease gene in experimental allergic encephalomyelitis (EAE), the principal autoimmune model of multiple sclerosis (MS). Previously, we showed that selective re-expression of H₁R by endothelial cells or T cells in H₁RKO mice significantly reduced or complemented EAE severity and cytokine responses, respectively. H₁R regulates innate immune cells, which in turn influences peripheral and central nervous system CD4⁺ T cell effector responses. Therefore, we selectively re-expressed H₁R in CD11b⁺ cells of H₁RKO mice to test the hypothesis that H₁R signaling in these cells contributes to EAE susceptibility. We demonstrate that transgenic re-expression of H₁R by H₁RKO-CD11b⁺ cells neither complements EAE susceptibility nor T cell cytokine responses highlighting the cell-specific effects of *Hrh1* in the pathogenesis of EAE and MS, and the need for cell-specific targeting in optimizing therapeutic interventions based on such genes.

Keywords

Histamine; Histamine H₁ receptor; Experimental allergic encephalomyelitis; Multiple sclerosis; Antigen presenting cells

1. Introduction

Professional antigen presenting cells (APCs), including dendritic cells (DCs), B cells, and macrophages, as well as specialized tissue resident macrophages such as microglia, provide the first line of defense against pathogens as they are able to process extracellular proteins and present them on MHC class II to CD4⁺ Th cells [1, 2]. Furthermore, APCs provide costimulation and secrete cytokines, which direct the differentiation of naïve CD4⁺ T cells towards Th1, Th2, or Th17 cells, thereby making APCs central to effective adaptive immunity [3]. APCs are also important in tolerance by providing cytokines that help in the

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generation of regulatory T cells [4–6]. However, MHC class II alleles represent the main genetic determinant in many chronic inflammatory diseases and the dysregulation of APC activity can result in the accumulation of self-reactive CD4⁺ T cells and influence susceptibility to autoimmunity [7].

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS). Even though the etiology is ill-defined, both experimental allergic encephalomyelitis (EAE), the autoimmune model of MS, and MS are considered to be neuroantigen driven, predominantly mediated by MHC class II-restricted CD4⁺ T cells capable of secreting either IFN- γ (Th1) or IL-17 (Th17), or both [8]. Much has been learned about the adaptive immune system in the pathogenesis of MS, but recent studies have suggested an important role for the innate immune system, mainly APCs, in the initiation and progression of MS by contributing to the pathogenic potential of T or B cells. DCs isolated from secondary progressive or relapsing remitting-MS patients and EAE have an activated phenotype, exhibit increased expression of activation markers CD40 and CD80, and decreased expression of programmed death ligand-1 [9]. Monocyte-derived DCs from MS patients secrete proinflammatory cytokines such as IFN γ , TNF α (to help Th1 differentiation), IL-6, and IL-23 (to help Th17 differentiation) [10–12]. New immunotherapies employed for MS and other autoimmune diseases not only target adaptive immune responses but also influence cells of the innate immune system [7, 13, 14]. Given the importance of APCs in MS pathology, it is pertinent to understand the role of physiological mediators, such as histamine (HA) [2-(4-imidazole) ethylamine], which modulate the functions of these cells and help direct the adaptive immune response [15].

HA is a biogenic monoamine that mediates a variety of physiological processes and regulates innate and adaptive immune responses [16]. Mast cells and basophils are the major sources of stored HA in the body but HA can also be secreted by other cell types, including DCs, neutrophils, macrophages, immature myeloid cells, and T cells [17]. HA plays an important role in the pathogenesis of MS and EAE [18]. In EAE, higher levels of HA within the cerebrospinal fluid correlate with the onset of disease and mast cell granule stabilizers can reduce EAE severity or alter the blood brain barrier (BBB) permeability [19]. Mast cells are present in MS lesions and mast cell activation is required for early onset and maximal EAE disease severity [20].

HA exerts its effects by binding to four receptors designated H₁R through H₄R. H₁R and H₂R are ubiquitously expressed, H₄R expression is confined mostly to hematopoietically derived cells although it has been shown recently that H₄R is also expressed functionally in the CNS [21], and H₃R is primarily expressed within the CNS tissue [22–24]. H₁R transcripts are highly expressed in chronic MS lesions [25] and the use of first generation antihistamines and H₁R antagonists correlated with decreased MS risk and was reported to stabilize the disease progression and improve neurological symptoms [26, 27]. In EAE, encephalitogenic T cell clones reactive to proteolipid protein have increased levels of H₁R transcripts. The treatment of mice with an H₁R antagonist during EAE can reduce clinical severity and pathology [20]. Furthermore, through positional candidate gene cloning, we identified the gene underlying *Bordetella pertussis* toxin induced HA sensitization (*Bphs*), which contributes to EAE and experimental allergic orchitis susceptibility, to be histamine H₁ receptor (*Hrh1*) [28].

Previously, we have shown that on the *Hrh1*-knockout background, H₁R re-expression in T cells regulates encephalitogenic responses [29]. In addition, we determined that selective overexpression of H₁R in endothelial cells protects mice from EAE and decreases BBB permeability [30]. However, while it is known that HA acting through H₁R affects maturation and differentiation of macrophages and DCs [16, 31–34] and can alter T cell

polarization by regulating cytokine production from maturing DCs [31, 35–37], no study has yet reported on the impact of H₁R signaling in these cell types during the pathogenesis of EAE. Therefore, we generated transgenic mice expressing H₁R specifically in CD11b⁺ cells to test the hypothesis that H₁R signaling in CD11b⁺ cells contributes to EAE susceptibility. In mice, CD11b is highly expressed in monocytes, macrophages, DCs, neutrophils, and microglia [38–40], and to a lesser extent in other cell types like NK cells and B cells. We chose to utilize the human *CD11b* promoter for our transgenic studies since CD11b⁺ cells primarily reflect APCs and has been well established and used in many studies to direct macrophage specific ablation and transgene expression [41–46]. Surprisingly, we found that re-expression of H₁R exclusively by CD11b⁺ cells did not restore EAE severity in H₁RKO mice. Moreover, selective re-expression of H₁R by CD11b⁺ cells does not affect encephalitogenic T cell responses. These results confirm our previous findings that H₁R signaling directly in CD4⁺ T cells contributes to EAE susceptibility rather than through CD11b⁺ cell mediated effects on CD4⁺ T cell encephalitogenic activity [29]. Our results highlight the cell-specific effects that an AID gene can play in the pathogenesis of complex diseases such as EAE and MS, and the need for cell-specific targeting in optimizing therapeutic interventions based on such genes.

2. Materials and methods

2.1 Animals

C57BL/6J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). B6.129P-*Hrh1^{tm1Wat}* (H₁RKO) [34] were maintained at the University of Vermont (Burlington, VT). H₁RKO mice were backcrossed to C57BL/6J background for >10 generations. For transgenic mouse generation, an HA-H₁R construct was made by deleting the methionine of the H₁R allele and adding an HA tag at the N terminus using TOPO cloning vector (Invitrogen). The HA-H₁R was then subcloned downstream of the human *CD11b/Itgam* promoter [46]. The linear DNA fragment containing the human *CD11b/Itgam* promoter, the HA-H₁R gene, and the human growth hormone (*hGH*) intron and polyadenylation signal was injected directly into fertilized C57BL/6J eggs at the University of Vermont transgenic/knockout facility. Mice were screened for *hGH* gene by PCR using *hGHF* 5' TAG GAA GAA GCC TAT ATC CCA AAG G 3', *hGHR* 5' ACA GTC TCT CAA AGT CAG TGG GG 3' primers. Proinsulin F 5' CTA GTT GCA GTA GTT CTC CAG 3' and proinsulin R 5' CCT GCC TAT CTT TCA GGT C 3' primers were used as internal control. Two founders were generated and were crossed to H₁RKO mice to establish H₁RKO-*CD11b^{H1R}*Tg mice. The experimental procedures used in this study were approved by the Animal Care and Use Committee of the University of Vermont.

2.2 Quantitative real-time RT-PCR (qRT-PCR)

To determine H₁R transgene expression in H₁RKO-*CD11b^{H1R}*Tg mice, single cell suspensions of spleen cells were plated in media and the nonadherent cells were removed after 3 h of plating. The adherent macrophages (>95%) were cultured for 24 h. CD11b⁺CD11c⁺ cells were isolated using EasySep mouse CD11c positive selection kit (StemCell technologies, BC, Canada) as recommended by the manufacturer. Microglial cells (CD45^{low/int}CD11b⁺) were FACS sorted from naïve animals as previously described [47]. Briefly, Mice were perfused with saline, and brain and spinal cord were removed. A single-cell suspension was obtained and passed through a 70-µm strainer. Mononuclear cells were obtained by Percoll gradient (37/70%) centrifugation, collected from the interphase, and washed. Cells were labeled with Live-Dead UV Blue dye (BD Pharmingen), followed by surface staining and FACS sorting for CD45^{low/int}CD11b⁺ cells. Total RNA was extracted from these cells using RNeasy RNA isolation reagent (Qiagen) as recommended by the manufacturer. cDNA generated from 500 ng of total RNA was used in qRT-PCR using the

SYBR green method. The sequences of *Hrh1* primers used were as follows: forward, 5'-CCAGAGCTTCGGAAGATAA-3'; reverse, 5'-ACCACAGCATGAGCAAAGTG-3'. *β2m* was used as reference gene, and relative mRNA levels were calculated using comparative threshold cycle (C_T) method.

2.3 Peritoneal exudate (PE) macrophages and LPS stimulation

Mice were immunized using the $1 \times \text{MOG}_{35-55}$ + CFA + PTX protocol. On day 7 after immunization, mice were administered an intraperitoneal injection of 1.5 ml of sterile thioglycolate (3%) and PE cells were collected by peritoneal lavage 4 days later [48]. The cells were suspended at a concentration of 1×10^6 macrophages/ml in media and the nonadherent cells removed after 3 h of plating. The adherent PE macrophages (>95%) were subsequently cultured in the presence and absence of 100ng/ml LPS for 24 h in media. Equivalent numbers of adherent cells were present amongst each of the strains studied. Supernatants were harvested, and the cytokines were analyzed by Bio-Plex multiplex analysis.

2.4 Induction and evaluation of EAE

Mice were immunized for the induction of EAE using a $2 \times$ or $1 \times$ immunization protocol [48, 49]. For the $2 \times$ protocol, animals were injected subcutaneously in the posterior right and left flank with a sonicated PBS/oil emulsion containing 100 μg of MOG_{35-55} and CFA (Sigma-Aldrich) supplemented with 200 μg of *Mycobacterium tuberculosis* H37Ra (Difco Laboratories). One week later, all mice received an identical injection of MOG_{35-55} -CFA. For the $1 \times$ immunization protocol, mice received a sonicated emulsion of 200 μg MOG_{35-55} and CFA containing 200 μg *Mycobacterium tuberculosis* H37RA by subcutaneous injections, immediately thereafter, each animal received 200 ng PTX (List Biological Laboratories) by intravenous injection. Mice were scored daily for clinical quantitative trait variables beginning at day 5 after injection as follows: 0, no clinical expression of disease; 1, flaccid tail without hind limb weakness; 2, hind limb weakness; 3, complete hind limb paralysis and floppy tail; 4, hind leg paralysis accompanied by a floppy tail and urinary or fecal incontinence; 5, moribund. Assessments of clinical quantitative trait variables and histological EAE lesions were performed as previously described [29].

2.5 Cytokine assays

For *ex-vivo* cytokine assays, spleen and draining lymph node cells (DLNs) were obtained from mice that were immunized previously with the $2 \times$ or $1 \times$ immunization protocols. Single cell suspensions at 1×10^6 cells/ml in RPMI 1640 medium (Cellgro Mediatech) plus 10% FBS (HyClone) were stimulated with 50 μg of MOG_{35-55} . Cell culture supernatants were recovered at 72 h and assayed for IFN- γ , IL-4, and IL-17 by ELISA (Biolegend). Supernatants were also analyzed for cytokines by Bio-Plex multiplex analysis (Bio-Rad).

2.6 Proliferation assay

Mice were immunized for EAE induction, and spleen and DLNs were harvested on d10. Single cell suspensions were prepared, and 5×10^5 cells/well in RPMI 1640 (10% FBS) were plated on standard 96-well U-bottom tissue culture plates and stimulated with 0, 1, 2, 10 and 50 μg of MOG_{35-55} for 72 h at 37°C. During the last 18 h of culture, 1 μCi of [^3H] thymidine (PerkinElmer) was added. Cells were harvested onto glass fiber filters and thymidine uptake was determined with a liquid scintillation counter.

2.7 Statistical analysis

Statistical analyses were performed using GraphPad Prism 5 software (GraphPad software Inc). Significance of differences was determined using one-way or two-way ANOVA as

described in the Figure Legends. For all analyses, $p = 0.05$ was considered significant except when a Bonferroni adjusted p -value was used to correct for multiple testing.

3. Results

3.1 Transgenic expression of H₁R in H₁RKO CD11b⁺ cells

In MS and EAE, APCs process and present CNS antigens to autoreactive T cells. Once T cells are activated in the periphery, they express chemokine receptors, which guide their extravasation and passage through the choroid plexus epithelium and endothelial cells of the BBB. In the CNS, primed T cells are restimulated by the resident APCs, resulting in an augmented inflammatory circuit, which culminates in neuropathology [50, 51]. In this study, we tested the hypothesis that H₁R signaling in CD11b⁺ cells contributes to EAE susceptibility. To directly address the hypothesis, we generated transgenic mice expressing H₁R under the control of the *CD11b* promoter (H₁RKO-*CD11b*^{H1R}Tg). Transgenic mice were generated directly on the wild-type (WT) C57BL/6J background. Two transgenic founders were obtained and crossed to H₁RKO mice to obtain H₁RKO mice expressing H₁R selectively in CD11b⁺ cells. The expression of the transgene was confirmed in CD11b⁺ cells, CD11b⁺CD11c⁺ (DCs) and CD45^{low/int}CD11b⁺ (microglia) cells from the two lines (H₁RKO-Tg5 and H₁RKO-Tg11) by qRT-PCR using H₁R specific primers. Relative to WT, H₁RKO-Tg5 mice had ~2 fold higher expression of H₁R transcripts. H₁R transcript levels in H₁RKO-Tg11 mice were comparable to WT. Two-way ANOVA looking at effect of Tg-line, cell type specific expression, and the interaction between Tg-line and cell type specific expression revealed that H₁R expression differs significantly between the Tg-lines but not among the cell types within the two Tg-lines (Tg-line effect, $p=0.03$; cell type effect, $p=0.70$; and interaction effect, $p=0.5$) (Fig. 1).

3.2 Expression of H₁R by CD11b⁺ cells regulates cytokine production upon LPS stimulation

To assess the functionality of the transgene during exposure to EAE immunizing conditions, we immunized WT, H₁RKO, H₁RKO-Tg5, and H₁RKO-Tg11 mice using the 1× myelin oligodendrocyte glycoprotein (MOG_{35–55}) + CFA + PTX protocol and 7 days post-immunization mice were administered thioglycolate intraperitoneally to recover peritoneal exudate (PE) macrophages, which express CD11b. These cells were stimulated in the presence or absence of LPS for 24 h and the supernatants assessed for cytokine production by multiplex assay. With the exception of IL-12 (p40), RANTES, G-CSF, and MCP-1, non-LPS stimulated PE macrophages either produced minimal or undetectable levels of cytokines. A panel of 9 cytokines produced by PE macrophages had no significant difference, either following immunization or *in-vitro* LPS stimulation (Supplementary Fig. 1). The results of 12 cytokine/chemokines that showed differential effects upon LPS stimulation are shown in the Fig. 2. Upon LPS stimulation, PE macrophages from H₁RKO mice produced significantly higher levels of the cytokines IL-3, IL-6, IL-12(p70), IL-17, Eotaxin, GM-CSF, and TNF- α when compared to WT mice. Importantly, re-expression of H₁R in CD11b⁺ cells clearly restored the production of these cytokines to the WT level. Despite significant differences in H₁R transgene expression in lines Tg5 and Tg11, in no instance were there differences in cytokine production by PE macrophages from the two lines. These results show that the transgene is functional and that H₁R signaling in CD11b⁺ cells can modulate cytokine production by PE macrophages upon LPS stimulation.

3.3 Transgenic expression of H₁R in H₁RKO CD11b⁺ cells fails to complement EAE severity

To assess whether H₁R signaling in CD11b⁺ cells influences EAE, we examined the susceptibility of WT, H₁RKO, H₁RKO-Tg5, and H₁RKO-Tg11 mice to EAE by

immunization using $1 \times \text{MOG}_{35-55} + \text{CFA} + \text{PTX}$ and $2 \times \text{MOG}_{35-55} + \text{CFA}$ protocols [48]. The disease course among the three strains was significantly different and consistent to our previous findings showing that H₁RKO mice exhibited a significantly less severe disease course than WT mice elicited by both immunization protocols [28, 29]. Surprisingly, however, the EAE disease course of both transgenic lines was not significantly different compared to H₁RKO mice ($\text{B6} > \text{H}_1\text{RKO} = \text{H}_1\text{RKO-CD11b}^{\text{H1R}} \text{Tg5} = \text{H}_1\text{RKO-CD11b}^{\text{H1R}} \text{Tg11}$) (Fig. 3A and 3B). Similar to the disease course, analysis of EAE-associated clinical trait variables following both immunization protocols revealed that cumulative disease score, day of onset, peak score, number of days affected, and overall severity index were significantly different between WT and H₁RKO mice. However, none of the EAE clinical trait variables were different between H₁RKO, H₁RKO-Tg5, and H₁RKO-Tg11 mice ($\text{B6} > \text{H}_1\text{RKO} = \text{H}_1\text{RKO-CD11b}^{\text{H1R}} \text{Tg5} = \text{H}_1\text{RKO-CD11b}^{\text{H1R}} \text{Tg11}$) (Tables I and II). These results suggest that the re-expression of H₁R selectively in CD11b⁺ cells does not complement EAE severity.

3.4 Expression of H₁R by CD11b⁺ cells does not affect encephalitogenic T cell responses

HA and H₁R play a significant role in T cell polarization, proliferation, and cytokine production [16]. It has been also shown that H₁R expression in APCs influences T cell activation and polarization [31, 35–37]. Therefore, to elucidate whether re-expression of H₁R in CD11b⁺ cells has any influence on the encephalitogenic T cells response, we examined *ex vivo* the MOG₃₅₋₅₅ specific response by spleen and draining lymph node (DLN) cells from $1 \times \text{MOG}_{35-55} + \text{CFA} + \text{PTX}$ and $2 \times \text{MOG}_{35-55} + \text{CFA}$ immunized WT, H₁RKO, H₁RKO-Tg5, and H₁RKO-Tg11 mice. Using a two-factor design, we detected a significant effect of immunization on the cytokine production among the strains. In accordance with our previous results [28–30], restimulated spleen and DLN cells from H₁RKO mice produced significantly less IFN- γ (Fig. 4A), higher levels of IL-4 (Fig. 4B) with no change in IL-17 (Fig. 4C) production compared to WT cells. However, restimulated cells from H₁RKO-Tg5 and H₁RKO-Tg11 mice produced similar levels of IFN- γ , IL-4, and IL-17 as H₁RKO mice (Fig. 4A, B, and C). Multiplex assay of 20 other cytokine/chemokines from restimulated cells revealed no significant effect of either the deletion of H₁R or re-expression of H₁R in CD11b⁺ cells (Supplementary Fig. 2). In MOG₃₅₋₅₅ specific *ex vivo* proliferation assays, all three strains responded equivalently in a dose dependent fashion (Fig. 4D). Therefore, in accordance with the EAE clinical disease trait data, these results indicate that H₁R signaling in CD11b⁺ cells minimally influences the MOG₃₅₋₅₅ specific encephalitogenic T cell response.

4. Discussion

Susceptibility to MS and EAE is polygenic in nature and conferred by MHC class II and non-MHC genes [52]. Previously, we identified *Hrh1* as a shared autoimmune susceptibility gene in EAE and autoimmune orchitis [28, 53–55] and human studies have also correlated the importance of H₁R in MS pathogenesis and disease progression [25–27]. HA, being a biogenic monoamine, plays a pivotal role in influencing many cell types [16]. HA binding to H₁R on macrophages and DCs has a profound effect on their maturation, differentiation, chemotaxis, antigen processing, as well as their ability to modulate the adaptive immune system by secreting cytokines that influence the generation of effector Th1 or Th2 cells [15]. Furthermore, DCs and macrophages play a pivotal role in the pathogenesis of EAE and MS. Here we asked whether H₁R signaling specifically in CD11b⁺ cells affected EAE susceptibility. Contrary to what is known about the role of H₁R in macrophages and DCs in other inflammatory and autoimmune conditions, our data demonstrate that despite modulating LPS-induced macrophage cytokine production *in-vitro*, H₁R signaling in CD11b⁺ cells does not complement EAE susceptibility. Similarly, the re-expression of H₁R

in CD11b⁺ cells does not influence antigen specific T cell responses compared to that of H₁RKO mice.

Macrophages and DCs functionally express H₁R, H₂R, and H₄R, but data for the expression of H₃R in these cell types is highly controversial [15]. HA, in combination with LPS, can influence DCs and macrophages to produce proinflammatory cytokines and chemokines [35]. Here we show that compared to WT mice, *in-vitro* LPS stimulated PE macrophages from immunized H₁RKO mice produced significantly higher levels of IL-3, IL-6, IL-12(p70), IL-17, Eotaxin, GM-CSF, and TNF- α and all of these cytokines were restored to WT levels in both H₁RKO-Tg5 and H₁RKO-Tg11 mice. These results clearly demonstrate that the H₁R transgene is functional and the failure of H₁RKO-Tg5 and H₁RKO-Tg11 mice to complement EAE susceptibility to the WT level is not due to a dysfunctional or nonfunctional H₁R transgene. In addition, we exclude the possibility that our results are due to random transgene insertion as we generated two founder lines of transgenic mice (H₁RKO-Tg5 and H₁RKO-Tg11), which exhibited similar EAE disease courses and ability to participate in *ex-vivo* T cell responses to MOG_{35–55}.

EAE can be passively induced in mice with the transfer of myelin specific T cells [56]. Myelin specific T cells are associated with the inflammatory lesions and in the cerebrospinal fluids of MS patients [57]. These observations led to the thought that the pathogenesis of MS and EAE is driven mainly by encephalitogenic CD4 T cells. Therefore, the role of innate immune cells, such as DCs or macrophages, in the pathogenesis of MS and EAE has been overlooked for many years despite the fact that MHC class II alleles (*DRB1*1501*) represent the majority of the genetic risk of developing MS [58]. However, in recent years, therapies targeting these cells are under investigation in EAE and other autoimmune disorders [3, 59].

We have previously reported decreased EAE susceptibility in H₁RKO mice [28] and the selective re-expression of H₁R in CD4⁺ T cells from H₁RKO mice fully complements EAE susceptibility. Moreover, we have shown that H₁R expression in naïve T cells is required for p38 MAPK activation and maximal IFN- γ production [29]. In addition, overexpression of H₁R specifically on endothelial cells strongly reduces EAE susceptibility and decreases BBB permeability in H₁RKO mice [30]. However, the absence of restored EAE susceptibility in transgenic mice expressing H₁R specifically in CD11b⁺ cells, including macrophages, DCs and microglia, suggests that the inflammatory response, either during disease induction or progression, is not affected by H₁R signaling in these cells. Whereas CD11b⁺ cells appear to represent ineffective targets, therapies designed to specifically inhibit or activate H₁R in T cells or endothelial cells, respectively, may be beneficial in the treatment of EAE and MS.

5. Conclusion

H₁R has been reported to influence innate immune cell maturation, differentiation, chemotaxis, cytokine production, which in turn influences CD4⁺ T cell effector responses. The observations made in the present study indicate that H₁R signaling in CD11b-expressing cells which includes antigen presenting cells is not required for susceptibility to EAE. These results confirm that the decreased encephalitogenic potential of T cells in H₁RKO mice is due to a T cell intrinsic defect, likely attributable to the requirement of H₁R for the full encephalitogenicity of T cells [29], and not due to an effect on CD11b⁺ cells, and further emphasize the cell-specific effects that an AID gene can play in the pathogenesis of complex diseases such as EAE and MS.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

APCs	antigen presenting cells
DCs	dendritic cells
HA	histamine
EAE	experimental allergic encephalomyelitis
MS	multiple sclerosis
BBB	blood brain barrier
PE	peritoneal exudate
DLN	draining lymph node
MOG_{35–55}	myelin oligodendrocyte glycoprotein
WT	wild type
SAID	shared autoimmune disease

References

1. Gandhi R, Laroni A, Weiner HL. Role of the innate immune system in the pathogenesis of multiple sclerosis. *J Neuroimmunol.* 2010; 221:7–14. [PubMed: 19931190]
2. Zozulya AL, Clarkson BD, Ortler S, Fabry Z, Wiendl H. The role of dendritic cells in CNS autoimmunity. *J Mol Med (Berl).* 2010; 88:535–544. [PubMed: 20217033]
3. Steinman RM. Dendritic cells: understanding immunogenicity. *Eur J Immunol.* 2007; 37(Suppl 1):S53–60. [PubMed: 17972346]
4. Yamazaki S, Iyoda T, Tarbell K, Olson K, Velinzon K, Inaba K, Steinman RM. Direct expansion of functional CD25+ CD4+ regulatory T cells by antigen-processing dendritic cells. *J Exp Med.* 2003; 198:235–247. [PubMed: 12874257]
5. Yamazaki S, Patel M, Harper A, Bonito A, Fukuyama H, Pack M, Tarbell KV, Talmor M, Ravetch JV, Inaba K, Steinman RM. Effective expansion of alloantigen-specific Foxp3+ CD25+ CD4+ regulatory T cells by dendritic cells during the mixed leukocyte reaction. *Proc Natl Acad Sci U S A.* 2006; 103:2758–2763. [PubMed: 16473944]
6. Tarbell KV, Yamazaki S, Olson K, Toy P, Steinman RM. CD25+ CD4+ T cells, expanded with dendritic cells presenting a single autoantigenic peptide, suppress autoimmune diabetes. *J Exp Med.* 2004; 199:1467–1477. [PubMed: 15184500]
7. Comabella M, Montalban X, Munz C, Lunemann JD. Targeting dendritic cells to treat multiple sclerosis. *Nat Rev Neurol.* 2010; 6:499–507. [PubMed: 20717105]
8. Greenstein JI. Current concepts of the cellular and molecular pathophysiology of multiple sclerosis. *Dev Neurobiol.* 2007; 67:1248–1265. [PubMed: 17514718]
9. Karni A, Abraham M, Monsonogo A, Cai G, Freeman GJ, Hafler D, Khoury SJ, Weiner HL. Innate immunity in multiple sclerosis: myeloid dendritic cells in secondary progressive multiple sclerosis

- are activated and drive a proinflammatory immune response. *J Immunol.* 2006; 177:4196–4202. [PubMed: 16951385]
10. Huang YM, Xiao BG, Ozenci V, Kouwenhoven M, Teleshova N, Fredrikson S, Link H. Multiple sclerosis is associated with high levels of circulating dendritic cells secreting pro-inflammatory cytokines. *J Neuroimmunol.* 1999; 99:82–90. [PubMed: 10496180]
 11. Vaknin-Dembinsky A, Balashov K, Weiner HL. IL-23 is increased in dendritic cells in multiple sclerosis and down-regulation of IL-23 by antisense oligos increases dendritic cell IL-10 production. *J Immunol.* 2006; 176:7768–7774. [PubMed: 16751425]
 12. Vaknin-Dembinsky A, Murugaiyan G, Hafler DA, Astier AL, Weiner HL. Increased IL-23 secretion and altered chemokine production by dendritic cells upon CD46 activation in patients with multiple sclerosis. *J Neuroimmunol.* 2008; 195:140–145. [PubMed: 18403025]
 13. Warren KG, Catz I, Ferenczi LZ, Krantz MJ. Intravenous synthetic peptide MBP8298 delayed disease progression in an HLA Class II-defined cohort of patients with progressive multiple sclerosis: results of a 24-month double-blind placebo-controlled clinical trial and 5 years of follow-up treatment. *Eur J Neurol.* 2006; 13:887–895. [PubMed: 16879301]
 14. del Pilar Martin M, Cravens PD, Winger R, Frohman EM, Racke MK, Eagar TN, Zamvil SS, Weber MS, Hemmer B, Karandikar NJ, Kleinschmidt-DeMasters BK, Stuve O. Decrease in the numbers of dendritic cells and CD4+ T cells in cerebral perivascular spaces due to natalizumab. *Arch Neurol.* 2008; 65:1596–1603. [PubMed: 18852339]
 15. Simon T, Laszlo V, Falus A. Impact of histamine on dendritic cell functions. *Cell Biol Int.* 2011; 35:997–1000. [PubMed: 21933149]
 16. Akdis CA, Simons FE. Histamine receptors are hot in immunopharmacology. *Eur J Pharmacol.* 2006; 533:69–76. [PubMed: 16448645]
 17. Baron JL, Madri JA, Ruddle NH, Hashim G, Janeway CA Jr. Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. *J Exp Med.* 1993; 177:57–68. [PubMed: 7678116]
 18. Tanaka S, Deai K, Konomi A, Takahashi K, Yamane H, Sugimoto Y, Ichikawa A. Expression of L-histidine decarboxylase in granules of elicited mouse polymorphonuclear leukocytes. *Eur J Immunol.* 2004; 34:1472–1482. [PubMed: 15114681]
 19. Logothetis L, Mylonas IA, Baloyannis S, Pashalidou M, Orolagos A, Zafeiropoulos A, Kosta V, Theoharides TC. A pilot, open label, clinical trial using hydroxyzine in multiple sclerosis. *Int J Immunopathol Pharmacol.* 2005; 18:771–778. [PubMed: 16388727]
 20. Gregory GD, Bickford A, Robbie-Ryan M, Tanzola M, Brown MA. MASTering the immune response: mast cells in autoimmunity. *Novartis Found Symp.* 2005; 271:215–225. discussion 225–231. [PubMed: 16605138]
 21. Pollack I, Hofbauer A. Histamine-like immunoreactivity in the visual system and brain of *Drosophila melanogaster*. *Cell Tissue Res.* 1991; 266:391–398. [PubMed: 1684918]
 22. Shiraishi M, Hirasawa N, Oikawa S, Kobayashi Y, Ohuchi K. Analysis of histamine-producing cells at the late phase of allergic inflammation in rats. *Immunology.* 2000; 99:600–606. [PubMed: 10792508]
 23. Leurs R, Smit MJ, Timmerman H. Molecular pharmacological aspects of histamine receptors. *Pharmacol Ther.* 1995; 66:413–463. [PubMed: 7494855]
 24. Yokoyama M, Yokoyama A, Mori S, Takahashi HK, Yoshino T, Watanabe T, Ohtsu H, Nishibori M. Inducible histamine protects mice from *P. acnes*-primed and LPS-induced hepatitis through H2-receptor stimulation. *Gastroenterology.* 2004; 127:892–902. [PubMed: 15362044]
 25. Alonso A, Jick SS, Hernan MA. Allergy, histamine 1 receptor blockers, and the risk of multiple sclerosis. *Neurology.* 2006; 66:572–575. [PubMed: 16505314]
 26. El Behi M, Dubucquoi S, Lefranc D, Zephir H, De Seze J, Vermersch P, Prin L. New insights into cell responses involved in experimental autoimmune encephalomyelitis and multiple sclerosis. *Immunol Lett.* 2005; 96:11–26. [PubMed: 15585303]
 27. Theoharides TC, Cochrane DE. Critical role of mast cells in inflammatory diseases and the effect of acute stress. *J Neuroimmunol.* 2004; 146:1–12. [PubMed: 14698841]

28. Bebo BF Jr, Yong T, Orr EL, Linthicum DS. Hypothesis: a possible role for mast cells and their inflammatory mediators in the pathogenesis of autoimmune encephalomyelitis. *J Neurosci Res.* 1996; 45:340–348. [PubMed: 8872894]
29. Hofstra CL, Desai PJ, Thurmond RL, Fung-Leung WP. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. *J Pharmacol Exp Ther.* 2003; 305:1212–1221. [PubMed: 12626656]
30. Lu C, Diehl SA, Noubade R, Ledoux J, Nelson MT, Spach K, Zachary JF, Blankenhorn EP, Teuscher C. Endothelial histamine H1 receptor signaling reduces blood-brain barrier permeability and susceptibility to autoimmune encephalomyelitis. *Proc Natl Acad Sci U S A.* 2010; 107:18967–18972. [PubMed: 20956310]
31. Caron G, Delneste Y, Roelandts E, Duez C, Bonnefoy JY, Pestel J, Jeannin P. Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. *J Immunol.* 2001; 167:3682–3686. [PubMed: 11564782]
32. O'Mahony L, Akdis M, Akdis CA. Regulation of the immune response and inflammation by histamine and histamine receptors. *J Allergy Clin Immunol.* 2011; 128:1153–1162. [PubMed: 21824648]
33. Pavlinkova G, Yanagawa Y, Kikuchi K, Iwabuchi K, Onoe K. Effects of histamine on functional maturation of dendritic cells. *Immunobiology.* 2003; 207:315–325. [PubMed: 14575147]
34. Szeberenyi JB, Laszlo V, Pallinger E, Orso E, Rothe G, Schmitz G, Falus A. Intracellular histamine content increases during in vitro dendritic cell differentiation. *Inflamm Res.* 2001; 50(Suppl 2):S112–113. [PubMed: 11411579]
35. Mazzoni A, Young HA, Spitzer JH, Visintin A, Segal DM. Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. *J Clin Invest.* 2001; 108:1865–1873. [PubMed: 11748270]
36. Jutel M, Watanabe T, Akdis M, Blaser K, Akdis CA. Immune regulation by histamine. *Curr Opin Immunol.* 2002; 14:735–740. [PubMed: 12413523]
37. Vanbervliet B, Akdis M, Vocanson M, Rozieres A, Benetiere J, Rouzaire P, Akdis CA, Nicolas JF, Hennino A. Histamine receptor H1 signaling on dendritic cells plays a key role in the IFN-gamma/IL-17 balance in T cell-mediated skin inflammation. *J Allergy Clin Immunol.* 2011; 127:943–953. e941–910. [PubMed: 21269673]
38. Beller DI, Springer TA, Schreiber RD. Anti-Mac-1 selectively inhibits the mouse and human type three complement receptor. *J Exp Med.* 1982; 156:1000–1009. [PubMed: 7153706]
39. Rosmarin AG, Weil SC, Rosner GL, Griffin JD, Arnaout MA, Tenen DG. Differential expression of CD11b/CD18 (Mo1) and myeloperoxidase genes during myeloid differentiation. *Blood.* 1989; 73:131–136. [PubMed: 2562920]
40. Anjuere F, Martin P, Ferrero I, Fraga ML, del Hoyo GM, Wright N, Ardavin C. Definition of dendritic cell subpopulations present in the spleen, Peyer's patches, lymph nodes, and skin of the mouse. *Blood.* 1999; 93:590–598. [PubMed: 9885220]
41. Schwartz JC, Arrang JM, Garbarg M, Pollard H, Ruat M. Histaminergic transmission in the mammalian brain. *Physiol Rev.* 1991; 71:1–51. [PubMed: 1846044]
42. Claiborne BJ, Selverston AI. Histamine as a neurotransmitter in the stomatogastric nervous system of the spiny lobster. *J Neurosci.* 1984; 4:708–721. [PubMed: 6142932]
43. Stuart AE. From fruit flies to barnacles, histamine is the neurotransmitter of arthropod photoreceptors. *Neuron.* 1999; 22:431–433. [PubMed: 10197524]
44. Gisselmann G, Plonka J, Pusch H, Hatt H. Unusual functional properties of homo- and heteromultimeric histamine-gated chloride channels of *Drosophila melanogaster*: spontaneous currents and dual gating by GABA and histamine. *Neurosci Lett.* 2004; 372:151–156. [PubMed: 15531107]
45. McCaman RE, Weinreich D. Histaminergic synaptic transmission in the cerebral ganglion of *Aplysia*. *J Neurophysiol.* 1985; 53:1016–1037. [PubMed: 2987431]
46. Farrant M, Kaila K. The cellular, molecular and ionic basis of GABA(A) receptor signalling. *Prog Brain Res.* 2007; 160:59–87. [PubMed: 17499109]
47. del Rio R, Noubade R, Saligrama N, Wall EH, Kremmentsov DN, Poynter ME, Zachary JF, Thurmond RL, Teuscher C. Histamine H4 receptor optimizes T regulatory cell frequency and

- facilitates anti-inflammatory responses within the central nervous system. *J Immunol.* 2012; 188:541–547. [PubMed: 22147765]
48. Jutel M, Watanabe T, Klunker S, Akdis M, Thomet OA, Malolepszy J, Zak-Nejmark T, Koga R, Kobayashi T, Blaser K, Akdis CA. Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. *Nature.* 2001; 413:420–425. [PubMed: 11574888]
 49. Noubade R, Milligan G, Zachary JF, Blankenhorn EP, del Rio R, Rincon M, Teuscher C. Histamine receptor H1 is required for TCR-mediated p38 MAPK activation and optimal IFN- γ production in mice. *J Clin Invest.* 2007; 117:3507–3518. [PubMed: 17965772]
 50. Holman DW, Klein RS, Ransohoff RM. The blood-brain barrier, chemokines and multiple sclerosis. *Biochim Biophys Acta.* 2011; 1812:220–230. [PubMed: 20692338]
 51. O'Connor KC, Bar-Or A, Hafler DA. The neuroimmunology of multiple sclerosis: possible roles of T and B lymphocytes in immunopathogenesis. *J Clin Immunol.* 2001; 21:81–92. [PubMed: 11332657]
 52. Zuvich RL, McCauley JL, Pericak-Vance MA, Haines JL. Genetics and pathogenesis of multiple sclerosis. *Semin Immunol.* 2009; 21:328–333. [PubMed: 19775910]
 53. Meeker ND, Stafford AN, Lunceford JK, Avner P, Ma RZ, Teuscher C. Physical mapping of the autoimmune disease susceptibility locus, Bphs: co-localization with a cluster of genes from the TNF receptor superfamily on mouse chromosome 6. *Mamm Genome.* 1999; 10:858–863. [PubMed: 10441735]
 54. Sudweeks JD, Todd JA, Blankenhorn EP, Wardell BB, Woodward SR, Meeker ND, Estes SS, Teuscher C. Locus controlling Bordetella pertussis-induced histamine sensitization (Bphs), an autoimmune disease-susceptibility gene, maps distal to T-cell receptor beta-chain gene on mouse chromosome 6. *Proc Natl Acad Sci U S A.* 1993; 90:3700–3704. [PubMed: 8475118]
 55. Teuscher C. Experimental allergic orchitis in mice. II. Association of disease susceptibility with the locus controlling Bordetella pertussis-induced sensitivity to histamine. *Immunogenetics.* 1985; 22:417–425. [PubMed: 4065933]
 56. Ben-Nun A, Wekerle H, Cohen IR. The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. *Eur J Immunol.* 1981; 11:195–199. [PubMed: 6165588]
 57. McFarland HF, Martin R. Multiple sclerosis: a complicated picture of autoimmunity. *Nat Immunol.* 2007; 8:913–919. [PubMed: 17712344]
 58. Oksenberg JR, Baranzini SE, Sawcer S, Hauser SL. The genetics of multiple sclerosis: SNPs to pathways to pathogenesis. *Nat Rev Genet.* 2008; 9:516–526. [PubMed: 18542080]
 59. Steinman RM. Dendritic cells in vivo: a key target for a new vaccine science. *Immunity.* 2008; 29:319–324. [PubMed: 18799140]

Figure 1.

Selective expression of H₁R in H₁RKO CD11b⁺ cells. *Hrh1* transgene expression in CD11b⁺, CD11b⁺CD11c⁺ (DCs), and CD45^{low/int}CD11b⁺ (microglial) cells were determined by qRT-PCR and analyzed using comparative Ct method with β 2-microglobulin as an endogenous control. Receptor expression levels are normalized to the WT level. Data are representative of 2 independent experiments ($n = 3$ per strain). Two-way ANOVA was used to examine the effect of Tg-line, cell type specific expression, and interaction between Tg-line and cell type specific expression. H₁R expression differed significantly between the two Tg-lines but not among cell types within line (Tg-line effect, $p=0.03$; cell type effect, $p=0.70$; and interaction effect, $p=0.5$). ** $p<0.01$ (Mann-Whitney test).

Figure 2.

Expression of H₁R by CD11b⁺ cells regulates cytokine responses. WT, H₁RKO, H₁RKO-*CD11b*^{H1R} Tg5, and H₁RKO-*CD11b*^{H1R} Tg11 ($n = 4-5$ per strain) mice were immunized using the $1 \times \text{MOG}_{35-55} + \text{CFA} + \text{PTX}$ protocol. On day 7 after immunization, mice were administered an intraperitoneal injection of 1.5 ml of sterile thioglycolate (3%) and peritoneal exudate (PE) cells were collected by lavage 4 days later. The cells were suspended at a concentration of 1×10^6 macrophages/ml in media and the nonadherent cells removed after 3 h of plating. The adherent PE macrophages (>95%) were subsequently cultured in the presence and absence of 100ng/ml LPS for 24 h in media. Supernatants were harvested, and the indicated cytokines [IL-1 α , IL-1 β , IL-2, IL-3, IL-5, IL-6, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, eotaxin-1/CCL11, G-CSF, GM-CSF, KC/CXCL1, MCP-1/CCL2, MIP-1 α /CCL3, MIP-1 β /CCL4, RANTES/CCL5, and TNF- α] were analyzed by Bio-Plex multiplex analysis. Significance of differences was determined by two-way ANOVA followed by Bonferroni multiple comparison test with a corrected p-value 0.0025.

Figure 3.

Transgenic expression of H₁R in H₁RKO CD11b⁺ cells fails to complement EAE severity. EAE was elicited in WT, H₁RKO, and H₁RKO-*CD11b*^{H1R} Tg mice using (A) 1× MOG₃₅₋₅₅ + CFA + PTX ($n = 19$ -WT, 52-H₁RKO, 40-H₁RKO-*CD11b*^{H1R} Tg5, and 20-H₁RKO-*CD11b*^{H1R} Tg11) or (B) 2× MOG₃₅₋₅₅ + CFA immunization protocol, ($n = 18$ -WT, 33-H₁RKO, 31-H₁RKO-*CD11b*^{H1R} Tg5, and 23-H₁RKO-*CD11b*^{H1R} Tg11). Regression analysis revealed that the disease course differed significantly among the strains ($p < 0.0001$) with being B6 > H₁RKO = H₁RKO-*CD11b*^{H1R} Tg5 = H₁RKO-*CD11b*^{H1R} Tg11. The significance of the differences in mean clinical scores at individual time points post-immunization was evaluated by two-way ANOVA followed by Bonferroni multiple comparisons with B6 > H₁RKO = H₁RKO-*CD11b*^{H1R} Tg5 = H₁RKO-*CD11b*^{H1R} Tg11 (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; and ****, $p < 0.0001$).

Figure 4.

Expression of H₁R by CD11b⁺ cells did not alter the antigen-specific cytokine responses of 1× MOG₃₅₋₅₅ + CFA + PTX or 2× MOG₃₅₋₅₅ + CFA immunized mice. Spleen and DLN cells were isolated from WT, H₁RKO, H₁RKO-*CD11b*^{H1R} Tg5, and H₁RKO-*CD11b*^{H1R} Tg11 ($n = 8-10$ per strain) mice that were immunized 10 d previously with the 1× MOG₃₅₋₅₅ + CFA + PTX or 2× MOG₃₅₋₅₅ + CFA protocols. Cells were isolated and restimulated *ex vivo* with 50 μg/mL MOG₃₅₋₅₅ for 72 h. Supernatants were harvested and (A) IFN- γ , (B) IL-4, and (C) IL-17 levels were determined by ELISA. Significance of differences was determined by two-way ANOVA followed by Bonferroni multiple comparisons (*, $p < 0.05$, **, $p < 0.01$, *** $p < 0.001$). (D) Cells from 2× MOG₃₅₋₅₅ + CFA immunized mice were restimulated *ex vivo* in the presence of various concentrations of MOG₃₅₋₅₅ for 72 h, and proliferation was determined by [³H]-thymidine incorporation. The mean cpm $\pm$ SD were calculated from triplicate wells, and the results shown are representative of two experiments. Significance of differences was determined by two-way ANOVA ([MOG₃₅₋₅₅] effect, $p < 0.0001$; strain effect, $p = 0.27$; interaction, $p = 0.64$).

Table 1

Clinical disease traits following immunization of C57BL/6J, H₁RKO, and H₁RKO- *CD11b^{HIR}* Tg mice with 1×MOG_{35–55} + CFA + PTX

Strain	Incidence ^a	Affected animals			
		CDS	DO	PS	DA
B6	18/19 (95)	56.2±4.6	13.1±0.3	3.9±0.3	18.0±0.3
H ₁ RKO	55/56 (98)	32.1±1.4	15.7±0.4	3.0±0.1	15.0±0.5
<i>CD11b^{HIR}</i> Tg5	38/40 (95)	30.3±2.2	16.2±0.3	3.1±0.1	13.7±0.5
<i>CD11b^{HIR}</i> Tg11	19/20 (95)	33.3±2.9	15.3±0.5	3.2±0.2	14.8±0.9
Overall	$\chi^2=0.99$, 3 p=0.80	H=31.2 p<0.0001	H=22.1 p<0.0001	H=11.1 p=0.01	H=28.0 p<0.0001
Post-hoc		B6>H1RKO=Tg5=Tg11 for all trait variables			

^aPercent affected. Animals were considered affected if clinical scores 1 were apparent for 2 or more consecutive days. CDS, cumulative disease score over 30 days of experiment; DA, days affected; DO, day of onset; PS, peak score; SI, severity index (cumulative disease score/days affected). Means ± SD are shown. The significance of differences for the trait values among the strains was assessed by χ^2 analysis (overall incidence) or the Kruskal–Wallis test (H), followed by Dunn's post hoc multiple comparisons. p values are as indicated.

Table II
Clinical disease traits following immunization of C57BL/6J, H₁RKO, and H₁RKO- *CD11b^{HIR}* Tg mice with 2× MOG_{35–55} + CFA

Strain	Incidence ^a	Affected Animals			
		CDS	DO	PS	DA
B6	18/18 (100)	37.6±2.9	16.6±0.7	3.2±0.2	14.2±0.7
H ₁ RKO	26/33 (79)	20.0±1.8	17.1±0.5	2.2±0.1	12.1±0.8
<i>CD11b^{HIR}</i> Tg5	22/29 (76)	17.2±2.7	18.1±0.5	2.5±0.2	11.8±0.9
<i>CD11b^{HIR}</i> Tg11	12/22 (55)	11.6±2.7	17.8±0.6	2.3±0.2	12.1±0.9
Overall	$\chi^2=11.5, 3$ p=0.009	H=28.7 p<0.0001	H=7.4 p=0.06	H=15.9 p=0.001	H=7.8 p=0.05
Post-hoc	B6>H ₁ RKO=Tg5=Tg11	B6>H ₁ RKO= Tg5=Tg11	B6>H ₁ RKO= Tg5=Tg11	B6>H ₁ RKO=Tg5=Tg11	B6>H ₁ RKO=Tg5=Tg11

^aPercent affected. Animals were considered affected if clinical scores 1 were apparent for 2 or more consecutive days. CDS, cumulative disease score over 30 days of experiment; DA, days affected; DO, day of onset; PS, peak score; SI, severity index (cumulative disease score/days affected). Means ± SD are shown. The significance of differences for the trait values among the strains was assessed by χ^2 analysis (overall incidence) or the Kruskal–Wallis test (H), followed by Dunn's post hoc multiple comparisons. p values are as indicated.