6-18-2008

Histamine Receptor H1 Signaling in Central Nervous System Autoimmmune Disease and Immune Deviation

Rajaumas Noubade
University of Vermont

HISTAMINE RECEPTOR H_1 SIGNALING IN CENTRAL NERVOUS SYSTEM AUTOIMMMUNE DISEASE AND IMMUNE DEVIATION

A Dissertation Presented

by

Rajkumar Noubade

to

The Faculty of the Graduate College

of

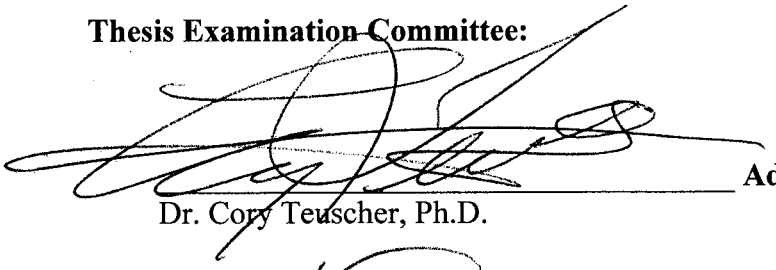
The University of Vermont

In Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
Specializing in Cell and Molecular Biology

February, 2008

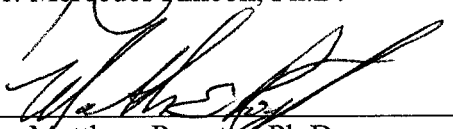
Accepted by the Faculty of the Graduate College, The University of Vermont, in partial fulfillment of the requirements for the degree of Doctor of Philosophy, specializing in Cell and Molecular Biology.

Thesis Examination Committee:

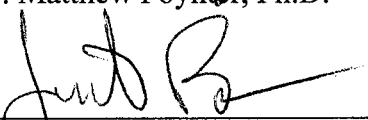


Advisor
Dr. Cory Teuscher, Ph.D.

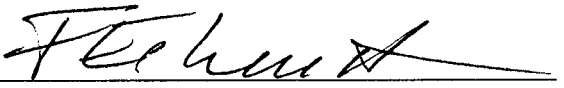
Dr. Mercedes Rincon, Ph.D.



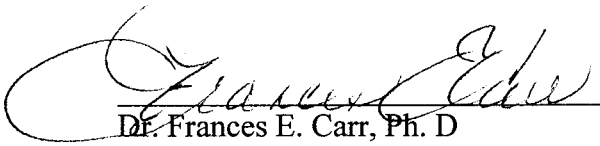
Dr. Matthew Poynter, Ph.D.



Dr. Jonathan Boyson, Ph.D.



Chairperson
Dr. Felix Eckenstein, Ph. D.



Vice President for Research and Dean of Graduate Studies
Dr. Frances E. Carr, Ph. D

Date: October 26, 2007

ABSTRACT

Multiple Sclerosis (MS) is a demyelinating disorder of the central nervous system affecting 0.1% of the population in the Northern hemisphere. MS is a complex disease that depends on genetic and environmental factors and is controlled by multiple genes that exert a modest effect in the overall disease outcome. The complex nature of the disease complicates the study of individual genes and their contribution in the disease process.

To investigate mechanisms underlying the development of diseases like MS and how disease course can be manipulated, animal models have been extensively used, with Experimental Allergic Encephalomyelitis (EAE) being the principle autoimmune model for MS. Even though EAE, like MS, is a complex disease and polygenic in nature, it can be reduced to monogenic intermediate or subphenotypes, which allows for identification of the causative gene and its mechanism.

One such subphenotype of EAE in mice, *Bordetella pertussis* toxin-induced histamine sensitization (Bphs) is controlled by *Hrh1*, gene encoding mouse histamine receptor H_1 (H_1R), wherein sensitized animals of susceptible strains die upon histamine challenge and resistant strains do not. Moreover, mice deficient in H_1R (H1RKO) show delayed onset and reduced severity in the clinical course of EAE. However, the mechanism by which H_1R and its polymorphisms regulate EAE is unknown.

As a disease susceptibility gene, *Hrh1* could act in different cell types and at several checkpoints in the disease process. This includes endothelial cells that regulate blood-brain barrier, antigen presenting cells or T cells, which regulate the cytokine production. Using transgenic mice expressing H_1R exclusively in T cells, this study shows that H_1R expression in T cells is sufficient to restore the EAE severity and the disease associated cytokine production of H1RKO mice to wild type levels.

H_1R from susceptible and resistant strains of mice differ by three amino acids. The P-V-P haplotype (H_1R^S) is associated with disease susceptibility whereas the L-M-S (H_1R^R) haplotype is associated with less severe disease. In this study, using transgenic mice, we show that reexpression of H_1R^S fully complements the clinical EAE and the disease-associated cytokine production of H1RKO mice to wild type levels, however, reexpression of H_1R^R fails to do so. These data suggest that H_1R^R is not functional relative to H_1R^S. Mechanistically, using 293T cells, we show that the two H_1R alleles exhibit differential cell surface expression and altered intracellular trafficking, with the H_1R^R allele being retained within the endoplasmic reticulum (ER). Moreover, we show that all three residues (L-M-S) comprising the H_1R^R haplotype are required for altered expression. Thus, polymorphisms influencing cell surface expression of H_1R regulate immune functions and autoimmune disease susceptibility.

CITATIONS

Material from this dissertation has been published in the following form:

Rajkumar Noubade, Roxana del Rio, Graeme Milligan, James F. Zachary, Elizabeth P. Blankenhorn, Mercedes Rincon and Cory Teuscher. (2007). Histamine receptor H_1 is required for TCR-mediated p38 MAP kinase activation and IFN-gamma production. Journal of Clinical Investigation, 117 (11), 1-13.

Material from this dissertation has been submitted for the publication of PNAS on

August 14, 2007 in the following form:

Rajkumar Noubade, Roxana del Rio, Benjamin McElvany, James F. Zachary, Jason M. Millward, Denisa D. Wagner, Elizabeth P. Blankenhorn, Cory Teuscher. (2007). Weibel Palade bodies negatively regulate blood brain barrier permeability and brain inflammation in experimental allergic encephalomyelitis. *PNAS.*

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 2 : Histamine receptor H_1 is required for TCR-mediated p38 MAP kinase activation and IFN-gamma production

CHAPTER 3 : Polymorphisms in murine histamine receptor H_1 lead to differential cell surface expression and influence autoimmune disease progression

APPENDIX A: Weibel-Palade bodies negatively regulate blood brain barrier permeability and brain inflammation in experimental allergic encephalomyelitis

LIST OF TABLES

CHAPTER 2 : Histamine receptor H_1 is required for TCR-mediated p38 MAP kinase activation and IFN-gamma production

CHAPTER 3 : Polymorphisms in murine histamine receptor H_1 lead to differential cell surface expression and influence autoimmune disease progression

CHAPTER 1: COMPREHENSIVE LITERATURE REVIEW

Multiple Sclerosis

Multiple Sclerosis (MS), a prototypic demyelinating inflammatory disease of the central nervous system (CNS) (Hafler, 2004), has been the most common neurological disorder in young adults since it was first noted by Jean Martin Charcoat in 1868 (Greenstein, 2007). More than 2.5 million people have been affected worldwide, with 350,000 individuals in the United States (US). The estimated prevalence rate in the year 2000, for the US Caucasian population was 1.91 per 1000 with an incidence rate of 7.3 per 100,000 (Berger et al., 2003). MS creates an economical burden to the individual, the health care system and the society. In 1994, the annual cost of MS in terms of direct care and loss of productivity in the US was estimated to be $34,000/ patient with a mean life-time cost of $2.2 million. This translates to a national cost of $6.8 billion/year. MS is more common in females than in males (~ 2:1); however, affected men generally have a delayed onset with worse prognosis (Kantarci and Wingerchuk, 2006).

A remarkable heterogeneity is seen in clinical MS with multiple forms identified: (i) relapsing-remitting MS, observed in 85-90% of patients, with full or partial recovery between relapses, with most developing into (ii) secondary progressive MS with progressive clinical deterioration and, (iii) primary progressive MS with neurological dysfunction from the onset without any clinical relapses (Hafler, 2004; Holmoy and Vartdal, 2007). The symptoms vary depending on the component of the CNS involved, brain or spinal cord, and include motor, sensory, autonomic and cognitive disabilities. About 50% of MS patients become dependent on a walking

aid by year 15 of the disease (Kantarci and Wingerchuk, 2006; Weinshenker et al., 1989).

Etiology of MS

One hundred and fifty years after its discovery, the etiology of MS is still unknown; however, genetic (Mackay and Myrianthopoulos, 1966; Sadovnick et al., 1988) as well as environmental factors (Dean et al., 1976; Kurtzke and Hyllested, 1987) have been implicated in its susceptibility. Considered to be a complex disease with multiple disease-susceptibility genes, MS lacks a clear pattern of inheritance. Hence it is believed that the disease is triggered by some kind of environmental factor in genetically susceptible individuals (Comabella and Martin, 2007; Hafler et al., 2005).

The support for the influence of genetic factors include: a 20-40 times greater risk in siblings and fraternal twins of patients, a 150-300 times greater risk in identical twins of patients and a lack of increased risk in adopted-relatives of patients (Allen et al., 1994; Ebers, 2005; Ebers et al., 1995; Hafler et al., 2005; Jersild et al., 1973; Kurtzke et al., 1982; Mackay and Myrianthopoulos, 1966; Risch and Merikangas, 1996). Familial studies have suggested that even phenotypic heterogeneity of MS has a genetic basis with greater similarity of clinical course in patients who are relatives (Kantarci et al., 2002).

The support for the involvement of environmental factors in MS include: non-infectious factors such as geography and migration, with lower MS risk among individuals migrating from high-risk to low-risk areas (Alter et al., 1978; Alter et al., 1966; Dean, 1967; Hammond et al., 2000; Kurtzke et al., 1985; Kurtzke et al., 1970); latitude gradient, with 3% higher risk in individuals born in the north (42^0 N and

above) than individuals born in the south latitude (37^0 N and below) (Hernan et al., 1999); exposure to sunlight; circulating levels of vitamin D, with low levels being associated to higher risk (Ascherio and Munger, 2007; Munger et al., 2004; Soilu-Hanninen et al., 2005; Soilu-Hanninen et al., 2007); and an incomplete concordance in monozygotic twins (Ebers, 2005; Weinshenker, 1996).

An infectious etiology with a variety of viral and bacterial agents associated with increased risk has been proposed, with Epstein-Barr virus (EBV) infection the most consistent and strongest risk factor (Ascherio and Munger, 2007; Coo and Aronson, 2004; Kurtzke, 1968, 1993; Marrie, 2004; Thacker et al., 2006). This multi-factorial etiology triggers a disease in MS patients characterized, neuropathologically, by discrete lesions (or plaques) mostly in the white matter of the CNS tissue, causing inflammatory infiltrates, demyelination, astrocytic proliferation (astrogliosis) and axonal damage. It is widely believed that the inflammatory infiltrates are pathogenically the primary factors and that MS is an autoimmune disease with an immune attack against myelin proteins (McFarland and Martin, 2007).

Myelin structure and functions

The myelin sheath is a multilamellar membrane, uniquely found and essential to the functioning of the vertebrate nervous system (Tzakos et al., 2005). In the CNS, the myelin sheath is formed by the wrapping of plasma membrane extensions of oligodendrocytes, specialized glial cells, in highly regular concentric layers around the axons. These concentric layers are practically fused together with very little or no cytoplasm (Kursula, 2006). High-resolution electron microscopic studies of myelin sheath have demonstrated periodic electron-dense and -light layers. Dense

layers, spaced between 150-170 A^0 apart, are formed by the apposition of the cytoplasmic surfaces of the plasma membrane extensions of the oligodendrocytes. The light layers, also known as intraperiodic lines, are formed by the apposition of the extracellular surfaces of the plasma membrane (Baumann and Pham-Dinh, 2001). The thickness of the myelin sheath varies with the length of the axon, with longer axons having thicker myelin (Waxman and Sims, 1984). The longitudinal organization of the myelin sheath is also unique, with three distinct anatomical and functional domains (Porter and Tennekoon, 2000).

The myelin sheath is segmented, forming internodes of about 150-200 μm length (Butt and Ransom, 1989). The internodes are separated by spaces where myelin is lacking and the axolemma is exposed to the extracellular milieu. These are called nodes of Ranvier and are enriched in voltage gated sodium channels (120,000/μm^2, the highest density in the nervous system) (Baumann and Pham-Dinh, 2001). Each successive myelin wrap, at its lateral margins, creates a loop containing some cytoplasm, called paranodal loops or paranodes (Porter and Tennekoon, 2000). The junction of paranodal loops with axons, known as juxtaparanodal region, is rich in potassium channels and thus segregates them from the voltage gated sodium channels in the nodes of Ranvier (Porter and Tennekoon, 2000; Rios et al., 2003). This organization is important for the normal conduction of electrical impulses along the axons (Kursula, 2006). Myelination alters the electrical properties of the axons because the myelin sheath has high resistance and low capacitance (Tolhurst and Lewis, 1992). Therefore, once an action potential is generated at one node of Ranvier, it flows down the axon quickly to the next node rather than leak back across the membrane. Thus, an

electrical impulse jumps from one node to the next node, a method of propagation known as saltatory conduction (Baumann and Pham-Dinh, 2001; Salzer, 2002). Myelin is very effective in increasing conduction velocity. An axon of about 1-5 μm in diameter can propagate an electrical impulse at about 20 meters per second if it is myelinated, while a non-myelinated axon needs to be 500-1000 μm thick in order to propagate an electrical impulse at the same rate (Hall, 1992).

Myelin constitutes about 40-50% of the CNS white matter on dry weight basis (Tzakos et al., 2005). It is a poorly hydrated structure containing about 40% water in contrast to the highly hydrated (80%) grey matter. Like all other cell membranes, it is composed of a lipid bilayer with intercalated proteins. However, in contrast to the other cell membranes, it is uniquely made of lipids (70% of the dry weight). The lipids are composed of cholesterol, phospholipids and glycolipids, with an enrichment of glycosphingolipids (Baumann and Pham-Dinh, 2001; Porter and Tennekoon, 2000). The low water content and lipid-rich composition of myelin contributes to its insulating properties and favor rapid nerve conduction velocity. When the myelin is damaged by diseases or when it is not formed due to genetic defects, it results in serious neurological conditions, including motor and sensory deficits (Porter and Tennekoon, 2000).

The myelin proteins, comprising the remainder 30% of the myelin dry weight, consist of a restricted set of proteins (Kursula, 2006). Most of these proteins are exclusively found in myelin and oligodendrocytes and are important in myelination and/or maintenance of myelin architecture (Tzakos et al., 2005). Myelin basic protein (MBP) is one of the most abundant proteins, constituting 30% of the total myelin

proteins, and is present at the cytoplasmic surfaces of the myelin membranes (Baumann and Pham-Dinh, 2001). MBP is essential for myelin compaction (Campagnoni and Macklin, 1988). Mutant mice, with a large deletion of the MBP gene (shiverer mice), lack the major dense line of myelin (Privat et al., 1979; Roach et al., 1985).

Proteolipid protein (PLP) and its splice variant, DM20, are the most abundant myelin proteins, constituting 50% of the total myelin proteins (Kursula, 2006). The absence of PLP/DM20 leads to a loosely wrapped myelin sheath and loss of intraperiodic lines, correlating with reduced physical stability (Boison et al., 1995). Therefore, PLP, along with MBP, is believed to cement the myelin sheath, like a zipper, by forming membrane junctions after myelin compaction (Boison et al., 1995; Klugmann et al., 1997) Further, the absence of PLP/DM20 leads to axonal damage and axonal degeneration, indicating that myelin plays a pivotal role in maintaining axonal integrity and function (Baumann and Pham-Dinh, 2001; Boison et al., 1995).

Myelin oligodendrocyte glycoprotein (MOG) is a minor myelin protein and is present on the outermost lamellae of the myelin sheath and is present in oligodendrocyetes, particularly on the processes (Tzakos et al., 2005). MOG is a surface marker for oligodendrocyte maturation and its presence correlates with late stages of maturation (Baumann and Pham-Dinh, 2001; Solly et al., 1996). The amino acid sequence of MOG is highly conserved among animal species, suggesting an important biological function (Tzakos et al., 2005) in the completion, compaction and/or maintenance of myelin (Johns and Bernard, 1999).

Myelin associated glycoprotein (MAG) is another minor glycoprotein important in myelin compaction (Tzakos et al., 2005). Absence of MAG leads to increased

cytoplasm content between lamellae and lower number of myelin wraps (Li et al., 1994; Montag et al., 1994). Other myelin proteins include connexin32, a gap junction protein, and 2'3'-cyclic nucleotide-3'-phosphodiesterase (CNPase) found in the cytoplasm of paranodes (Tzakos et al., 2005).

Immunology of MS

MS is considered to be a coordinated immunological attack against myelin proteins in the CNS (Ferber et al., 1996). A large body of literature provides evidence that the immune system is involved in the disease process (McFarland and Martin, 2007). Even though specific self-antigen(s) has not been definitively demonstrated, it is generally accepted that CD4 T cells reactive to major constituents of the myelin sheath, MBP (Berger et al., 2003; Bielekova et al., 2000; Bielekova et al., 2004), PLP (Bielekova et al., 2004) and MOG (Genain et al., 1999; Olsson et al., 1992; Soderstrom et al., 1993) mediate the autoimmune pathology of the disease. Myelin-specific T cells are easily detected in normal individuals (Sospedra and Martin, 2005). The triggers that cause these cells to attack myelin are largely unknown.

A role of infectious agents has long been proposed to break the tolerance to myelin components in genetically susceptible individuals through molecular mimicry and bystander activation (Fujinami and Oldstone, 1985) (Lang et al., 2002; Tejada-Simon et al., 2003; Wucherpfennig and Strominger, 1995) (Anthony et al., 1997; Waldner et al., 2004). Environmental factors, described earlier, are also considered to trigger the activation of autoreactive CD4 T cells in genetically susceptible individuals. In this regard, histamine elicited by environmental factors or generated during an ongoing infection could act as a mediator of such a disease-inducing trigger. The myelin-

reactive CD4 T cells penetrate CNS through the blood-brain barrier (BBB), formed by specialized endothelial cells that are connected through tight junctions (Minagar and Alexander, 2003).These endothelial cells are typically ensheathed by basal lamina and astrocytic end-feet processes (Kim et al., 2006)

Astrocytes are the most abundant glial cells in CNS (Minagar et al., 2002). They are critical for the development, structural support and the maintenance of BBB. In co-culture systems, astrocytes upregulate the several proteins of tight junction structure that connects the endothelial cells (Dehouck et al., 1990; Rubin et al., 1991). The BBB endothelial cells exhibit an apical or luminal polarization of transporters like p-glycoprotein, glucose transporter 1 (GLUT1), thus forming a transport barrier (Abbott et al., 2006). Astrocytes up-regulate the expression and the polarized localization of these transporters (Kim et al., 2006). BBB also acts as a metabolic barrier and astrocytes upregulate several of the BBB-specific enzymes such as monoamine oxidase, superoxide dismutase, that support the protective and detoxifying roles of BBB (Haseloff et al., 2005). The perivascular end-feet of the astrocytes are highly specialized with high density of orthogonal array of particles containing the water channel aquaporin 4 and the potassium channel Kir4.1. The polarity of these proteins, which are anchored to the basal lamina through a proteoglycan protein called agarin, contributes to the integrity of BBB (Minagar et al., 2002). Moreover, astrocytes secrete several factors such as basic fibroblast growth factor, glial-derived neurotrophic factor, angiopoetin 1 and TGFβ, that induce several aspects of BBB (Kim et al., 2006).

The entry of T cells into the CNS is a multistep process involving the induction of adhesion molecules such as vascular cell adhesion molecule (VCAM) on the

endothelial cells, mostly by cytokines including IFN-γ, TNF-α and IL-23 released during the inflammatory process. The interaction of these adhesion molecules with their binding partners, such as VLA-4, on the surface of activated CD4 T cells allows the CD4 T cells to adhere to the endothelial cells and diapedese into the CNS (Brocke et al., 1993; Butcher et al., 1999). Matrix metalloproteases (MMP), particularly MMP-2 and -9, surround the inflamed BBB endothelial cells and degrade the basal membrane as well as the extracellular matrix of parenchyma, thus enabling the T cells to spread in the CNS (Anthony et al., 1997; Clements et al., 1997; Lindberg et al., 2001; Pedotti et al., 2003).

After gaining access to the white matter, the CD4 T cells re-encounter the myelin antigens presented to them by the resident antigen presenting cells (APCs), particularly microglial cells (Minagar et al., 2002). Activated T cells produce several cytokines, which in turn activate more APCs and thus set up a pro-inflammatory loop that provides an infiltrate rich in activated T cells, macrophages and other cells of hematopoietic origin such as B cells and mast cells. The activated macrophages attack myelin and phagocytose large chunks of the myelin sheath (Ferber et al., 1996; Sospedra and Martin, 2005) and produce toxic materials such as nitric oxide (Brenner et al., 1997; Conlon et al., 1999). There is a myelin-directed cytotoxic T cell response, an auto-antibody response and an activation of the complement cascade (Compston et al., 1986; Keegan et al., 2005; Lalive et al., 2006; Laurell and Link, 1972; Lucchinetti et al., 1996; Morgan et al., 1984).

This concerted attack of T and B cells, complement cascade, inflammatory mediators including cytokines and nitric oxide on the myelin sheath results in areas

of severe demyelination. In addition, there is loss of myelin-producing oligodendroglial cells, an increase in the number of fibrous scar tissue-forming astrocytes, and permanent axonal damage (Conlon et al., 1999; Sospedra and Martin, 2005; Trapp et al., 1998), thus resulting in the pathophysiological defects observed in the affected individual.

The cytokine-producing phenotype of myelin-specific T cells determines the ability of these cells to cause inflammation in the CNS. Organ-specific autoimmune diseases such as MS are thought to be primarily mediated by Th1 type of CD4 T cells. These cells are differentiated in the presence of interleukin (IL)-12 and are characterized by the production of large amounts of IFN-γ, TNF-α and IL-2 (Ferber et al., 1996; McFarland and Martin, 2007; Sospedra and Martin, 2005). Increased levels of IFN-γ, TNF-α in the serum (Hohnoki et al., 1998) and of IL-12 in cerebrospinal fluid (CSF) (Dormond et al., 2002) of MS patients have been observed. However, these cytokines worsened the disease upon systemic administration (Panitch et al., 1987; Sharief and Hentges, 1991). The disease-enhancing effect of these cytokines has been associated with their ability to enhance the expression of adhesion molecules on the vascular endothelium (Ferber et al., 1996). Recently, IL-17 and IL-23 are being considered to be the important proinflammatory cytokines in autoimmune diseases (McFarland and Martin, 2007). Accordingly, an augmentation of *IL-17* mRNA in mononuclear cells of CSF and in brain tissues of MS patients is observed (Dormond et al., 2002; Matusevicius et al., 1999). IL-23 is present in both active and chronic lesions (Lundmark et al., 2007). Myeloid dendritic cells from MS patients express higher IL-23 than those from normal individuals (Vaknin-Dembinsky et al., 2006). All

these proinflammatory cytokines, in addition to orchestrating the inflammation, play an important role in the demyelination process by activating phagocytic cells and by inducing apoptosis of myelin producing cells, which lead to impaired saltatory conduction along the axon and pathological effects (Pouly et al., 2000; Selmaj et al., 1991).

Genetics of MS

The evidence for genetic factors in MS susceptibility is compelling. Several approaches including genetic linkage, candidate gene association and gene expression studies have been used (Becanovic et al., 2004; Fernald et al., 2005). However, all have failed to demonstrate a clear mode of inheritance in the disease. Consequently, it has been concluded that MS is polygenic and affected by multiple genes, each exerting a modest effect in the overall disease outcome (Sawcer, 2006). The most consistent association has been with the major histocompatibility complex (MHC) class II or human leukocyte antigen (HLA), specifically HLA-DRB1*1501-DQB1*0602 haplotypes located on chromosome 6 (Hafler et al., 2007; Haines et al., 1996; Haines et al., 1998). In spite of its strong linkage, HLA association explains only about 50% of the genetic etiology of MS (Ebers and Sadovnick, 1994). Evidence for additional linkages to several chromosomes outside HLA has been observed, supporting the complexity. Candidate gene studies have suggested more than 100 such non-MHC genes, a conservative estimate suggesting about 30 genes, but no consensus has been accepted (Becanovic et al., 2006; GAMES, 2001, 2003).

Recently, using a large-scale genomewide association scan, The International Multiple Sclerosis Genetic Consortium has identified IL-2 receptor alpha (IL-2RA)

and IL-7 receptor alpha (IL-7RA) alleles, along with those in the HLA locus, as risk factors for MS (Hafler et al., 2007). The risk conferred by polymorphisms in IL-7RA is confirmed by two additional, independent studies (Gregory et al., 2007; Lundmark et al., 2007). However, risk contributed by IL-2RA and IL-7RA is minimal and explains only 0.2% of the variance (Peltonen, 2007). Moreover, the approaches used in these studies have little statistical power to detect rare variants that could confer a relatively large genetic risk (Hafler et al., 2007; Sawcer, 2006). Overall, it has been difficult to identify genes associated with MS because of the genetic complexity of the disease, the genetic diversity of the human population, the relatively small sample sizes, and the environmental influence and possible variations in the disease diagnosis (Andersson and Karlsson, 2004).

Animal models of MS

Due to the complex genetic architecture of MS, to investigate mechanisms of disease development and disease manipulation, animal models have been extensively used,. The most important advantage of animal models, compared to humans, is the better control of genetic background and environment. In addition, large numbers of animals can be studied. The disease can be deliberately induced and animals can be genetically manipulated. Animal models have the potential to significantly reduce the genetic complexity inherent in autoimmune diseases into intermediate or subphenotypes, such as histamine sensitivity. In addition, animal models permit the refinement of candidate regions to an interval small enough to allow identification of the causative gene using classical positional cloning and candidate gene screening. Thus, even though animal models do not completely display all the disease parameters found in the human

disease they model, specific traits of animal models reflect a particular pathway and give better mechanistic understanding of a particular stage of the human disease (Andersson and Karlsson, 2004).

There are two major strategies of discovering genetic contribution to the development of disease using animal models. The first one, "gene-to-disease" pursues a hypothesis-based role of a particular gene in the disease development and is performed using gene-knock out and transgenic mice. The other, "disease-to-gene" strategy, is an unbiased, hypothesis-free approach aimed at identifying the disease-relevant part of the genome. This strategy involves the use of hybrids of one susceptible strain of mice crossed with a resistant strain. Development of the disease is studied in these mice followed by a marker assisted genome screen. Statistical linkage analysis is used to relate disease development to a genetic variant at a defined genetic location. Subsequent refinement will yield identification of the candidate gene, which can further be investigated for genetic polymorphisms between the parental strains and the influence of these polymorphisms on the disease development process. Both the strategies of genetic analysis of susceptibility genes have their advantages and are complementary to each other (Andersson and Karlsson, 2004).

Animal models of MS-experimental allergic encephalomyelitis (EAE)

Animal models that simulate features of MS provide a powerful tool for investigating the pathogenesis of the disease. Several mouse models, virus-induced and/or autoimmune, have been developed that reflect clinical and pathological attributes. The viral models for MS include the Theiler's murine encephalomyelitis virus (TMEV) (Lipton and Dal Canto, 1976), recombinant- TMEV (Olson et al., 2001), murine

hepatitis virus (MHV) (Matthews et al., 2002), Semliki forest virus (Mokhtarian and Swoveland, 1987) and Sindbis virus (Mokhtarian et al., 1989). All these neurotropic viruses induce demyelination either by directly infecting the neurons or by activating autoreactive T cells though molecular mimicry (Ercolini and Miller, 2006; Grigoriadis and Hadjigeorgiou, 2006).

EAE is the principle autoimmune animal model for MS. With 70 years of history, it is one of the most endured animal models of a human disease (Pedotti et al., 2003). EAE was originally developed by Rivers and colleagues as a model for neuroparalytic accidents in patients who received anti-rabies vaccine, by inducing disseminated encephalomyelitis in monkeys by repeated injections of rabbit brain extracts (Rivers et al., 1933). The introduction of adjuvants, such as Fruend's complete adjuvant (CFA), greatly facilitated the induction of the disease with a single or fewer injections of antigen (Fruend et al., 1947). Subsequently, the disease has been induced in several animals including non-human primates (Genain et al., 1995), guinea pigs (Fruend et al., 1947), rats (Lipton and Freund, 1952), rabbits (Morrison, 1947), hamsters (Tal et al., 1958), goats (Lumsden, 1949), sheep (Innes, 1951), dogs (Thomas et al., 1950), and mice (Olitsky et al., 1950). It is now well established that the experimental disease is mediated by T cells reactive to components of the myelin sheath. This prototypical model for cell-mediated autoimmune disease in general is the best available animal model for human CNS inflammatory demyelinating disease. EAE simulates several features of MS including its pathology, histopathology and pathogenesis making it a powerful tool in the investigation of the pathophysiology (Baxter, 2007; Blankenhorn et al., 2000; Gold et al., 2006; Martin and McFarland, 1995; Palakal et al., 2007).

EAE can be induced in genetically susceptible animals by inoculation with either crude CNS tissue-homogenate or their components, such as PLP, MBP, MOG, MAG, myelin oligodendrocyte basic protein, or the encephalitogenic peptides in an appropriate adjuvant (Encinas et al., 1996; Kuchroo et al., 2002; Trotter et al., 1987; Zamvil et al., 1985). CFA containing *Mycobacterium tuberculosis* H37RA, and *Bordetella pertussis* toxin (PTX) are the most commonly used adjuvants in disease induction. EAE can also be induced by adoptive transfer of CD4 T cells from immunized animals into naïve mice, which underscores the importance of these cells in the immuno-etiology of the disease (Baron et al., 1993; Bernard et al., 1976; Bernard and Mackay, 1983; Krueger et al., 2005; Langrish et al., 2005).

The development of EAE in the immunized mice occurs in two distinct stages, the induction phase and the effector phase. During the induction or the initial priming phase, up to day 10 post immunization, APCs present the immunized-component of the myelin sheath to CD4 T cells as "foreign" antigen in lymph node and/or spleen and activate them (Powell et al., 1990; Sayed and Brown, 2007).

Optimal activation of T cells requires two signals (Bretscher, 1999). The first signal is delivered by the interaction of T cell receptor (TCR) with antigen presented by MHC molecule on the surface of APCs. The second signal consists of engagement of costimulatory molecules such as CD28, inducible costimulatory molecule (ICOS), programmed death pathway 1 (PD-1), and CD154 expressed on T cells with CD80/CD86, ICOS ligand, PD-1 ligand 1 (PD1-L1)/PD-1 ligand 2 (PD-L2) and CD40 respectively, expressed on APCs (Agata et al., 1996; Hutloff et al., 1999; McAdam et al., 1998; Sharpe and Freeman, 2002). The critical regulatory role in EAE of ICOS and optimal T cell

activation is supported by complete absence of EAE in CD28-deficient mice (Chitnis et al., 2001; Oliveira-dos-Santos et al., 1999), blockade of disease by administration of anti-CD28 antibodies (Perrin et al., 1999), resistance of CD80/CD86-deficient mice to the induction of EAE (Chang et al., 1999; Girvin et al., 2000), exacerbation of disease in ICOS-deficient (Chitnis et al., 2001) and anti-PD1 antibody treated mice (Salama et al., 2003). Another costimulatory molecule, CTLA-4, is expressed on activated CD4 T cells and its interaction with CD80/CD86 is a negative regulator of T cell activation (Karandikar et al., 1996). Administration of anti-CTLA4 blocking antibody during the priming phase of EAE exacerbates the disease (Karandikar et al., 1996). In addition, factors such as histamine play a critical role during priming of autoreactive T cells (Chapter 2). In the presence of proper secondary signals, autoreactive CD4 T cells get activated and differentiated into distinct lineages such as Th1 or Th17 cells, defined by the unique set of cytokines they produce upon re-activation (Furuzawa-Carballeda et al., 2007).

During the effector phase of the disease, the activated autoreactive T cells leave the secondary lymphoid organs, traffic to the CNS and persist there to orchestrate the inflammatory events. In healthy individuals, the traffic of lymphocytes into the CNS is very low and tightly regulated by a highly specialized structure called the blood-brain-barrier (BBB) formed by endothelial cells connected though tight junctions (Engelhardt, 2006). Therefore, loss of BBB integrity is a critical checkpoint in the pathogenesis of CNS inflammatory diseases (Noubade et al., 2007)(Appendix A of this thesis). Only activated T cells, not naïve T cells, can penetrate the BBB, a process mediated by adhesion molecules, chemokines and their respective chemokine receptors (Engelhardt

and Ransohoff, 2005; Hickey et al., 1991; Matejuk et al., 2002; Wekerle et al., 1987). Activated T cells express adhesion molecules such as lymphocyte function associated antigen 1 (LFA-1) and very late antigen 4 (VLA-4). Endothelial cells are induced to express adhesion molecules such as intracellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), platelet/endothelial cell adhesion molecule 1 (PECAM-1) on their surface, mostly by cytokines such as IFN-γ and TNF-α (Baron et al., 1993; Butter et al., 1991; Graesser et al., 2002; Wilcox et al., 1990). ICAM-1 and VCAM-1 are ligands for LFA-1 and VLA-4, respectively. Interaction of the adhesion molecules on T cells with their binding partners on endothelial cells (Baron et al., 1993; Yednock et al., 1992) and the degradation of the type IV collagen of the basement membrane underlying the endothelial cells by MMPs, results in extravasation of T cells through BBB endothelial cells into the CNS tissue. Type IV collagen is present only in the endothelial cell basement membrane and has distinct binding sites important for T cell binding to the basement lamina of endothelial cells (Sacca et al., 2003).

MMPs are a family of proteolytic enzymes present at low levels in a normal CNS but most of them, particularly MMP-2 and -9, are elevated during EAE. MMPs also assist T cells to spread in the white matter by degrading the brain parenchyma (Agrawal et al., 2006; Anthony et al., 1998; Clements et al., 1997; Dwyer et al., 1998; Harrington et al., 2005; Kieseier et al., 1998; Pagenstecher et al., 1998; Toft-Hansen et al., 2004). Once within the CNS, the T cells are re-activated when the normally-expressed myelin antigens are presented by the resident microglial cells and/or astrocytes (Matsumoto et al., 1992; Constantinescu et al., 2005; Stuve et al., 2002). The re-activation of CD4 T cells is necessary for them to be retained within the CNS and exert their effector functions.

Otherwise, the T cells exit rapidly or undergo apoptosis (Hickey, 2001).

The re-activation of CD4 T cells results in the increase of a number of pro-inflammatory molecules including MMPs, adhesion molecules, chemokines such as RANTES, MCP1, MIP1α, MIP1β, osteopontin (Butterfield et al., 1999; Chabas et al., 2001; Dogan and Karpus, 2004; Glabinski et al., 1995; Godiska et al., 1995), and cytokines such as IFN-γ, TNF-α, IL-1, IL-6, IL-12, IL-17, IL-22 and IL-23. These cytokines, particularly INF-γ and IL-23, activate macrophages and microglial cells, which in turn upregulate their MHC class II molecules, re-present myelin antigen to CD4 T cells, and thus set up an inflammatory loop (Becher et al., 2003; Constantinescu et al., 2005; Ferber et al., 1996; Gutcher and Becher, 2007; Kuchroo et al., 1993). Consequently, there is a sustained breach of BBB integrity and rapid, massive infiltration of cells including CD4 T cells, CD8 T cells, macrophages, B cells, monocytes, mast cells and neutrophils, into the CNS white matter.

The activated macrophages phagocytose myelin and myelin-producing cells leading severe demyelination (Ferber et al., 1996; Pender, 1987). Myelin-reactive CD8 T cells (Chabas et al., 2001; Huseby et al., 2001), B cells, myelin-specific antibodies (Iglesias et al., 2001; Lebar et al., 1986; Svensson et al., 2002), complement proteins that form membrane attack complexes at the surface of oligodendrocytes (Jegou et al., 2007; Piddlesden et al., 1993), oposonization of myelin by autoantibodies and complement proteins (Jegou et al., 2007), all contribute to the demyelination. Proteolytic enzymes (Guyton et al., 2005) and toxic products, such as reactive oxygen and nitrogen intermediates (MacMicking et al., 1992), are released by infiltrated cells. Increasing evidence suggests that inflammatory mediators released from the

infiltrated cells cause a considerable axonal loss (Park et al., 2005). All these events result in an ascending paralytic disease, that begins with weakness and loss of tone in the tail and progresses to complete paralysis of forelimbs, hind limbs, fecal and urinary incontinence, moribund state in some animals, and occasional mortality.

CD4 T cell subsets and their cytokines in EAE

Historically, CD4 T cells of Th1 type, characterized by their ability to secrete IFN-γ, are believed to be sufficient to orchestrate the inflammatory events and initiate myelin destruction in CNS. IL-12 drives the differentiation of CD4 T cells into Th1 type cells (Harrington et al., 2006). Mice deficient in IL-12p40 are resistant MBP-induced EAE (Segal et al., 1998). CD4 T cells differentiated *in vitro* in the presence of IL-12 induce disease in naïve recipients upon adoptive transfer (Baron et al., 1993; Bernard et al., 1976; Bernard and Mackay, 1983). Administration of anti-IL-12p40 antibodies suppresses EAE in adoptive transfer recipients (Leonard et al., 1995). IL-18 also promotes Th1 differentiation (Okamura et al., 1995). Mice deficient in IL-18 are resistant to EAE (Shi et al., 2000) and anti-IL-18 antibodies significantly reduce the IFNg production and disease development during MBP-induced EAE in rats (Wildbaum et al., 1998). The number of Th1 CD4 T cells in inflammatory lesions correlate with disease severity (Merrill et al., 1992). Encephalitogenic MBP- and PLP-specific CD4 T cells clones are Th1 type (Ando et al., 1989; Baron et al., 1993; Kuchroo et al., 1993). Mice deficient in T-bet, the key transcription factor for the development of Th1 CD4 T cells (Szabo et al., 2000) do not develop EAE (Bettelli et al., 2004). Silencing T-bet by RNA interference ameliorated EAE (Gocke et al., 2007). TNF-α is upregulated in CNS during EAE (Juedes et al., 2000). The

encephalitogenicity of MBP-specific T cell clones is strongly correlated with the TNF-α production (Powell et al., 1990). However mice deficient in TNF-α, IFN-γ or IFN-γR are susceptible to EAE (Ferber et al., 1996; Frei et al., 1997; Willenborg et al., 1996). Similar observations were made in mice deficient in IL-12, particularly IL-12p35 (Becher et al., 2002; Ghosh et al., 2002).

In contrast, mice deficient in IL-23, a member of IL-12 family, were completely resistant to EAE (Cua et al., 2003; Langrish et al., 2005). Further, these EAE-induced IL-23-deficient mice completely lacked IL-17- positive cells in the CNS while IFN-γ-positive cells were present. These discrepancies led to the identification of a distinct subset of CD4 T cells called IL-17 producing-Th17 cells (Harrington et al., 2005; Krueger et al., 2005). IL-17-deficient mice are resistant to EAE (Komiyama et al., 2006). Subsequently, in addition to IL-17, the Th17 CD4 T cells have been shown to produce IL-1β, IL-6, TNF-α, IL-22, GM-CSF (Furuzawa-Carballeda et al., 2007). The presence of IL-6, TGF-β and IL-1β is essential for the generation of these cells and mice deficient in IL-6 as well as IL-1R are resistant to EAE (Okuda et al., 1998; Schiffenbauer et al., 2000). Additionally, IL-23-derived Th17 CD4 T cells induced EAE in naïve recipients upon adoptive transfer (Krueger et al., 2005; Langrish et al., 2005). However, adding complexity to the Th17 pathway is the observation that mice deficient in IL-17E, also known as IL-25, have increased IL-23 expression and are hypersusceptible to EAE (Kleinschek et al., 2007). Thus the relative contribution of Th1 versus Th17 CD4 T cells to the development of EAE remains to be elucidated.

CD8 T cells in EAE

Initial studies suggested that CD8 T cells may play a protective role in EAE when a significant reduction in disease relapses was observed in CD8 knock-out or CD8 T cell-depleted mice (Jiang et al., 1992; Koh et al., 1992). However, several lines of evidence such as predominance of CD8 over CD4 T cells in the brain of MS patients and the close association of CD8 T cells with MS lesions (Booss et al., 1983; Cabarrocas et al., 2003; Hauser et al., 1986; Neumann et al., 2002; Skulina et al., 2004) led to a more careful analysis of these cells in EAE pathogenesis. One criticism for CD4 T cell predominance in EAE has been that when EAE is induced by immunizing the mice with myelin antigens in appropriate adjuvant, the antigens are presented by MHC class II molecules and thus activate CD4 T cells rather than by MHC class I molecules (Ji and Goverman, 2007). Therefore, when MHC class I restricted MBP-specific CD8 T cells were adoptively transferred to naïve recipients, it resulted in severe EAE with extensive demyelination (Huseby et al., 2001). More recently, CD8 T cells generated from mice immunized in the traditional way with myelin antigens in CFA also induced a severe EAE when adoptively transferred to naïve recipients, demonstrating the pathogenic role of CD8 T cells in EAE (Abdul-Majid et al., 2003; Ford and Evavold, 2005; Ji and Goverman, 2007; Sun et al., 2001).

B cells and autoantibodies in EAE

Generally EAE is thought to be a T cell-mediated disease. It does not require B cells and antibodies, as B cell-deficient mice develop severe disease (Lyons et al., 1999; Wolf et al., 1996). Antibody titer and disease severity do not directly correlate (Cross et al., 2001). However, the most important diagnostic marker for early MS,

particularly in patients with normal brain scans, is the presence of oligoclonal antibodies and plasma cells in CSF (Paolino et al., 1996). But the heterogeneity of antigen specificity of these antibodies questioned whether there are consequences of MS-related antigen or represent MS-unrelated B cell responses. A very small proportion of these antibodies were found to be against myelin antigens but their contribution to the actual disease process is unknown (Cross et al., 2001; Ziemssen and Ziemssen, 2005).

B cells, plasma cells and myelin-specific antibodies are present in MS plaques and areas of demyelination (Genain et al., 1999). However, evidence that autoantibodies cause demyelination came from observations that administration of antimyelin antibodies enhanced demyelination in rats and non-human primates (Genain et al., 1999; Schluesener et al., 1987). In mice, it was found that B cells are critical in EAE induction with MOG protein but not peptide (Lyons et al., 1999). Even though autoantibodies that recognize many myelin proteins such as MBP and PLP have been identified to promote demyelination and potentiate EAE (Cross et al., 2001; Endoh et al., 1986), antibodies to MOG are considered to be more critical because MOG is expressed on the outer surface of the myelin sheath (Gardinier et al., 1992).

Transgenic mice producing high titers of anti-MOG antibodies did not develop spontaneous EAE but developed an early and exacerbated disease upon induction, indicating that the autoantibodies can modify the disease course and pathogenesis (Litzenburger et al., 1998). Anti-MOG antibodies capable of inducing EAE require glycosylated epitopes on the surface of oligodendrocytes (Marta et al., 2005) and their demyelinating ability depends on the activation of complement cascade rather than direct cell mediated cytotoxicity (Urich et al., 2006). Additionally, B cells function as antigen

presenting cells. The autoantibodies increase myelin opsonization and subsequent phagocytosis by macrophages and microglial cells and thus contribute to demyelination (Jegou et al., 2007; Ziemssen and Ziemssen, 2005).

Genetic susceptibility of EAE

The observation that different strains of mice differ significantly in their susceptibility to EAE was made in the first study of EAE in mice when it was noted that Swiss mice were susceptible to EAE induced by brain-tissue homogenate in Fruend's adjuvant, while the Rockefeller Institute strain of mice did not develop the disease (Olitsky and Yager, 1949). Subsequently, a large number of studies analyzing the genetic control of susceptibility and resistance to EAE have been carried out using inbred strains of mice and, to date, a total of 40 quantitative trait loci (QTL) have been identified (Baker et al., 1995; Blankenhorn et al., 2000; Butterfield et al., 1999; Butterfield et al., 1998; Encinas et al., 1996; Encinas et al., 2001; Fillmore et al., 2003; Karlsson et al., 2003; Mazon Pelaez et al., 2005; Sundvall et al., 1995; Teuscher et al., 2006a). A genomic region on chromosome 17 containing MHC genes, like in MS, has been the strongest and consistently linked region in EAE. Also, this was the first QTL to be identified (Fritz et al., 1985).

A large number of non-MHC loci have also been identified to control EAE susceptibility in mice. Among these, in addition to loci that control clinical disease parameters, such as incidence (Baker et al., 1995; Bakker et al., 2002; Butterfield et al., 1998; Encinas et al., 1996; Sundvall et al., 1995), disease onset (Butterfield et al., 1998; Mazon Pelaez et al., 2005) and disease severity (Baker et al., 1995; Butterfield et al., 1998; Mazon Pelaez et al., 2005), genes that control sub-phenotypes of the disease,

such as histopathological lesion-severity either in the brain (Blankenhorn et al., 2000; Butterfield et al., 1999; Karlsson et al., 2003) or spinal cord (Baker et al., 1995; Blankenhorn et al., 2000; Butterfield et al., 1998; Karlsson et al., 2003), weight loss (Encinas et al., 1996; Encinas et al., 2001), demyelination (Blankenhorn et al., 2000), inflammation (Encinas et al., 2001; Mazon Pelaez et al., 2005) and paralysis (Encinas et al., 2001), have been identified. QTLs controlling disease-subtypes, such as acute progressive, remitting-relapsing, chronic non-remitting and monophasic non-remitting/non-relapsing EAE (Butterfield et al., 1999; Karlsson et al., 2003), and those controlling electro-pathophysiological changes of neurons that reflect the extent of demyelination (Mazon Pelaez et al., 2005) have been reported.

EAE and MS are sexually dimorphic diseases with more females affected than males. Accordingly, most of the loci identified are gender specific (Butterfield et al., 1999; Fillmore et al., 2004; Fillmore et al., 2003). The effect of the Y-chromosome, reflecting parent-of-origin, has also been documented (Teuscher et al., 2006a). In addition, extrinsic factors such as the physical structure of the antigen-CFA containing particles (on the surface against buried within) of the emulsion (Fillmore et al., 2003), age and season (Fillmore et al., 2004; Teuscher et al., 2006a), use of pertussis toxin (Blankenhorn et al., 2000) have been shown to override genetic checkpoints, demonstrating the role of gene-environmental interactions in the disease susceptibility. Thus different loci are linked to different aspects of the disease development process and may reflect the heterogeneity observed in MS patients. The study of these loci in isolation and their contribution to disease development may help in understanding the inherent heterogeneity of the disease. One such locus is *Bordetella pertussis* induced histamine

sensitization (*Bphs*), controlling susceptibility to histamine-induced death in PTX sensitized mice (Sudweeks et al., 1993), has been identified to be *Hrh1*, the gene encoding histamine receptor H_1 protein (Ma et al., 2002).

Identification of histamine receptor H_1 as a susceptibility gene in EAE

Anaphylactic-like hypovolemic shock syndromes can be induced in mice by injecting vasoactive amines such as histamine or serotonin or a mixture of both (Bergman and Munoz, 1965, 1968; Harris and Fulton, 1958; Iff and Vaz, 1966). Inbred strains of mice varied in their sensitivity to these agents and the variation was genetically determined (Bergman and Munoz, 1968; Iff and Vaz, 1966; Parfentjev, 1955; Tokuda et al., 1963). Subsequently, it was found that products from *Bordetella pertussis* significantly enhanced this sensitivity to vasoactive amine treatment and that inbred strains of mice differ in their susceptibility to the enhancing effect. The *B. pertussis* product was later identified as PTX (Bergman and Munoz, 1968; Black et al., 1988; Munoz, 1957; Munoz, 1963; Vaz et al., 1977). SJL/J is the prototypic susceptible mouse strain and C3H/HeJ and CBA/J are the prototypic resistant strains of mice to the PTX-induced histamine sensitivity. The susceptibility of inbred strains to the histamine-sensitizing effects of PTX was found to be under the control of a single autosomal dominant gene (Wardlaw, 1970). The strains of mice susceptible for Bphs developed EAE, while the strains resistant to Bphs did not (Linthicum, 1982). However, it is noteworthy that there are some exceptional strains of mice, suggesting that additional genes control the disease susceptibility. *Bphs* is also associated with susceptibility to experimental allergic orchitis (Teuscher, 1985).

Using microsatellite and random amplified polymorphic DNA (RAPD)

markers and backcross populations of susceptible SJL/J and resistant C3H/HeJ and CBA/J strains, the *Bphs* locus was mapped to a 33 centimorgan (cM) region of mouse chromosome 6 (Sudweeks et al., 1993). The candidate interval was further refined to an interval to include fewer genes (Meeker et al., 1999). For positionally cloning *Bphs*, a panel of interval specific congenic lines was generated by introgressing the susceptible SJL/J allele onto the resistant C3H/HeJ background. Studies of histamine sensitivity in the congenic lines established that *Bphs* resided in a region containing *Hrh1* (Ma et al., 2002). The identity of *Hrh1* as *Bphs* was further confirmed by the complete resistance of histamine receptor H_1 knock out (H1RKO) mice to Bphs. Further, H1RKO mice exhibited a reduced severity and delayed onset of EAE compared to the wild type (WT) mice (Ma et al., 2002), indicating that histamine, acting through histamine receptor H_1, regulates EAE.

Histamine and histamine receptor H_1

Histamine [2-(4-imidazole) ethylamine] is a ubiquitously distributed biogenic amine that mediates diverse physiological processes including neurotransmission and brain functions, secretion of pituitary hormones, and regulation of gastrointestinal and circulatory functions (Parsons and Ganellin, 2006). Additionally, histamine is a potent mediator of inflammation and a regulator of innate and adaptive immune responses (Akdis and Simons, 2006).

Mast cells and basophils are the major sources of stored histamine (Code and Mitchell, 1957; Riley and West, 1953). The granule-stored histamine from these cells is rapidly released upon various immunological and non-immunological stimuli. Mast cell-deficient mice were induced to synthesize histamine upon phorbol ester stimulation

(Taguchi et al., 1982). This "nascent" or "inducible" histamine is proposed to be synthesized by the induction of L-histidine decarboxylase (HDC), the rate-limiting enzyme for histamine synthesis in cells such as activated monocytes/macrophages and neutrophils (Ghosh et al., 2002; Kahlson and Rosengren, 1968; Shiraishi et al., 2000; Takamatsu et al., 1996; Tanaka et al., 2004). T cells, B cells and dendritic cells also synthesize "inducible" histamine (Aoi et al., 1989; Kubo and Nakano, 1999; Szeberenyi et al., 2001). As these cells lack storage vesicles, histamine synthesized is immediately released. The HDC activity is modulated by a variety of stimuli during infections and inflammation (Schneider et al., 2002).

Histamine exerts its pleiotropic effect through four receptors that are designated as histamine receptor H_1, H_2, H_3, and H_4, according to the chronological order of their discovery (Hill et al., 1997; Parsons and Ganellin, 2006). H_1R is widely distributed on a variety of tissues and cell types including: mammalian brain; gastrointestinal tract; genitourinary system; cardiovascular system; adrenal medulla; hepatocytes; nerve cells; airway and vascular smooth muscle cells; endothelial cells; eosinophils; monocytes neutrophils; dendritic cells; and lymphocytes (both T and B cells) (Hill et al., 1997; Parsons and Ganellin, 2006; Smit et al., 1999). Biochemical characterization of the H_1R protein using photoaffinity binding studies and gel electrophoresis under reducing conditions has revealed a molecular weight of 56kDa in mice, rats and guinea pig brain (Ruat et al., 1988; Ruat and Schwartz, 1989; Ruat et al., 1990; Smit et al., 1999).

Purification of H_1R protein has not been successful thus far. H_1R was first cloned from bovine adrenal medulla, which yielded an intron-less gene (Yamashita et al., 1991). This enabled subsequent cloning of H_1R from several species including mouse, which

mapped to chromosome 6 (Inoue et al., 1996). The deduced amino acid sequence represented a 488 amino acid protein with calculated molecular weight of 56kDa. Modeling of the protein revealed the presence of seven transmembrane domains, characteristic of G protein coupled receptors (GPCRs). A striking feature of the proposed structure was a very large third intracytoplasmic loop (212 amino acids) and relatively short intracellular C-terminal tail (17 amino acids). The histamine binding pocket is formed between the third (TM3) and fifth (TM5) transmembrane domains (Hill et al., 1997; Jongejan et al., 2005; Smit et al., 1992). Similar to H_1R, H_2R is also expressed on a variety of cell types, while H_3R expression is restricted mostly to neuronal cells in the brain and some peripheral tissues. H_4R is expressed exclusively on cells of hematopoietic origin (Parsons and Ganellin, 2006).

GPCRs transduce the external signal of ligand binding by activating heterotrimeric G proteins, which in turn couple to a variety of second messenger signaling pathways (Fredholm et al., 2007). H_1R couples to second messenger signaling pathways via the activation of G proteins belonging to $G\alpha_{q/11}$ sub-family (Parsons and Ganellin, 2006). Generally, activation of H_1R leads to stimulation of phospholipase C, resulting in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to form inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), which causes calcium mobilization from intracellular stores and activation of protein kinase C (PKC), respectively (Hill et al., 1997). In addition, H_1R signaling also mediates other signaling pathways such as the production of phospholipase A2 and arachidonic acid (Leurs et al., 1994), cGMP and nitric oxide (Hill, 1992; Leurs et al., 1995; Satoh and Inui, 1984; Toda, 1987), and the activation of NF-κB (Bakker et al., 2001), STAT1 (Sakhalkar et al.,

2005), and STAT4 (Engelhardt, 2006). H_1R-mediated PKCα stimulation activates MAP kinase pathways, particularly MEK1 (Lipnik-Stangelj and Carman-Krzan, 2004; Megson et al., 2001), ERK MAP kinase and p38 MAP kinase (Robinson and Dickenson, 2001; Steffel et al., 2005) (Chapter 2). Even though H_1R is the first histamine receptor to be identified and a large number of studies on histamine and H_1R have been published in the last decade (Simons, 2004), little is known about the cell-type specific H_1R signaling pathways.

H_2R signaling is mediated via the $G_{\alpha s}$ subfamily of G proteins and primarily leads to increased cAMP production and calcium mobilization (Alewijnse et al., 1998; Del Valle and Gantz, 1997; Leurs et al., 1994; Smit et al., 1996). H_3R is coupled to $G_{\alpha i/o}$ subfamily of G proteins and leads to inhibition of cAMP and accumulation of calcium (Krueger et al., 2005). H_4R signaling is also mediated by coupling to $G_{\alpha i/o}$ subfamily of G proteins and induces calcium mobilization, inhibits cAMP production and activates MAP kinases (Buckland et al., 2003; Hofstra et al., 2003; Morse et al., 2001).

In endothelial cells, H_1R-mediated calcium mobilization and PKC activation promotes cytoskeletal changes to induce cell shape change (Lum and Malik, 1994). Additionally, H_1R-mediated signals lead to disassembly of VE-cadherin complexes that regulate endothelial barrier function (Gao et al., 2000; Winter et al., 1999). These effects result in increased vascular permeability. H_1R signaling also increases expression of adhesion molecules such as ICAM-1, VCAM-1 and P-selectin on endothelial cells (Gonzalez-Scarano et al., 1987; Kubes and Kanwar, 1994; Yamaki et al., 1998).

In dendritic cells, H_1R provides positive signals for enhanced antigen presentation capacity by upregulating several co- stimulatory molecules such as CD80 and

CD86 by increasing the production of proinflammatory cytokines such as IL-1, IL-6, IL-8, MCP-1 and MIP-1α and Th1 priming activity of these cells (Caron et al., 2001; Mazzoni et al., 2001; Meretey et al., 1991). In contrast, H_2R acts as a negative signal for many of these functions. H_1R induces intracellular calcium flux, actin polymerization and chemotaxis by immature dendritic cells (Mazzoni et al., 2001). H_1R is upregulated in monocyte-derived macrophages and leads to calcium mobilization and enhanced IL-8 production (Triggiani et al., 2007). Treatment of macrophages, isolated from lung parenchyma, with H_1R blockers led to lower IL-6 production (Triggiani et al., 2001).

In B cells, H_1R signals enhance anti-IgM mediated proliferation and antibody production against a T cell-independent antigen, TNP-ficoll, indicating that H_1R signals are important in B cell receptor-triggered responses (Banu and Watanabe, 1999). H1RKO mice produce higher ova-specific IgG1 and IgE compared to the WT mice, indicating that H_1R suppresses humoral responses (Banu and Watanabe, 1999; Jutel et al., 2001).

Studies using total splenocytes have shown that H_1R regulates antigen-specific T-cell effector functions and modulates production of the cytokines IFN-γ and IL-4 (Bakker et al., 2002; Banu and Watanabe, 1999; Bryce et al., 2006; Jutel et al., 2001) Chapter two of this thesis will demonstrate that H_1R in purified CD4 T cells regulates IFN-γ and IL-4 production.

Histamine and histamine receptor H_1 in MS and EAE

The first observation of the role of histamine and histamine receptors in EAE came from the use of pharmacological anti-histaminic agents to block the development of the disease (Linthicum, 1982). Subsequently, several studies used these drugs, particularly H_1R blockers, to reduce the pathology of EAE (Chabas et al., 2001;

Dietsch and Hinrichs, 1989; Dimitriadou et al., 2000; El Behi et al., 2007; Pedotti et al., 2003; Waxman et al., 1984). The genetic susceptibility to EAE development was originally thought to be a function of MHC genes and genes controlling hypersensitivity to histamine (Linthicum, 1982). An analysis of CSF from several MS patients showed a 60% higher histamine content than the control group, while histamine-N-methyltransferase, a histamine metabolizing enzyme, was lower than the control group (Tuomisto et al., 1983). Microarray analysis of chronic plaques in MS patients revealed relative overexpression of H_1R transcripts (Dormond et al., 2002). Administration of anti-H_1R agents either reduced the risk of MS (Alonso et al., 2006) or improved the neurological symptoms (Logothetis et al., 2005). H1RKO mice exhibit milder disease than WT mice (Bakker et al., 2002)(chapter 2 and 3). Mice deficient in H_2R also develop an attenuated disease (Teuscher et al., 2004). EAE is significantly enhanced in H_3R-deficient (Teuscher et al., 2007) and H_4R-deficient mice (Teuscher, unpublished data). Mice deficient in histidine decarboxylase, and therefore in histamine, develop more severe disease than WT mice (Musio et al., 2006). All these findings indicate a regulatory role for histamine in the pathogenesis of EAE.

Mast cells and basophils are the major source of histamine in the body (Mekori and Metcalfe, 2000). It has long been known that mast cells accumulate at the site of inflammatory demyelination in the brain and spinal cord both in animal models and in MS patients (Bebo et al., 1996; Brenner et al., 1994; Dietsch and Hinrichs, 1989; Ibrahim et al., 1996; Kermode et al., 1990; Olsson, 1974; Orr, 1988). Mast cell numbers and/or distribution correlated with MS lesion and EAE susceptibility (Brenner et al., 1994). Mast cell-stabilizing drugs have been shown to improve disease symptoms in EAE

(Brosnan and Tansey, 1984; Dietsch and Hinrichs, 1989; Seeldrayers et al., 1989). Mast cell-deficient mice exhibited delayed onset and reduced disease severity compared to the WT mice. The disease was restored upon reconstitution of these mice with bone marrow derived-mast cells (Secor et al., 2000), indicating a pathologic role for mast cells in EAE. Interestingly, reconstituted mast cells were present only in peripheral tissues but not in the brain and spinal cord, an observation confirmed by another independent study (Tanzola et al., 2003). The number of reconstituted mast cells in the spleen decreased with increased disease severity. While no mast cells were detected in the lymph node of naïve reconstituted animals, a large number of them were present in the draining inguinal lymph node in diseased animals (Tanzola et al., 2003). These findings suggest that mast cells act in the periphery, rather than the CNS, and therefore influence EAE during the induction phase rather than the effector phase. In addition, immunized mast cell-deficient mice had lower frequency of IFN-γ positive cells in the draining lymph node than WT mice. *Ex-vivo* stimulated T cells from these mice produced significantly lower IFN-γ and IL-4 than WT mice (Secor et al., 2000) (Tanzola et al., 2003). These results suggest that mast cells, and therefore histamine, provide a permissible microenvironment for the optimal induction of autoreactive T cells in the secondary lymphoid organs.

Due to its activity in multiple cell types including endothelial cells, antigen presenting cells and T cells, histamine acting through H_1R can function at several critical checkpoints during both induction and effector phases of EAE. In Chapter 2, it is demonstrated that H_1R exerts its effects during the induction of encephalitogenic T cells and that expression of H_1R in T cells is sufficient to restore clinical disease severity and cytokine production in H1RKO mice to the WT levels, independent of its actions in

other cell types important in the disease process. Further, in Chapter 3, it is established that even though the resistant allele of H_1R differs only by three amino acids from the susceptible allele, it confers resistance to disease due to lack of cell surface expression.

In endothelial cells, as described before, H_1R signaling leads to vasodilation, increased vascular permeability and thus affects BBB integrity. H_1R signaling also acts as a secretagogue for the regulated release of the stored factors from endothelial-specific storage vesicles called Weibel-Palade bodies (WPBs) (Hattori et al., 1989). The WPBs contain several vasoactive factors such as von Willebrand factor, P-selectin, and IL-8, the syntheses of which are induced by inflammatory signals including PTX (Rondaij et al., 2006). When these factors are released, they act on the endothelial cells in an autocrine fashion. The direct vasodilatory effects of histamine combined with the autocrine effects of the WPB contents are likely to result in shock and the death observed during the effector phase of Bphs. However, in Appendix A, it is shown that the release of WPB contents do not mediate the shock observed during the Bphs.

CHAPTER 2: HISTAMINE RECEPTOR H_1 IS REQUIRED FOR TCR MEDIATED P38 MAP KINASE ACTIVATION AND IFN-GAMMA PRODUCTION

Rajkumar Noubade[1], Roxana del Rio[1], Graeme Milligan[2], James F. Zachary[3], Elizabeth P. Blankenhorn[4], Mercedes Rincon[1] and Cory Teuscher[1]

[1]Departments of Medicine and Pathology, University of Vermont, Burlington, VT 05405;

[2]Molecular Pharmacology Group, Davidson Building, University of Glasgow, Glasgow G12 8QQ Scotland, UK

[3]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL 61802;

[4]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19129

Address correspondence to: Dr. Cory Teuscher
Immunobiology Program
C317 Given Medical Building
University of Vermont
Burlington, VT 05405
(802) 656-3270
C.Teuscher@uvm.edu

Text pages: 27
Figures: 7
Tables: 0

Running title: H_1R requirement for p38 MAP kinase activation and IFN-γ production.

Key words: Histamine receptor 1, Interferon-γ, p38 MAP kinase, Autoimmunity, EAE/MS,

Acknowledgments: Supported by the National Institutes of Health Grants AI45666 and NS36526.

Abstract

Histamine H_1 receptor (H_1R) is a shared susceptibility gene in experimental allergic encephalomyelitis (EAE) and orchitis (EAO), two classical T-cell mediated models of organ-specific autoimmune diseases. Here we show that expression of H_1R in CD4 T cells is required for IFNγ production but is dispensable for proliferation. H_1R ligation is necessary for TCR-mediated activation of p38 MAP kinase, a known regulator of IFNγ expression. Importantly, selective expression of H_1R in CD4 T cells fully complements both IFNγ production and EAE susceptibility of H_1R deficient mice. Thus, the presence of H_1R in CD4 T cells and its interaction with histamine regulates early TCR signals that lead to Th1 differentiation and autoimmune disease.

Introduction

Histamine [2-(4-imidazole) ethylamine] is a ubiquitous mediator of diverse physiological processes including neurotransmission and brain functions, secretion of pituitary hormones, and regulation of gastrointestinal and circulatory functions (Parsons and Ganellin, 2006). Additionally, histamine is a potent mediator of inflammation and a regulator of innate and adaptive immune responses (Akdis and Simons, 2006). Histamine exerts its effect through four receptors that belong to the seven-transmembrane G protein-coupled receptor family and are designated histamine H_1, H_2, H_3, and H_4 receptor, according to the chronological order of their discovery (1, 3).

H_1R couples to second messenger signaling pathways via the activation of heterotrimeric $G\alpha_{q/11}$ family of G proteins (Parsons and Ganellin, 2006). Generally, activation of H_1R leads to stimulation of phospholipase C, resulting in the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) to form inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG), which causes calcium mobilization from intracellular stores and activation of protein kinase C (PKC), respectively (Hill et al., 1997). In addition, H_1R signaling also mediates other signaling pathways such as the production of cGMP, arachidonic acid and nitric oxide (Leurs et al., 1995), and the activation of NF-κB (Bakker et al., 2001), STAT1 (Sakhalkar et al., 2005), STAT4 (Engelhardt, 2006) and MAP kinase pathway (Lipnik-Stangelj and Carman-Krzan, 2004; Megson et al., 2001; Robinson and Dickenson, 2001). However, even though H_1R is the first histamine receptor to be identified and a large number of studies on histamine and H_1R have been published in the last decade (Simons, 2004), little is known about the cell-type specific H_1R signaling pathways.

In the immune system, histamine has been reported to be a potent modulator of innate and adaptive immune responses. Histamine, acting through H_1R, affects the maturation of dendritic cells and alters their T cell-polarizing activity (Caron et al., 2001). It regulates antigen-specific T-cell effector functions and the related antibody isotype response (Banu and Watanabe, 1999). H_1R signaling in splenocytes has been reported to modulate cytokine secretion by these cells (Bakker et al., 2002; Banu and Watanabe, 1999; Bryce et al., 2006; Jutel et al., 2001) but no study has addressed the role of H_1R in purified CD4 T cells.

We have previously demonstrated that *Hrh1* (encoding the mouse H_1R protein) is a shared susceptibility gene in experimental allergic orchitis (EAO) and encephalomyelitis (EAE), the autoimmune model of multiple sclerosis (MS) (Bakker et al., 2002). In both MS and EAE, CD4 T cells secreting IFNγ (Th1) (Baron et al., 1993) and/or IL-17 (Th17) (Krueger et al., 2005) are necessary and sufficient for eliciting EAE pathology and clinical signs. The relative contributions of each of these cytokines to the development of EAE *in vivo* are debated, because conflicting evidence exists on the importance of IFNγ *vs.* IL-17. On the one hand, the importance of IL-17 is established in studies showing that EAE is diminished in IL-23-deficient but not IL-12-deficient animals (with no expression of the Th17-promoting or Th1-promoting cytokines, respectively) (Cua et al., 2003), and severe EAE is observed in IFNγ knockout mice and IFNγR knockout mice (Ferber et al., 1996; Willenborg et al., 1996). These findings contrast with studies showing that either CD4 Th1 cells (Baron et al., 1993) or CD4 Th17 cells (Langrish et al., 2005) can transfer EAE to naïve recipients. Recent studies reporting the predominant presence of a pre-Th1, $IFN\gamma^+/IL\text{-}17^+$ CD4 T cell subtype, early

after induction of EAE with encephalitogenic myelin oligodendrocyte glycoprotein 35-55 (MOG_{35-55}) peptide (Suryani and Sutton, 2007) may help resolve these apparent inconsistencies. Nevertheless, IFNγ alone or in conjunction with IL-17, is well-established as a cytokine of relevance in EAE immunopathology.

We have previously shown that H_1R deficient (H1RKO) mice exhibit a significant delay in the onset of EAE and a reduction in the severity of the clinical signs compared to wild-type (WT) mice (Bakker et al., 2002). This phenotype is associated with an immune deviation of the elicited CD4 T cell population from a Th1 response to a Th2 response with no detectable difference in IL-17 secretion, suggesting that the CD4 Th1 cells and the IFNγ produced by them play an important role in the pathology of the disease. In this report, we have studied the mechanism underlying the immune deviation, and show that it is directly due to H_1R regulation of cytokine responses in CD4 T cells, and not to H_1R expression in antigen presenting cells (APCs). In this study, we also show that H_1R is expressed in unstimulated CD4 T cells but is rapidly downregulated upon activation. H_1R is required for the activation of the p38 MAP kinase signaling pathway and for IFNγ production in response to TCR stimulation in CD4 T cells. Finally, H_1R mediated signaling in CD4 T cells, independent of APCs, regulates the encephalitogenic Th1 effector cell response in EAE.

Results

H_1R expression is required for IFNγ production by CD4 T cells

MOG_{35-55} peptide-immunized H1RKO splenocytes produce less IFNγ and more IL-4 than the splenocytes from immunized WT mice (Bakker et al., 2002). However, it is not clear whether this immune deviation is due to the lack of H_1R signaling in CD4 T cells or in APCs. To investigate the role of H_1R in regulating IFNγ production and Th1 differentiation, CD4 T cells were purified from WT and H1RKO mice and activated with anti-CD3 and anti-CD28 monoclonal antibodies (mAbs) in the presence of recombinant IL-12 and neutralizing anti-IL-4 mAb. After 4 days, Th1 effector cells were extensively washed, counted and equal number of cells were re-stimulated with anti-CD3 mAb for 24 hours. Th1 effector cells from H1RKO mice produced considerably less IFNγ than WT Th1 cells (Fig. 1A). We also examined the production of IL-4 upon re-stimulation of Th2 effector cells generated in presence of IL-4 and anti-IFNγ mAb. A marginal increase in IL-4 production was observed in cells from H1RKO mice compared to cells from WT mice (Fig. 1B). Recent studies have established IL-17 as an important cytokine in EAE (20). Consequently, we examined IL-17 production by Th17 cells generated in the presence of IL-6 and TGF-β and anti-IFNγ and anti-IL-4 mAbs. There was no difference in IL-17 production by Th17 differentiated cells from H1RKO and WT mice (Fig. 1C). Moreover, we examined the role of H_1R in non-polarized effector cells, generated by stimulating cells in the absence of exogenous cytokines for 4 days. Effector cells were then re-stimulated with anti-CD3 mAb for 24 hours. CD4 T effector cells from H1RKO mice produced significantly less IFNγ than those from WT mice (Fig. 1D). Thus, under

these conditions, IFNγ production in H1RKO effector CD4 T cells is impaired.

IFNγ production by CD4 T cells contributes to their differentiation into Th1 effector cells (Robinson and O'Garra, 2002). To examine the role that H_1R signaling plays in this process, purified CD4 T cells from H1RKO and WT mice were stimulated with anti-CD3 and anti-CD28 mAbs for different periods of time and IFNγ production was quantified. CD4 T cells from H1RKO mice produced significantly lower IFNγ than those from WT mice at all time points examined (Fig. 1E). In contrast, no difference in IL-2 production between WT and H1RKO CD4 T cells was observed (Fig. 1F). Furthermore, proliferation was comparable between WT and H1RKO CD4 T cells (Fig. 1G). Taken together, these results demonstrate that H_1R expression in CD4 T cells plays a critical role in regulating IFNγ production during the activation and differentiation of these cells.

H_1R gene expression is downregulated early upon TCR activation

In order to demonstrate that the reduced secretion of IFNγ by CD4 T cells is due to the absence of a functional H_1R in these cells, we carried out H_1R complementation in to H1RKO CD4 T cells by retroviral transduction. We generated a retroviral construct using the pEGZ-HA vector where H_1R was subcloned downstream of a hemagglutinin (HA) tag and upstream of IRES-EGFP. To confirm that the HA-H_1R could be properly expressed we transiently transfected HEK293T cells with the pEGZ-HA-H_1R construct and examined its expression by Western blot analysis using anti-HA mAb. A band corresponding to the HA-H_1R size (~55 kDa) was present only in HA-H_1R transfected cells (Fig. 2A). To demonstrate that the HA-H_1R was expressed on the cytoplasmic membrane, the HA-H_1R transfected HEK293T cells were stained using anti-

HA mAb and examined by confocal microscopy. HA-H_1R was expressed on the cytoplasmic membrane only in HA-H_1R transfected cells (Fig. 2B).

H_1R coupling to second messenger pathways is primarily via $G\alpha_{q/11}$ (Parsons and Ganellin, 2006). The ability of the transfected HA-H_1R to activate $G\alpha_{11}$ was tested in a [^{35}S] GTPγS binding assay. When membrane fractions from transfected HEK293 cells were used in the [^{35}S] GTPγS binding assay, HA-H_1R was capable of activating $G\alpha_{11}$ in response to histamine (Fig. 2C). Taken together, these results show that HA-H_1R is properly expressed and is functional.

To perform retroviral transduction, CD4 T cells were isolated from H1RKO and WT mice, activated with anti-CD3 and anti-CD28 mAbs for 16 hours and transduced with either pEGZ or pEGZ-HA-H_1R retroviruses. Expression of HA-H_1R in transduced CD4 T cells was confirmed by confocal microscopy and flow cytometry (data not shown). After 2 days, transduced CD4 T cells were isolated by cell sorting based on EGFP expression and equal numbers of cells were activated with anti-CD3 mAb for an additional 24 hours. Both pEGZ and pEGZ-HA-H_1R transduced CD4 T cells from H1RKO mice produced significantly lower levels of IFNγ than those from WT mice (Fig. 3A). These results indicate that the expression of H_1R in activated CD4 T cells does not restore the IFNγ production in H_1R deficient cells.

Retroviral transduction requires prior activation of CD4 T cells for at least 16 hours to induce cell cycling. Thus, if H_1R is normally required during the early phase of activation concomitant with TCR engagement, the retroviral transduction would not rescue the H1R deficiency. Our results above (Fig.1C) indicated that the IFNγ production was already reduced at 36 hours in H1RKO CD4 T cells compared to the WT cells.

We therefore examined IFNγ production by H1RKO CD4 T cells earlier during the activation with anti-CD3 and anti-CD28 mAbs. Although lower levels of IFNγ were present in WT CD4 T cells at 24 hours of activation, H1RKO CD4 T cells still produced significantly less IFNγ (Fig. 3B) indicating that H_1R plays a role early during the activation of CD4 T cells.

H_1R expression during mouse T cell activation has not been investigated. We therefore analyzed the H_1R gene expression in WT CD4 T cells stimulated for different periods of time with anti-CD3 and anti-CD28 mAbs. Relative levels of H_1R mRNA were examined by conventional and quantitative real time RT-PCR analysis. CD4 T cells markedly downregulated H_1R mRNA expression by 24 hours after activation (Fig. 3C and 3D), further indicating that H_1R plays a role early (< 24 hours) after TCR engagement and that it is not required for IFNγ production by CD4 T cells once they are activated.

Selective H_1R expression in T cells in transgenic mice restores IFNγ production

To examine the role of H_1R during the initial activation of CD4 T cells, we generated transgenic mice expressing H_1R under the control of distal *lck* promoter, which drives expression in T cells (Wildin et al., 1991). Transgenic mice were generated directly on the C57BL/6J background. Two transgenic founders were identified and crossed to H1RKO mice to obtain H1RKO mice expressing H_1R selectively in T cells (H1RKO-Tg mice). The expression of the transgene in CD4 T cells from two lines (H1RKO-Tg-1 and H1RKO-Tg-3) was confirmed by RT-PCR using transgene-specific primers (Fig. 4A). We examined the surface expression of the transgene in CD4 T cells by immuno-staining using anti-HA mAb and confocal microscopy (Fig. 4B). The

transgene was expressed in CD4 T cells from both transgenic lines. No differences in the total numbers or distribution of T cell subpopulations in the thymus and peripheral lymphoid tissues were observed among WT, H1RKO and either of the H1RKO-Tg lines (data not shown).

We then examined whether the expression of H_1R in H1RKO CD4 T cells restored IFNγ production. CD4 T cells from WT, H1RKO and H1RKO-Tg mice were stimulated with anti-CD3 and anti-CD28 mAbs and IFNγ levels quantified. The levels of IFNγ secreted by CD4 T cells from H1RKO-Tg-3 were comparable to WT CD4 T cells and those from H1RKO-Tg-1 remained slightly lower than the WT CD4 T cells but significantly higher than the levels in H1RKO CD4 T cells (Fig. 4C). Analyses at different periods of time after activation confirmed that the transgenic expression of H_1R in H1RKO CD4 T cells fully restores IFNγ production (Fig. 4D).

We also studied the IFNγ production from Th1 polarized and non-polarized effector cells from H1RKO-Tg mice. CD4 T cells from WT, H1RKO and H1RKO-Tg mice were differentiated in the absence of exogenous cytokines (non-polarized) or in the presence of recombinant IL-12 and anti IL-4 mAb (Th1). After 4 days, effector cells were re-stimulated with anti-CD3 mAb for 24 hours and IFNγ production was measured. Both Th1 polarized (Fig. 4E) and non-polarized CD4 effector T cells (Fig. 4F) from H1RKO-Tg mice produced significantly more IFNγ than the H1RKO effectors. Furthermore, the levels of IFNγ in H1RKO-Tg cells were comparable to those in WT CD4 T cells. Together, these data demonstrate that the presence of H_1R at the time of activation of CD4 T cells under both polarizing and non-polarizing conditions regulates IFNγ

production and Th1 differentiation.

Impaired activation of p38 MAP kinase by TCR ligation in H1RKO CD4 T cells

In order to dissect the molecular mechanism of H_1R signaling in regulating IFNγ production by CD4 T cells, we examined the signaling pathways that have been previously associated with H_1R in other cell types. NF-κB has been shown to be activated through H_1R in green monkey kidney cells (Bakker et al., 2001) and has been associated with regulation of IFNγ expression in CD4 T cells (Aronica et al., 1999). Therefore, we performed an electrophoretic mobility shift assay (EMSA) to examine NF-κB DNA binding. CD4 T cells from WT and H1RKO mice were stimulated with anti-CD3 and anti-CD28 mAbs for different periods of time. There was no difference in NF-κB activation between WT and H1RKO CD4 T cells (Fig. 5A). STAT1 is also known to regulate IFNγ expression in CD4 T cells (Ramana et al., 2002) and it has recently been shown that H_1R signaling regulates STAT1 phosphorylation in splenocytes (Sakhalkar et al., 2005). Therefore we examined activation of STAT1 by Western blot analysis in stimulated CD4 T cells. STAT1 phosphorylation was undetected at early time points up to 3 hours of activation in both WT and H1RKO cells (data not shown). Phospho-STAT1 was detected after 3 hours of activation but there was no difference in STAT-1 phosphorylation between WT and H1RKO CD4 T cells (Fig. 5B). Although H_1R signaling has also been reported to regulate STAT4 phosphorylation in splenocytes (Engelhardt, 2006), phospho-STAT4 was not detected in WT and H1RKO CD4 T cells after activation with anti-CD3 and anti-CD28 mAbs (data not shown).

H_1R ligation has recently been shown to lead to the phosphorylation of p38 MAP kinase in DDT_1MF-2 cells (Robinson and Dickenson, 2001) and in human aortic

endothelial cells (Steffel et al., 2005). Activation of p38 MAP kinase pathway is required for IFNγ production and Th1 differentiation (Rincon et al., 1998). We therefore examined the activation of p38 MAP kinase by Western blot analysis. CD4 T cells from WT and H1RKO mice were stimulated with anti-CD3 and anti-CD28 mAbs for different periods of time. p38 MAP kinase was activated in WT CD4 T cells but was markedly impaired in H1RKO CD4 T cells (Fig. 5C). In contrast, no difference in ERK MAP kinase activation was observed between WT and H1RKO CD4 T cells (Fig. 5D). As has been reported by us previously (Weiss et al., 2000), activation of JNK MAP kinase could not be detected at the earlier time points in both WT and H1RKO CD4 T cells stimulated with anti-CD3 and anti-CD28 mAbs (data not shown). We further examined the activation of p38 MAP kinase by TCR ligation in H1RKO-Tg CD4 T cells. Unlike H1RKO CD4 T cells, the levels of phospho p38 MAP kinase in H1RKO-Tg CD4 T cells were equivalent to those in the WT CD4 T cells (Fig. 5E). Thus, TCR mediated activation of p38 MAP kinase required the presence of H_1R in CD4 T cells.

Activation of p38 MAP kinase by TCR is mediated by histamine/H_1R binding

To understand the mechanism by which H_1R could regulate TCR mediated p38 MAP kinase activation, we examined if histamine itself could activate the p38 MAP kinase in CD4 T cells. Histamine is already present at low concentrations (about 10^{-7}M) in the serum used for the culture medium. Therefore, we assessed p38 MAP kinase phosphorylation in response to histamine using a medium prepared with previously dialyzed serum to deplete histamine (Banu and Watanabe, 1999). CD4 T cells from WT and H1RKO mice were resuspended in the histamine-free medium and treated with histamine. p38 MAP kinase was activated by histamine in WT CD4 T cells but not

in H1RKO CD4 T cells (Fig. 6A), indicating that histamine activates this pathway in CD4 T cells through H_1R.

Since histamine was present in the normal medium used to activate CD4 T cells with anti-CD3 and anti-CD28 mAbs (Fig. 5C and 5E), it was possible that the activation of p38 MAP kinase by TCR ligation was co-dependent upon histamine signaling through the H_1R. To test this possibility, we examined p38 MAP kinase activation upon anti-CD3 and anti-CD28 mAb stimulation in histamine-free medium. TCR ligation failed to activate p38 MAP kinase in both WT and H1RKO CD4 T cells in histamine-free medium (Fig. 6B). In contrast, the absence of histamine did not affect TCR-mediated ERK activation (Fig.8) or the intracellular calcium mobilization (data not shown) in WT CD4 T cells. To further demonstrate the selective requirement for histamine in TCR-mediated p38 MAP kinase activation, WT and H1RKO CD4 T cells were stimulated in histamine-free medium with anti-CD3 and anti-CD28 mAbs in the presence of histamine. TCR-mediated p38 MAP kinase activation was restored by histamine in WT CD4 T cells but not in H1RKO CD4 T cells (Fig. 6C), indicating that binding of histamine to H_1R was required for activation of p38 MAP kinase upon TCR ligation. Interestingly, the levels of phospho-p38 MAP kinase in WT CD4 T cells treated anti-CD3 and anti-CD28 mAbs and histamine were similar to the levels obtained when the cells were treated with histamine alone (Fig. 6C). The inability of TCR to activate p38 MAP kinase in H_1R deficient cells in normal medium (Fig. 5C), the inability of TCR ligation to activate p38 MAP kinase in the histamine-free medium (Fig. 6B) and the inability of TCR to further increase p38 MAP kinase activation when histamine was added to the histamine free medium strongly suggest that the activation of p38 MAP kinase observed upon TCR ligation is dependent

upon concomitant H_1R signaling.

Although the precise mechanism by which p38 MAP kinase regulates IFNγ production in CD4 T cells remains unclear, recent studies have suggested that the activation of the this MAP kinase pathway is required for T-bet expression (Engelhardt, 2006; Jones et al., 2003) and T-bet regulates IFNγ production (Szabo et al., 2000). We therefore examined T-bet expression by Western blot analysis during activation of WT and H1RKO CD4 T cells. T-bet levels were lower in activated H1RKO CD4 T cells compared to the WT CD4 T cells (Fig. 6D). Thus, the impairment in p38 MAP kinase activation in the absence of H_1R reduces the T-bet expression and thereby IFNγ production by CD4 T cells during TCR activation.

In order to demonstrate that the reduced p38 MAP kinase activation in H1RKO CD4 T cells in responsible for the lower IFNγ production by these cells, we crossed H1RKO mice with the previously described distal $MKK6_{Glu}$-Tg mice (Rincon et al., 1998). These mice express a constitutively active form of MKK6, a specific upstream activator of p38 MAP kinase, under the control of *dlck* promoter to drive the expression in T cell lineage. Thus p38 MAP kinase is constitutively and selectively active in T cells in these mice. Anti-CD3 and anti-CD28 mAb stimulated CD4 T cells from H1RKO-$MKK6_{Glu}$-Tg mice produced significantly more IFNγ than CD4 T cells from littermate H1RKO mice (Fig. 6E), indicating that the diminished activation of p38 MAP kinase in H1RKO CD4 T cells is responsible for the reduced IFNγ production by these cells.

H_1R signaling directly in CD4 T cells regulates encephalitogenic Th1 effector responses

As a shared autoimmune disease susceptibility gene, *Hrh1* has been shown to control numerous disease associated subphenotypes, including blood brain barrier permeability, antigen presentation and delayed type hypersensitivity responses (Caron et al., 2001; Gao et al., 2003). To assess whether or not H_1R signaling in CD4 T cells influences EAE by regulating encephalitogenic Th1 responses, we examined the susceptibility of H1RKO and H1RKO-Tg mice to EAE using the classical MOG_{35-55}+CFA+PTX model and the 2× MOG_{35-55}+CFA model (Teuscher et al., 2006a), which does not use PTX as an ancillary adjuvant. Regression analysis (Teuscher et al., 2006a) revealed that the clinical disease courses elicited by both induction protocols fit a Sigmoidal curve and that compared to H1RKO mice the clinical course of EAE is significantly more severe in the transgenic mice [MOG_{35-55}+CFA+PTX model: (overall $F = 66.1$; $p < 0.0001$) with WT ($F = 132.1$; $p < 0.0001$), H1RKO-Tg-1 ($F = 127.5$; $p < 0.0001$), and H1RKO-Tg-3 ($F = 83.3$; $p < 0.0001$) mice significantly greater than H1RKO mice; 2× MOG_{35-55}+CFA model: (overall $F = 8.9$; $p < 0.0001$) with WT ($F = 226.9$; $p < 0.0001$), H1RKO-Tg-1 ($F = 134.0$; $p < 0.0001$) and H1RKO-Tg-3 ($F = 215.8$; $p < 0.0001$) mice significantly greater than H1RKO mice].

An analysis of EAE associated clinical quantitative trait variables (Butterfield et al., 1998) revealed that the mean day of onset (DO), cumulative disease score (CDS), number of days affected (DA), overall severity index (SI) and the peak score (PS) were significantly different among the strains immunized with either MOG_{35-55}+CFA+PTX (Table 1) or 2× MOG_{35-55}+CFA (Table 2). Post hoc multiple comparisons of each

trait variable revealed that WT = H1RKO-Tg-1 = H1RKO-Tg-3 > H1RKO. Additionally, compared to H1RKO mice both MOG_{35-55}+CFA+PTX (Fig. 7C) and 2× MOG_{35-55}+CFA (Fig. 7D) immunized H1RKO-Tg-1 and H1RKO-Tg-3 mice exhibited significantly more severe overall CNS pathology (Blankenhorn et al., 2000) which was equivalent in severity to that seen in WT mice. Therefore, H_1R expression in CD4 T cells alone is capable of complementing EAE susceptibility in H_1R deficient animals.

We also examined cytokine production following *ex-vivo* stimulation of splenocytes from mice immunized with MOG_{35-55}+CFA+PTX and 2× MOG_{35-55}+CFA. The H_1R transgene fully complemented IFNγ production by H1RKO CD4 T cells and restored IL-4 production to WT levels (Fig. 7E and 7F). In contrast, no significant differences in TNFα or IL-17 production were detected among WT, H1RKO and H1RKO-Tg mice. Together, these data indicate that H_1R signaling in CD4 T cells complements EAE severity independently of TNFα and IL-17 production.

Discussion

Although H_1R has been previously shown to play a role in regulating encephalitogenic Th1 immune response in EAE (Bakker et al., 2002), it was unclear whether this was caused by the deficiency of H_1R in CD4 T cells or APCs. In this study, we show that the presence of H_1R in CD4 T cells is essential for the activation of p38 MAP kinase and IFNγ production by these cells and the lack of H_1R in CD4 T cells is responsible for the increased EAE resistance of H1RKO mice. These findings also explain the likely cause of the Th2 deviation and aberrant IL-4 production seen in the H1RKO (Bakker et al., 2002), a result we confirmed in the present study. This deviation could logically be due to the impairment in p38 MAP kinase activation that reduces the T-bet expression and thereby IFNγ production by CD4 T cells during TCR activation. Without H_1R, naïve T cells cannot be driven into the full Th1 developmental pathway, and the result is an unbalanced immune repertoire that is generally thought to be protected from signs of EAE (Shaw et al., 1997).

Even though the expression of H_1R in CD4 T cells has been reported (Sachs et al., 2000) it was unknown how H_1R is regulated during the activation phase of CD4 T cells. Here we show, for the first time, that the H_1R gene expression is silenced early after the activation of CD4 T cells. Modulation of H_1R signaling, like other GPCRs, is complex and includes receptor desensitization, internalization and the subsequent down-regulation (McCreath et al., 1994; Smit et al., 1996). Desensitization of H_1R is induced by both agonist specific (homologous) and agonist non-specific (heterologous) pathways, mainly involving PKC-mediated phosphorylation of H_1R (Fujimoto et al., 1999; Miyoshi et al., 2004). PKC activation has been shown to inhibit H_1R both at the protein level as

well as at the gene expression level (Miyoshi et al., 2006; Pype et al., 1998; Yoshimura et al., 2005). Because TCR ligation leads to potent activation of PKC (Acuto and Cantrell, 2000), silencing of H_1R expression in activated CD4 T cells may be a consequence of PKC activation. Although the transcriptional regulation of H_1R promoter is not well understood, H_1R-mediated signaling has been shown to be necessary for continued H_1R expression (Miyoshi et al., 2006; Yoshimura et al., 2005). Thus, the loss of H_1R gene expression in activated CD4 T cells in mice may be a mechanism to turn off possible subsequent histamine signals in these cells. In humans, H_1R expression is reported to increase in Th1 differentiated cells (Jutel et al., 2001). However, H_1R mRNA was rapidly downregulated even during the Th1 differentiation of mouse CD4 T cells (data not shown). These apparently contradictory results may be explained by the different origin of the T cells (mouse *vs*. human) or by other differences in the culture conditions used.

H_1R has been previously implicated in the regulation of IFNγ production. H_1R-deficient splenocytes have been shown to produce lower IFNγ when activated by anti-CD3 and-CD28 mAbs or by specific antigen (Bakker et al., 2002; Banu and Watanabe, 1999; Bryce et al., 2006; Jutel et al., 2001) but no studies have addressed the role of H_1R in isolated CD4 T cells. Here we show that H_1R expression in CD4 cells is essential specifically for IFNγ production by these cells but not for IL-2 production or proliferation. A previous report showed hypoproliferation of total splenocytes from H_1R deficient mice in response to anti-CD3 mAb (Banu and Watanabe, 1999). However, the low proliferative response could be due to the H_1R deficiency in cells other than CD4 T cells, such as antigen presenting cells (e.g. macrophages or dendritic cells) that also express H_1R. Although CD4 T cells also express H_2R and H_4R, in addition to H_1R,

the selective restoration of the IFNγ response in CD4 T cells from H1RKO-Tg mice clearly demonstrates that signaling through H_1R is necessary for regulation of IFNγ production in these cells.

Several studies have shown that p38 MAP kinase is activated in CD4 T cells or total T cells upon TCR activation. Co-stimulatory molecules (such as CD28, 4-1BB, ICOS, CD30) also contribute to the activation of p38 MAP kinase during activation (Dodeller and Schulze-Koops, 2006). While most studies agree on the role of p38 MAP kinase on IFNγ production and Th1 differentiation, recent studies have questioned the requirement of TCR-mediated p38 MAP kinase activation. Instead, they propose that activation of this pathway by cytokines such as IL-12 or IL-18 is probably more relevant (Berenson et al., 2006). To date, the effect of other components also present in the milieu during TCR activation has not been addressed. Here we show, for the first time, that activation of p38 MAP kinase by TCR/CD28 ligation is dependent on the presence of histamine and its binding to H_1R. A previous study has shown the requirement of H_1R for ZAP-70 activation in H1RKO total splenocytes in conjunction with the hypoproliferative defect in these cells (Banu and Watanabe, 1999). However, here we show that in CD4 T cells, H_1R is not required for other key signaling pathways such as ERK activation (Fig.8), NF-κB activation (Fig. 5A) or calcium mobilization (data not shown), as well as for IL-2 production and proliferation. Thus, deficiency of H_1R in CD4 T cells appears to selectively impair the activation of the p38 MAP kinase pathway, but the mechanism remains to be investigated further. p38 MAP kinase is normally activated through the upstream MAPKK, MKK3 and MKK6 (and MKK4 in response to some stimuli) (Kyriakis and Avruch, 2001). It has been shown that GADD45 proteins interact

with MEKK4, an upstream kinase of MKK3 and MKK6 and thus activate p38 MAP kinase (Takekawa and Saito, 1998). An alternative pathway for activation of p38 MAP kinase through its autophosphorylation has also been recently proposed (Salvador et al., 2005). H_1R signaling is mediated by $G\alpha_{q/11}$ protein, which is also associated with TCR signaling through CD3ε (Stanners et al., 1995). Thus, it is possible the H_1R through $G\alpha_{q/11}$ could regulate GADD45 members (α, β and γ) and lead to p38 MAP kinase activation through either the classical or alternative pathway in CD4 T cells.

Epidemiological data indicate that the use of sedating H_1R antagonists is associated with decreased MS risk (Alonso et al., 2006); and in a small pilot study, patients with relapsing-remitting or relapsing-progressive MS given the H_1R antagonist hydroxyzine remained stable or improved neurologically (Logothetis et al., 2005). Additionally, microarray analysis revealed that the H_1R is overexpressed in the chronic plaques of MS patients (Dormond et al., 2002). Historically, the role of histamine in autoimmune inflammatory disease of the CNS has been viewed as a mediator of the effector or inflammatory phase of the disease (Bebo et al., 1996). However, recent data showing that EAE and neuroantigen specific T effector cell responses are significantly different in histamine- and histamine receptor-deficient mice compared to WT mice revealed that histamine plays a role during the induction phase and priming of autoreactive effector T cells (Bakker et al., 2002; Fillmore et al., 2004; Musio et al., 2006). In this regard, our results show that H_1R signaling in T cells regulates Th1 effector functions, but not Th17 effector functions, and EAE severity, independent of APCs and other hematopoietically-derived cells. Moreover, our results demonstrate that H_1R signaling in CD4 T cells regulates the encephalitogenic Th1 effector responses

during the priming of naïve antigen-specific CD4 T cells. Taken together, this suggests that pharmacological targeting of the H_1R may be useful early in the treatment of MS and other autoimmune inflammatory diseases in which molecular mimicry, bystander activation (with or without epitope spreading), and viral persistence play a role in perpetuating immunopathology as a consequence of continual priming of pathogenic adaptive immune responses (Fujinami et al., 2006).

Materials and methods

Mice

C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). B6.129P-*Hrh1*^{tm1Wat} (H1RKO) (Banu and Watanabe, 1999) mice were maintained in the animal facility at the University of Vermont (Burlington, VT). The experimental procedures used in this study were approved by the Animal Care and Use Committee of the University of Vermont.

For transgenic mouse generation, an HA-H_1R construct was made by deleting the methionine of the Bphs-susceptible H_1R allele (Bakker et al., 2002) and adding an HA tag at the N-terminus using TOPO cloning vector (Invitrogen, Carlsbad, CA). The HA-H_1R was then subcloned downstream of the distal *lck* promoter (Wildin *et al.*, 1991). The linear DNA fragment containing the distal *lck* promoter, the HA-H_1R 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 by DNA slot blot using a *Bam*HI–*Sac*I 0.5 kb fragment from the *hGH* gene as a probe. Two founders were generated and were crossed to H1RKO mice to establish transgenic mouse lines in H1RKO background (H1RKO-Tg mice). Distal *lck* $MKK6_{Glu}$ transgenic mice (Rincon et al., 1998) were crossed to H1RKO mice to generate H1RKO-$MKK6_{Glu}$ transgenic mice.

Cell preparation and culture conditions

CD4 T cells were isolated from spleen and lymph nodes by negative selection for CD8-, MHC class II-, NK1.1- and CD11b-positive cells using magnetic beads from Qiagen, Valencia, CA, as previously described (Rincon et al., 1998). Purified

CD4 T cells were stimulated with plate bound anti-CD3 (5 μg/ml) and soluble anti-CD28 (1 μg/ml) monoclonal antibodies (mAbs) from BD Pharmingen (Franklin Lakes, NJ). Th1 polarized CD4 effector T cells were generated by culturing the CD4 T cells (1×10^6 cells/ml) with anti-CD3 and anti-CD28 mAbs in presence of 4 ng/ml of recombinant IL-12 (R&D systems, Minneapolis, MN) and 10 μg/ml of anti IL-4 mAb (BD Pharmingen, San Diego, CA). Th2 polarized CD4 effector T cells were generated by activating cells (1×10^6/ml) with anti-CD3 and anti-CD28 mAbs in presence of 30 ng/ml of recombinant IL-4 (R&D systems, Minneapolis, MN) and 10 μg/ml of anti IFNγ-4 mAb. Effector Th17 CD4 T cells were generated by activating CD4 T cells (1×10^6 cells/ml) with anti-CD3 and anti-CD28 mAbs in presence of 1 ng/ml of TGFβ (Peprotech Inc, Rocky Hill, NJ) and 30 ng/ml of IL-6 (R&D systems, Minneapolis, MN) and 10 μg/ml of anti-IFNγ and 10 μg/ml of anti IL-4 mAbs. After 4 days, the cells were extensively washed, counted and equal number of cells were restimulated with anti-CD3 mAb. After 24 hours, the supernatants were collected and IFNγ, IL-4 and IL-17 were analyzed by ELISA. Non-polarized effector cells were generated by culturing CD4 T cells with anti-CD3 and anti-CD28 mAbs in the absence of exogenous cytokines for four days. The cells were then extensively washed, counted and equal numbers of cells were restimulated with anti-CD3 mAb. After 24 hours, the supernatants were collected and IFNγ was analyzed by ELISA.

Histamine dihydrochloride was obtained from Sigma-Aldrich (St. Louis, MO). RPMI prepared with 10% Fetalclone © bovine serum (Hyclone, Logan,UT), serum dialyzed twice with 10,000 kDa molecular cutoff, was used as histamine free medium.

Cytokine production

ELISAs were performed on the cell culture supernatants as described previously (Fillmore et al., 2004), using the primary antibodies: anti-IFNγ, anti-IL-2, anti-IL-4 and anti-IL17 mAbs and their corresponding biotinylated mAbs (BD Pharmingen, San Diego, CA). Other ELISA reagents included: Horseradish peroxidase-conjugated avidin D (Vector Laboratories, Burlingame, CA), TMB microwell peroxidase substrate and stop solution (Kirkegaard and Perry Laboratories, Gaithersburg, MD) and recombinant IFNγ, IL-4 and IL-2 (R&D Systems, Minneapolis, MN) used as standards.

For cytokine analysis in ex-vivo stimulated splenocytes from mice immunized with the classical MOG_{35-55}+CFA+PTX model and the 2x MOG_{35-55}+CFA model, single cell suspensions were prepared @ $1x10^6$ cells/ml in RPMI medium and stimulated with 50 μg/ml of MOG_{35-55}. Cell culture supernatants were recovered at 72 hours and cytokine levels were measured by ELISA using anti-IFNγ, anti-IL-4 and anti-IL17 mAbs and their corresponding biotinylated mAbs (BD Pharmingen, San Diego, CA). TNFα ELISA kit was from (BD Pharmingen, San Diego, CA).

Proliferation Assays

CD4 T cells (2.5×10^5 cells/well) were activated with anti-CD3 and anti-CD28 mAbs for 72 h and proliferation was determined by ^{3}[H]-thymidine incorporation during the last 18 h of culture.

Quantitative Real-Time Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from CD4 T cells using RNeasy RNA isolation reagent (Qiagen, Valencia, CA) as recommended by the manufacturer. cDNA generated from 1 μg total RNA was used in quantitative real time RT-PCR using the SYBR green

method. The sequences of *Hrh1* primers used were F: 5'-CCAGAGCTTCGGGAAGATAA-3' and R: 5'ACCACAGCATGAGCAAAGTG-5'. β-2-microglobulin was used as reference gene and relative mRNA levels were calculated using comparative C_T method. For conventional RT-PCR, the cDNA was amplified by PCR and visualized on 1% gel. The primers mentioned above were used for *Hrh1* and the primers used for *Hprt1* were F: 5'-GTTGGATACAGGCCAGACTTTGTTG-3'and R:5'-GAGGGTAGGCTGCCTATAGGCT-3". To study the transgene expression in H1RKO-Tg mice, the cDNA prepared as explained above was amplified using a forward primer in *Hrh1* (5"-CTCCCGGACCACAGACTCAGA-3') and a reverse primer in the 3rd exon of *hGH* (5'-GACGGAGGTCTGGGGGTTCTG) and the PCR product was visualized on 1% agarose gel.

Retroviral transduction experiments

The retroviral vector plasmid pEGZ-HA was a generous gift from Dr. Ingolf Berberich (University of Wurzburg, Wurzburg, Germany) and packaging vectors pHIT123 and pHIT 60 were generous gifts from Dr. Alan Klingsman (Oxford University, Oxford. UK). Two restriction sites, *BamHI* and *EcoRI* were inserted into the mouse H_1R cDNA by PCR and cloned such that the second codon is in frame with the HA tag of pEGZ generating an HA-H_1R fusion protein. pEGZ is a bicistronic system with IRES-EGFP. EGFP served as a marker for transfected cells.

The retroviral vector plasmids, pEGZ-HA-H_1R or the empty pEGZ and the packaging vectors pHIT60 and pHIT123 were transiently transfected into human embryonic kidney fibroblasts expressing the SV40 large T antigen (HEK293T) cells using the calcium phosphate method. After two days, the retrovirus containing

supernatants were used to transduce (by centrifugation at 800g for 3 hours at 32^0C) CD4 T cells previously activated with anti-CD3 and anti-CD28 mAbs for 16 hours. The transduced CD4 T cells were cultured in presence of 50 U/ml of IL-2 for two days and were sorted using a FACSAria instrument (BD Pharmingen, San Diego, CA), based on their EGFP expression. Equal numbers of EGFP positive cells were restimulated with anti-CD3 mAb and 24 hours later IFNγ was measured in the supernatant by ELISA.

Confocal microscopy

HEK293T cells were transfected with pEGA-HA-H_1R or empty pEGZ control vector (5 μg total DNA) using the calcium phosphate method. Cells were fixed, permeabilized and stained using an anti-HA mAb (Cell Signaling Technologies, Danvers, MA) followed by an incubation with Alexa-568 anti-mouse antibody (Molecular Probes, Eugene, Oregon). TOPRO-3 nuclear stain (Molecular Probes, Eugene, Oregon) was used as a nuclear marker. Cells were examined by confocal microscopy using Zeiss LSM 510 META Confocal Laser Scanning Imaging System (Carl Ziess Microimaging Inc, Thronwood, NY)

Cell lysates and Western blotting

Whole-cell lysates were prepared from $1x10^6$-$5x10^6$ cells in Triton lysis buffer and were then separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes as described previously (Farley et al., 2006). Primary antibodies used for Western blot analysis include anti-HA (Abcam Inc. Cambridge, MA), anti-p38, anti-phospho-p38, anti-phospho-STAT1, anti-phospho-STAT4, anti-phospho-ERK, anti-ERK, anti-phospho-JNK, anti-JNK (Cell Signaling Technologies. Danvers, MA), anti-T-bet (a gift from Dr. L. Glimcher, Harvard

University School of Public Health, Boston, MA) and anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA).

[^{35}S]GTPγS binding assay

The HA-H_1R cDNA was subcloned into pcDNA3 using restriction sites *EcoRI*/*BamHI*. The [^{35}S]GTPγS binding experiments were initiated by the addition of 50 fmols of receptor to an assay buffer (20mM HEPES (pH 7.4), 3mM MgCl2, 100mM NaCl, 1μM GDP, 0.2mM ascorbic acid, and 100nCi [^{35}S]GTPγS) containing 100μM histamine. Non-specific binding was determined in the above condition with the addition of 100μM GTPγS. Reactions were incubated for 15 min at 30^{0}C and were terminated by the addition of 500μl of ice-cold buffer containing 20mM HEPES (pH 7.4), 3mM $MgCl_2$, 100mM NaCl and 0.2mM ascorbic acid. The samples were centrifuged at 16,000 x g for 10 minutes at 4^{0}C. The resulting pellets were re-suspended in solubilization buffer (100mM Tris, 200mM NaCl, 1mM EDTA, and 1.25% Nonidet P-40) plus 0.2% SDS. Samples were precleared with Pansorbin for 1 hour, followed by immunoprecipitation with C-terminal $G_{\alpha 11}$ antiserum. Finally, the immunocomplexes were washed with solubilization buffer and bound [^{35}S]GTPγS was estimated by liquid scintillation-spectrometry.

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts were prepared from anti-CD3 and anti-CD28 mAbs treated CD4 T cells as previously described (Berenson et al., 2006). Binding reactions for electrophoretic gel mobility shift assay were carried out at room temperature using 2 μg nuclear proteins and [^{32}P]dCTP-end labeled double-stranded oligonucleotide probes containing NF-κB binding site from the mouse κ intron enhancer (Sense 5'-

GATCAGAGGGGACTTTCCGAGGGAT-3' and anti-sense 5'-GATCCCTCGGAAAGTCCCCTCTGAT-3'). Samples were separated by electrophoresis under non-denaturing conditions and exposed to film for autoradiography.

Induction and Evaluation of EAE

Mice were immunized for the induction of EAE using either the MOG_{35-55}-complete Freund's adjuvant (CFA) double-inoculation (Butterfield et al., 1998) or the MOG_{35-55}-CFA+PTX single-inoculation protocols (Teuscher et al., 2006a). For the double-injection protocol mice were injected subcutaneously with an emulsion of 100 μg of MOG_{35-55} and an equal volume of CFA containing 200 μg of *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Detroit, MI) in the posterior right and left flank; one week later all mice were similarly injected at two sites on the right and left flank anterior of the initial injection sites. Animals immunized using the MOG_{35-55}-CFA+PTX single-inoculation protocol received an emulsion of 200 μg MOG_{35-55} and equal volume of CFA containing 200 μg of *Mycobacterium tuberculosis* H37RA by subcutaneous injections distributed equally in the posterior right and left flank and scruff of the neck. Immediately thereafter, each animal received 200 ng PTX (List Biological Laboratories, Campbell, CA) by intravenous injection. Mice were scored daily starting at day 5 post-injection as previously described (Teuscher et al., 2006a). Clinical quantitative trait variables including disease incidence and mean day of onset (DO), cumulative disease score (CDS), number of days affected (DA), overall severity index (SI) and the peak score (PS) were generated as previously described (Butterfield et al., 1998).

Brains and SC were dissected from calvaria and vertebral columns, respectively, and fixed by immersion in 10% phosphate- buffered formalin (pH 7.2). Following

adequate fixation, brain and SC were trimmed and representative transverse section embedded in paraffin, sectioned at 5 μm, and mounted on glass slides. Sections were stained with hematoxylin and eosin for routine evaluation and Luxol fast blue-periodic acid Schiff for demyelination. Sections from representative areas of the brain and SC were scored in a semi-quantitative fashion for the various histopathologic parameters as previously described (Blankenhorn et al., 2000). An overall CNS pathology index (PI) for each lesions was obtained by calculating the average scores for the lesions observed in the brain and spinal cord.

Statistical analysis

The statistical analyses, as indicated in the figure legends, were performed using GraphPad Prism 4 software (GraphPad software Inc, San Diego, CA).

References

Acuto, O., and Cantrell, D. (2000). T cell activation and the cytoskeleton. Annu Rev Immunol *18*, 165-184.

Akdis, C.A., and Simons, F.E. (2006). Histamine receptors are hot in immunopharmacology. Eur J Pharmacol *533*, 69-76.

Alonso, A., Jick, S.S., and Hernan, M.A. (2006). Allergy, histamine 1 receptor blockers, and the risk of multiple sclerosis. Neurology *66*, 572-575.

Aronica, M.A., Mora, A.L., Mitchell, D.B., Finn, P.W., Johnson, J.E., Sheller, J.R., and Boothby, M.R. (1999). Preferential role for NF-kappa B/Rel signaling in the type 1 but not type 2 T cell-dependent immune response in vivo. J Immunol *163*, 5116-5124.

Bakker, R.A., Schoonus, S.B., Smit, M.J., Timmerman, H., and Leurs, R. (2001). Histamine H(1)-receptor activation of nuclear factor-kappa B: roles for G beta gamma- and G alpha(q/11)-subunis in constitutive and agonist-mediated signaling. Mol Pharmacol *60*, 1133-1142.

Banu, Y., and Watanabe, T. (1999). Augmentation of antigen receptor-mediated responses by histamine H1 receptor signaling. J Exp Med *189*, 673-682.
Baron, J.L., Madri, J.A., Ruddle, N.H., Hashim, G., and Janeway, C.A., Jr. (1993). Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. J Exp Med *177*, 57-68.

Bebo, B.F., Jr., Yong, T., Orr, E.L., and Linthicum, D.S. (1996). Hypothesis: a possible role for mast cells and their inflammatory mediators in the pathogenesis of autoimmune encephalomyelitis. J Neurosci Res *45*, 340-348.

Berenson, L.S., Yang, J., Sleckman, B.P., Murphy, T.L., and Murphy, K.M. (2006). Selective requirement of p38alpha MAPK in cytokine-dependent, but not antigen receptor-dependent, Th1 responses. J Immunol *176*, 4616-4621.

Bryce, P.J., Mathias, C.B., Harrison, K.L., Watanabe, T., Geha, R.S., and Oettgen, H.C. (2006). The H1 histamine receptor regulates allergic lung responses. J Clin Invest *116*, 1624-1632.

Butterfield, R.J., Blankenhorn, E.P., Roper, R.J., Zachary, J.F., Doerge, R.W., and Teuscher, C. (2000). Identification of genetic loci controlling the characteristics and severity of brain and spinal cord lesions in experimental allergic encephalomyelitis. Am J Pathol *157*, 637-645.

Butterfield, R.J., Sudweeks, J.D., Blankenhorn, E.P., Korngold, R., Marini,

J.C., Todd, J.A., Roper, R.J., and Teuscher, C. (1998). New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. J Immunol *161*, 1860-1867.

Caron, G., Delneste, Y., Roelandts, E., Duez, C., Bonnefoy, J.Y., Pestel, J., and Jeannin, P. (2001a). Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. J Immunol *167*, 3682-3686.

Caron, G., Delneste, Y., Roelandts, E., Duez, C., Herbault, N., Magistrelli, G., Bonnefoy, J.Y., Pestel, J., and Jeannin, P. (2001b). Histamine induces CD86 expression and chemokine production by human immature dendritic cells. J Immunol *166*, 6000-6006.

Cua, D.J., Sherlock, J., Chen, Y., Murphy, C.A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T.*, et al.* (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature *421*, 744-748.

Dodeller, F., and Schulze-Koops, H. (2006). The p38 mitogen-activated protein kinase signaling cascade in CD4 T cells. Arthritis Res Ther *8*, 205.

Farley, N., Pedraza-Alva, G., Serrano-Gomez, D., Nagaleekar, V., Aronshtam, A., Krahl, T., Thornton, T., and Rincon, M. (2006). p38 mitogen-activated protein kinase mediates the Fas-induced mitochondrial death pathway in CD8+ T cells. Mol Cell Biol *26*, 2118-2129.

Ferber, I.A., Brocke, S., Taylor-Edwards, C., Ridgway, W., Dinisco, C., Steinman, L., Dalton, D., and Fathman, C.G. (1996). Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). J Immunol *156*, 5-7.

Fujimoto, K., Ohta, K., Kangawa, K., Kikkawa, U., Ogino, S., and Fukui, H. (1999). Identification of protein kinase C phosphorylation sites involved in phorbol ester-induced desensitization of the histamine H1 receptor. Mol Pharmacol *55*, 735-742.

Fujinami, R.S., von Herrath, M.G., Christen, U., and Whitton, J.L. (2006). Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. Clin Microbiol Rev *19*, 80-94.

Gao, J.F., Call, S.B., Fillmore, P.D., Watanabe, T., Meeker, N.D., and Teuscher, C. (2003). Analysis of the role of Bphs/Hrh1 in the genetic control of responsiveness to pertussis toxin. Infect Immun *71*, 1281-1287.

Hill, S.J., Ganellin, C.R., Timmerman, H., Schwartz, J.C., Shankley, N.P., Young, J.M., Schunack, W., Levi, R., and Haas, H.L. (1997). International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev *49*, 253-278.

Jones, D.C., Ding, X., Zhang, T.Y., and Daynes, R.A. (2003). Peroxisome proliferator-

activated receptor alpha negatively regulates T-bet transcription through suppression of p38 mitogen-activated protein kinase activation. J Immunol *171*, 196-203.

Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, O.A., Malolepszy, J., Zak-Nejmark, T., Koga, R., Kobayashi, T., Blaser, K., and Akdis, C.A. (2001). Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature *413*, 420-425.

Koch, A., Raidl, M., Lux, M., Muller, K., Buning, H., Humme, S., and Erdmann, E. (2006). IL-12-induced T-bet expression and IFNgamma release in lymphocytes from asthmatics-Role of MAPkinases ERK-1/-2, p38(MAPK) and effect of dexamethasone. Respir Med.

Kyriakis, J.M., and Avruch, J. (2001). Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev *81*, 807-869.

Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., McClanahan, T., Kastelein, R.A., and Cua, D.J. (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med *201*, 233-240.

Leurs, R., Smit, M.J., and Timmerman, H. (1995). Molecular pharmacological aspects of histamine receptors. Pharmacol Ther *66*, 413-463.
Lipnik-Stangelj, M., and Carman-Krzan, M. (2004). Histamine-stimulated nerve growth factor secretion from cultured astrocytes is blocked by protein kinase C inhibitors. Inflamm Res *53 Suppl 1*, S57-58.

Liu, Z., Kharmate, G., Patterson, E., and Khan, M.M. (2006). Role of H(1) receptors in histamine-mediated up-regulation of STAT4 phosphorylation. Int Immunopharmacol *6*, 485-493.

Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., *et al.* (2002). Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. Nat Med *8*, 500-508.

Logothetis, L., Mylonas, I.A., Baloyannis, S., Pashalidou, M., Orologas, A., Zafeiropoulos, A., Kosta, V., and Theoharides, T.C. (2005). A pilot, open label, clinical trial using hydroxyzine in multiple sclerosis. Int J Immunopathol Pharmacol *18*, 771-778.

Ma, R.Z., Gao, J., Meeker, N.D., Fillmore, P.D., Tung, K.S., Watanabe, T., Zachary, J.F., Offner, H., Blankenhorn, E.P., and Teuscher, C. (2002). Identification of Bphs, an autoimmune disease locus, as histamine receptor H1. Science *297*, 620-623.

McCreath, G., Hall, I.P., and Hill, S.J. (1994). Agonist-induced desensitization of histamine H1 receptor-mediated inositol phospholipid hydrolysis in human umbilical

vein endothelial cells. Br J Pharmacol *113*, 823-830.

Megson, A.C., Walker, E.M., and Hill, S.J. (2001). Role of protein kinase Calpha in signaling from the histamine H(1) receptor to the nucleus. Mol Pharmacol *59*, 1012-1021.

Miyoshi, K., Das, A.K., Fujimoto, K., Horio, S., and Fukui, H. (2006). Recent advances in molecular pharmacology of the histamine systems: regulation of histamine H1 receptor signaling by changing its expression level. J Pharmacol Sci *101*, 3-6.

Miyoshi, K., Kawakami, N., Horio, S., and Fukui, H. (2004). Homologous and heterologous phosphorylations of human histamine H1 receptor in intact cells. J Pharmacol Sci *96*, 474-482.

Musio, S., Gallo, B., Scabeni, S., Lapilla, M., Poliani, P.L., Matarese, G., Ohtsu, H., Galli, S.J., Mantegazza, R., Steinman, L., and Pedotti, R. (2006). A key regulatory role for histamine in experimental autoimmune encephalomyelitis: disease exacerbation in histidine decarboxylase-deficient mice. J Immunol *176*, 17-26.

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol *6*, 1133-1141.

Parsons, M.E., and Ganellin, C.R. (2006). Histamine and its receptors. Br J Pharmacol *147 Suppl 1*, S127-135.

Pype, J.L., Dupont, L.J., Mak, J.C., Barnes, P.J., and Verleden, G.M. (1998a). Regulation of H1-receptor coupling and H1-receptor mRNA by histamine in bovine tracheal smooth muscle. Br J Pharmacol *123*, 984-990.

Pype, J.L., Mak, J.C., Dupont, L.J., Verleden, G.M., and Barnes, P.J. (1998b). Desensitization of the histamine H1-receptor and transcriptional down-regulation of histamine H1-receptor gene expression in bovine tracheal smooth muscle. Br J Pharmacol *125*, 1477-1484.

Ramana, C.V., Gil, M.P., Schreiber, R.D., and Stark, G.R. (2002). Stat1-dependent and -independent pathways in IFN-gamma-dependent signaling. Trends Immunol *23*, 96-101.

Rincon, M., Enslen, H., Raingeaud, J., Recht, M., Zapton, T., Su, M.S., Penix, L.A., Davis, R.J., and Flavell, R.A. (1998). Interferon-gamma expression by Th1 effector T cells mediated by the p38 MAP kinase signaling pathway. Embo J *17*, 2817-2829.

Robinson, A.J., and Dickenson, J.M. (2001). Activation of the p38 and p42/p44 mitogen-activated protein kinase families by the histamine H(1) receptor in DDT(1)MF-2 cells. Br J Pharmacol *133*, 1378-1386.

Robinson, D.S., and O'Garra, A. (2002). Further checkpoints in Th1 development.

Immunity *16*, 755-758.

Sachs, B., Hertl, M., and Merk, H.F. (2000). Histamine receptors on lymphocytes: distribution and functional significance. Skin Pharmacol Appl Skin Physiol *13*, 313-323.

Sakhalkar, S.P., Patterson, E.B., and Khan, M.M. (2005). Involvement of histamine H1 and H2 receptors in the regulation of STAT-1 phosphorylation: inverse agonism exhibited by the receptor antagonists. Int Immunopharmacol *5*, 1299-1309.

Salvador, J.M., Mittelstadt, P.R., Guszczynski, T., Copeland, T.D., Yamaguchi, H., Appella, E., Fornace, A.J., Jr., and Ashwell, J.D. (2005). Alternative p38 activation pathway mediated by T cell receptor-proximal tyrosine kinases. Nat Immunol *6*, 390-395.

Shaw, M.K., Lorens, J.B., Dhawan, A., DalCanto, R., Tse, H.Y., Tran, A.B., Bonpane, C., Eswaran, S.L., Brocke, S., Sarvetnick, N., *et al.* (1997). Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis. J Exp Med *185*, 1711-1714.

Simons, F.E. (2004). Advances in H1-antihistamines. N Engl J Med *351*, 2203-2217.

Smit, M.J., Timmerman, H., Hijzelendoorn, J.C., Fukui, H., and Leurs, R. (1996). Regulation of the human histamine H1 receptor stably expressed in Chinese hamster ovary cells. Br J Pharmacol *117*, 1071-1080.

Stanners, J., Kabouridis, P.S., McGuire, K.L., and Tsoukas, C.D. (1995). Interaction between G proteins and tyrosine kinases upon T cell receptor.CD3-mediated signaling. J Biol Chem *270*, 30635-30642.

Steffel, J., Akhmedov, A., Greutert, H., Luscher, T.F., and Tanner, F.C. (2005). Histamine induces tissue factor expression: implications for acute coronary syndromes. Circulation *112*, 341-349.

Suryani, S., and Sutton, I. (2007). An interferon-gamma-producing Th1 subset is the major source of IL-17 in experimental autoimmune encephalitis. J Neuroimmunol *183*, 96-103.

Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., Fathman, C.G., and Glimcher, L.H. (2000). A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell *100*, 655-669.

Takekawa, M., and Saito, H. (1998). A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. Cell *95*, 521-530.

Teuscher, C., Noubade, R., Spach, K., McElvany, B., Bunn, J.Y., Fillmore, P.D., Zachary, J.F., and Blankenhorn, E.P. (2006). Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A *103*, 8024-8029.

Teuscher, C., Poynter, M.E., Offner, H., Zamora, A., Watanabe, T., Fillmore, P.D., Zachary, J.F., and Blankenhorn, E.P. (2004). Attenuation of Th1 effector cell responses and susceptibility to experimental allergic encephalomyelitis in histamine H2 receptor knockout mice is due to dysregulation of cytokine production by antigen-presenting cells. Am J Pathol *164*, 883-892.

Weiss, L., Whitmarsh, A.J., Yang, D.D., Rincon, M., Davis, R.J., and Flavell, R.A. (2000). Regulation of c-Jun NH(2)-terminal kinase (Jnk) gene expression during T cell activation. J Exp Med *191*, 139-146.

Wildin, R.S., Garvin, A.M., Pawar, S., Lewis, D.B., Abraham, K.M., Forbush, K.A., Ziegler, S.F., Allen, J.M., and Perlmutter, R.M. (1991). Developmental regulation of lck gene expression in T lymphocytes. J Exp Med *173*, 383-393.

Willenborg, D.O., Fordham, S., Bernard, C.C., Cowden, W.B., and Ramshaw, I.A. (1996). IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. J Immunol *157*, 3223-3227.

Yang, T.T., Ung, P.M., Rincon, M., and Chow, C.W. (2006). Role of the CCAAT/enhancer-binding protein NFATc2 transcription factor cascade in the induction of secretory phospholipase A2. J Biol Chem *281*, 11541-11552.

Yoshimura, S., Mishima, R., Miyoshi, K., Fujimoto, K., Murata, Y., Kitamura, Y., Takeda, N., and Fukui, H. (2005). Histamine H1 receptor-mediated histamine H1 receptor gene expression. Inflamm Res *54 Suppl 1*, S42-43.

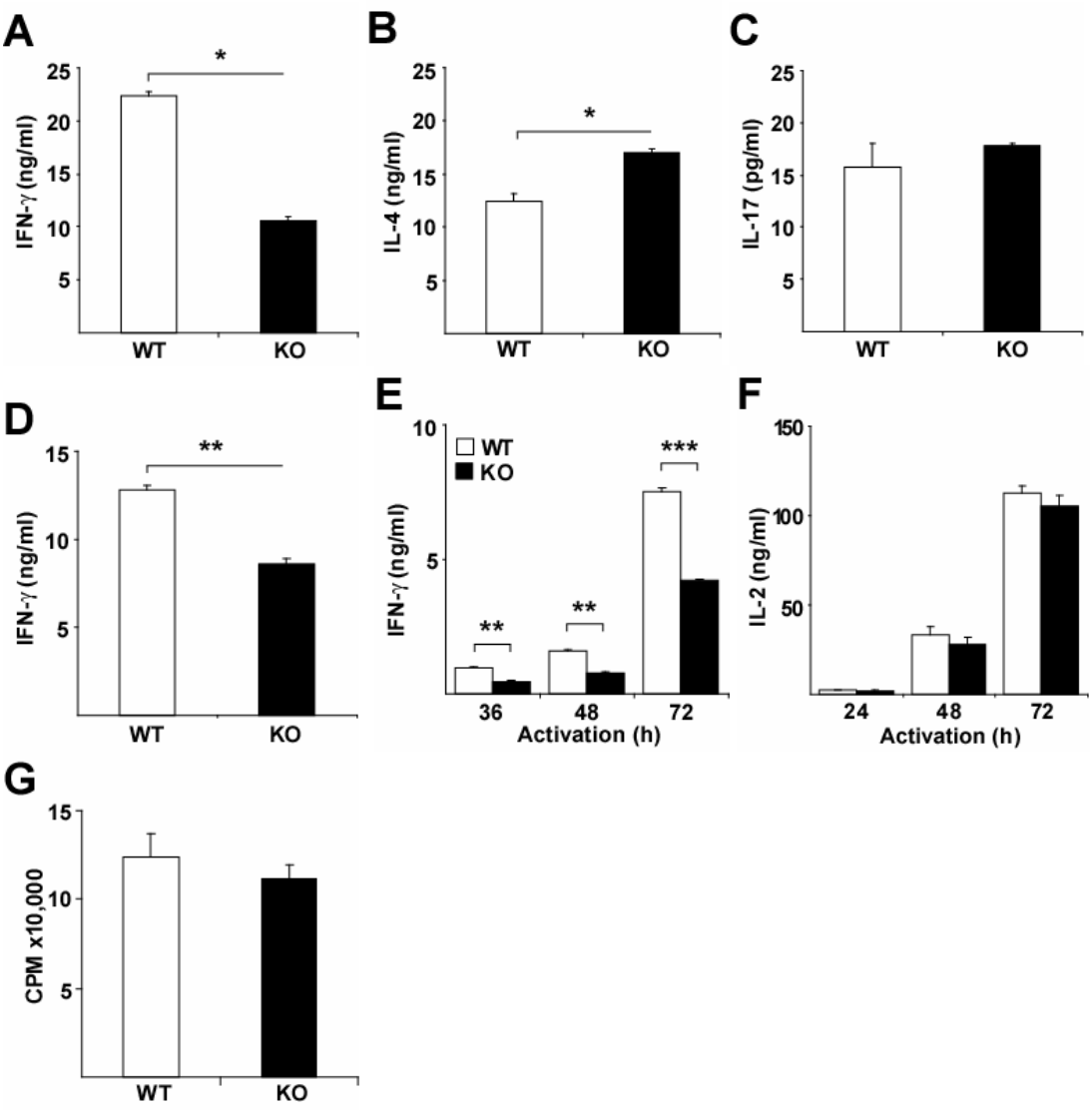


Figure 1. H_1R is required for IFNγ production by CD4 T cells.

Purified CD4 T cells from WT and H1RKO mice were activated with anti-CD3 (5μg/ml) and anti-CD28 (1μg/ml) mAbs either in presence of IL-12 (4ng/ml) and anti-IL-4 mAb (10μg/ml) (**A**), or IL-4 (30ng/ml) and anti-IFNγ mAb (10μg/ml) (**B**) or TGF-β (1ng/ml), IL-6 (30ng/ml) and anti-IFNγ (10μg/ml) and anti-IL4 mAbs (10μg/ml) (**C**). After 4 days, cells were restimulated with anti-CD3 mAb (5μg/ml) for 24h. IFNγ (**A**), IL-4 (**B**) or IL-17 (**C**) production was determined by ELISA in triplicate. *, $p < 0.05$, compared with H1RKO cells (Student's t-test). (**D**) CD4 T cells were activated with anti-CD3 (5μg/ml) and anti-CD28 (1μg/ml) mAbs. After 4 days, cells were restimulated with anti-CD3 mAb (5μg/ml) for 24h and IFNγ production was determined by ELISA.**, $p = 0.002$ compared with H1RKO cells (Student's t-test). (**E** and **F**) CD4 T cells were stimulated as in (**D**) for the indicated periods of time. Supernatants were analyzed for IFNγ (**E**) and IL-2 (**F**) by ELISA. Significance of differences in cytokine production were assessed by two-way ANOVA ($F = 168.8$; $p < 0.0001$) followed by post-hoc comparisons using one-way ANOVA (** $p < 0.01$; *** $p < 0.001$). (**G**) CD4 T cells from WT and H1RKO mice were stimulated as in (**E**) and 18h ^{3}[H]-thymidine incorporation measured in total 72h culture. All the data are representative of at least two independent experiments.

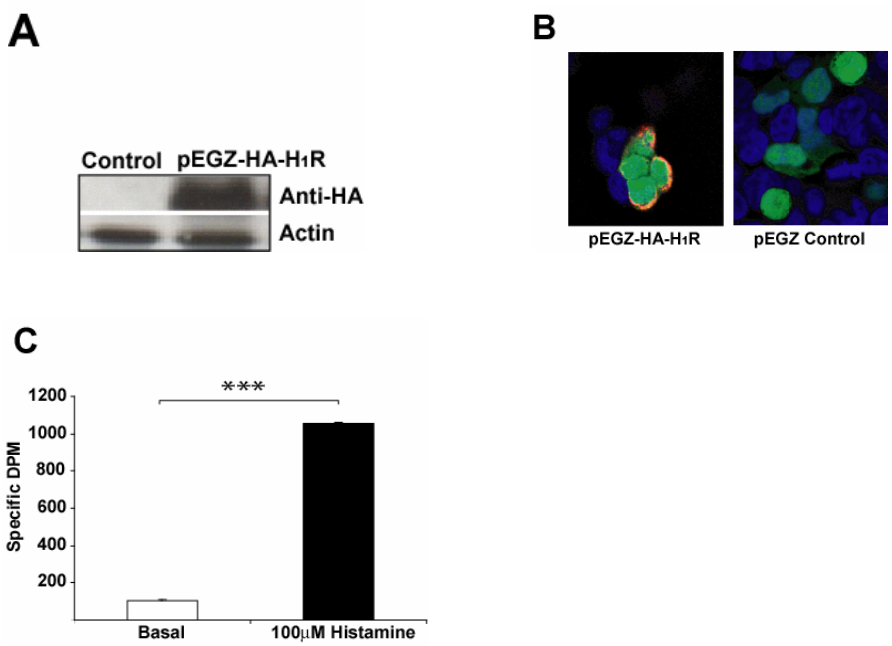


Figure 2. Expression and function of HA-H_1R in HEK293T cells.

(**A**) HEK293T cells were transfected with empty pEGZ (control) and pEGZ-HA-H_1R plasmids and the expression of HA-H_1R was determined by Western blot using an anti-HA mAb. The data is representative of at least three independent experiments. (**B**) HEK293T cells were transfected as in (**A**), fixed, permeabilized and stained with an anti-HA mAb (red) and Topro-nuclear dye (blue). EGFP expression (green) represents transfected cells. Cells were visualized by confocal microscopy. The data are representative of at least three independent experiments. (**C**) HEK293 cells were transfected with pHA-H_1R-$G_{\alpha 11}$ fusion construct, membrane fractions generated and were used in absence (basal) or presence of 10^{-4}M histamine in [^{35}S] GTPγS binding assay. Samples were then used in immuno-precipitation using $G_{\alpha 11}$ antiserum and the bound [^{35}S] GTPγS was measured by liquid-scintillation spectrometry.

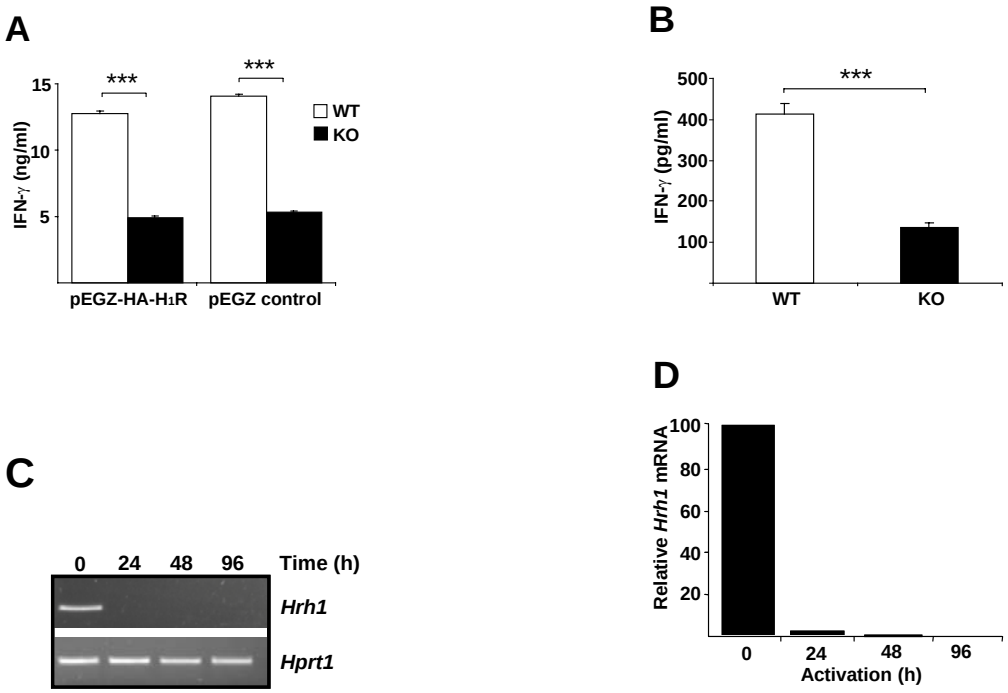


Figure 3. H_1R expression is downregulated upon activation in CD4 T cells.

(**A**) CD4 T cells from WT and H1RKO mice were stimulated in the presence of anti-CD3 and anti-CD28 mAbs for 16h and then retrovirally transduced with pEGZ-HA-H_1R or with empty pEGZ control plasmids. Transduced, sorted EGFP+ cells were then re-stimulated with anti-CD3 mAb and 24h later the supernatants were harvested for determination of IFNγ by ELISA in triplicate. The data presented is representative of two independent experiments. ***, $p < 0.0001$ compared with H1RKO cells (Student's t-test). (**B**) Freshly isolated CD4 T cells from WT and H1RKO were activated with anti-CD3 and anti-CD28 mAbs. After 24h, IFNγ production was determined by ELISA. The results shown are representative of at least three independent experiments. ***, $p < 0.001$ compared with H1RKO cells (Student's t-test). (**C** and **D**) CD4 T cells were isolated from WT mice and stimulated with anti-CD3 and anti-CD28 mAbs. Cells were harvested at the indicated time point, total RNA was isolated and used to examine H_1R expression by conventional RT-PCR with HPRT as the endogenous control (**C**) and by quantitative real time RT-PCR relative to β2-microglobulin as the endogenous control (**D**). Data presented as expression relative to the unstimulated CD4 T cells. The data are representative of at least three independent experiments.

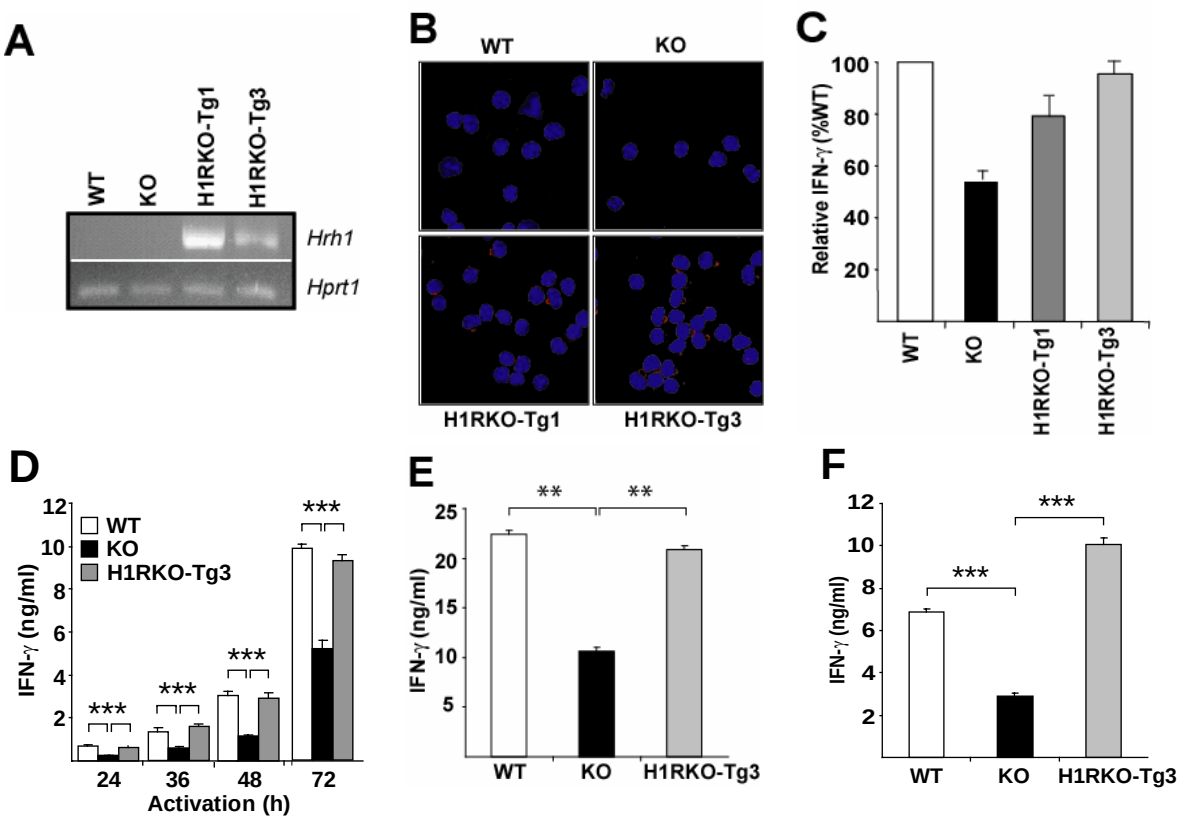


Figure 4. Transgenic expression of H_1R in H1RKO CD4 T cells complements IFN-γ production.

(**A**) H_1R transgene expression was analyzed by RT-PCR in CD4 T cells from WT, H1RKO mice and the two independent lines of H_1R transgenic mice crossed with H1RKO mice (H1RKO-Tg-1 and H1RKO-Tg-3). (**B**) CD4 T cells were stained with anti-HA mAb (red) and visualized by confocal microscopy. Nuclear stain Topro (blue) is shown. (**C**) CD4 T cells were activated with anti-CD3 and anti-CD28 mAbs for 72 h and IFNγ was determined by ELISA. Data are expressed as IFNγ production relative to that by WT cells (set as 100%). (**D**) CD4 T cells from WT, H1RKO and H1RKO-Tg-3 were stimulated as in (**C**) for the indicated periods of time and IFNγ was determined by ELISA. Statistical analysis using two-way ANOVA ($F=55.1$; $p<0.0001$) followed by post-hoc comparisons using one-way ANOVA was performed (**, $p<0.01$; ***, $p<0.001$). (**E**) CD4 T cells were activated with anti-CD3 and anti-CD28 mAbs in presence of IL-12 (4ng/ml) and anti-IL-4 mAb (10μg/ml). After 4 days, cells were restimulated and IFNγ production was determined. Statistical analysis using one-way ANOVA ($F=25.4$; $p<0.001$) followed by Bonferroni's post-hoc comparisons with H1RKO cells was performed (**, $p<0.01$). (**F**) CD4 T cells were activated with anti-CD3 and anti-CD28 mAbs. After 4 days, cells were restimulated with anti-CD3 mAb for 24h and IFNγ production was determined by ELISA. Significance of differences was determined as in (**E**) (***, $p < 0.001$). The data presented are representative of at least three independent experiments.

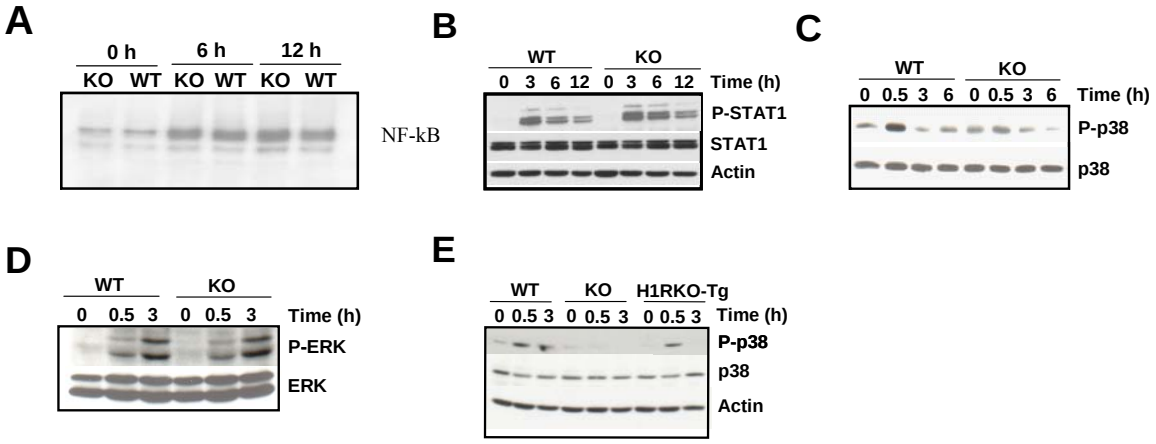
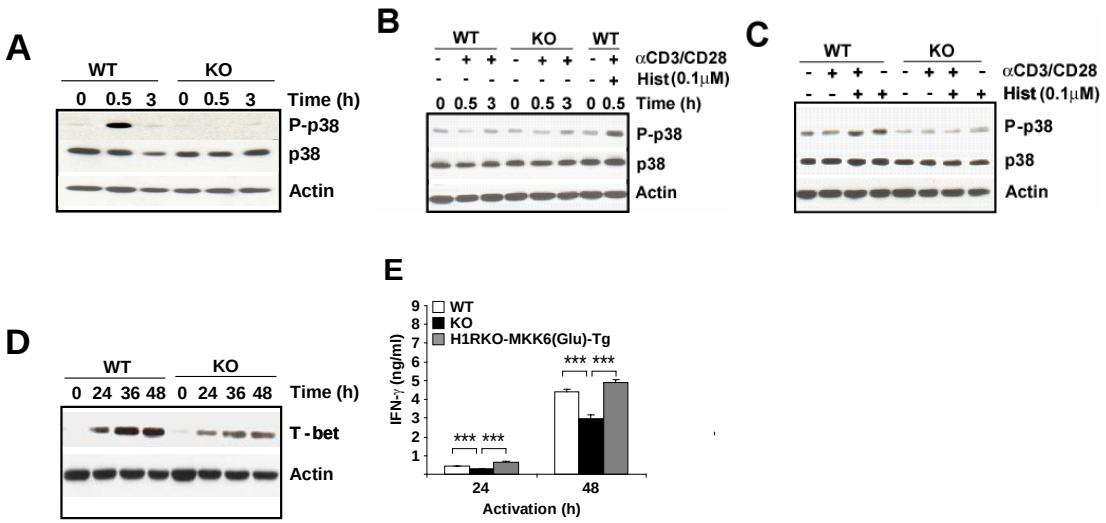


Figure 5. Activation of p38 MAP kinase by TCR ligation requires H_1R signals.

(**A**) Purified CD4 T cells from WT and H1RKO mice were stimulated with anti-CD3 and anti-CD28 mAbs for the indicated periods of time, nuclear extracts were prepared and analyzed for NF-κB DNA binding by EMSA. (**B**) CD4 T cells from WT and H1RKO mice were stimulated with anti-CD3 and anti-CD28 mAbs for the indicated periods of time, whole cell lysates were prepared and analyzed for phospho-STAT1 (P-STAT1) and total STAT1 by Western blot analysis. Actin was used as loading control. (**C**) CD4 T cells from WT and H1RKO were treated with anti-CD3 and anti-CD28 mAbs for the indicated periods of time, whole cell lysates were prepared and analyzed for phospho-p38 MAP kinase and total p38 by Western blot analysis. (**D**) CD4 T cells from WT and H1RKO were activated with anti-CD3 and anti-CD28 mAbs for the indicated periods of time, whole cell lysates were prepared and analyzed for phospho-ERK and total ERK by Western blot analysis. (**E**) CD4 T cells from WT, H1RKO and H1RKO-Tg-3 mice were stimulated with anti-CD3 and anti-CD28 mAbs for the indicated periods of time and whole cell lysates were analyzed for phospho-p38, total p38 and actin by Western blotting. All the results presented are representative of at least two independent experiments.

Figure 6. Activation of p38 MAP kinase by TCR ligation is mediated by histamine/H_1R binding.



(**A**) CD4 T cells from WT and H1RKO mice were treated with histamine (10^{-7} M) for the indicated periods of time in the histamine-free medium. Whole cell extracts were used to analyze phospho-p38, total p38 and actin by Western blotting. (**B**) CD4 T cells were isolated from WT and H1RKO mice and stimulated with anti-CD3 and anti-CD28 mAbs in the histamine free-medium for the indicated periods of time. CD4 T cells stimulated in 10^{-7}M histamine (Hist) containing medium are shown as positive control for p38 MAP kinase activation. Phospho-p38, total p38 and actin are shown. (**C**) CD4 T cells from WT and H1RKO were incubated with anti-CD3 and anti-CD28 mAbs, 10^{-7}M histamine or both in the histamine-free medium for 30 minutes and whole cell lysates were analyzed for phospho-p38, total p-38 and actin by Western blotting. (**D**) CD4 T cells from WT and H1RKO mice were stimulated with anti-CD3 and anti-CD28 mAbs for the indicated periods of time and whole cell lysates were analyzed for T-bet expression by Western blot. Actin is shown as loading control. (**E**) Purified CD4 T cells from WT, H1RKO and H1RKO-MKK6$_{Glu}$ transgenic mice were stimulated with anti-CD3 and anti-CD28 mAbs for the indicated periods of time and supernatants were analyzed for IFNγ production by ELISA in triplicate. Significance of differences were determined by Student's t-test (*** $p < 0.001$). All the data are representative of at least two independent experiments.

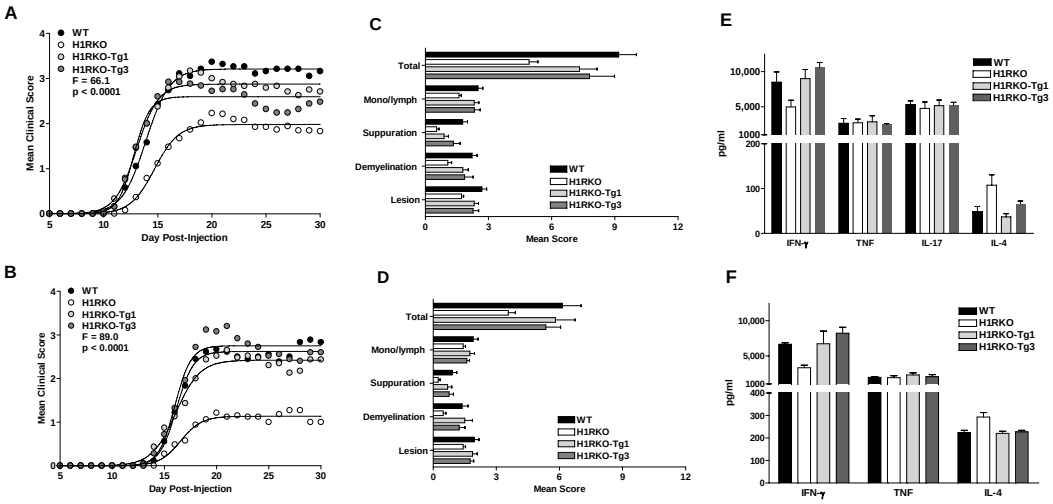


Figure 7. H_1R signaling directly in CD4 T cells regulates encephalitogenic Th1 effector responses.

Clinical EAE course, severity of CNS pathology and *ex-vivo* cytokine responses of WT, H1RKO and H1RKO-Tg mice were compared following immunization with MOG_{35-55}+CFA+PTX (**A, C** and **E**) and 2× MOG_{35-55}+CFA (**B, D** and **F**). Cytokine production was assessed by stimulating splenocytes with MOG_{35-55} on D10 post-injection, supernatants collected and quantified by ELISA in triplicate. The significance of differences in the course of clinical disease, clinical disease traits, CNS pathology indices (PI) and cytokine responses were assessed by regression analysis (63), Chi-square test, or ANOVA followed by post hoc multiple comparisons. With the exception of disease incidence, and TNF-α and IL-17 production, significant differences among the strains were detected for all parameters at $p < 0.0001$ with C57BL/6J = H1RKO Tg-1 = H1RKO Tg-3 > H1RKO.

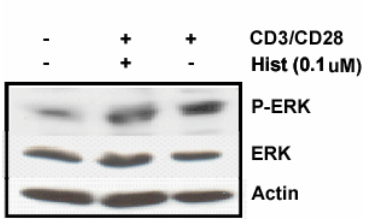


Figure 8. Absence of histamine does not affect TCR-mediated ERK activation.

WT CD4 T cells were activated with anti-CD3 and anti-CD28 mAbs for 30 min in presence or absence of 10^{-7}M histamine and whole cell lysates were analyzed for phospho-ERK, total ERK and actin by Western blotting.

Strain	Incidence	Day of onset	Cumulative disease score	Days affected	Severity index	Peak score
C57BL/6J	19/19	13.1±0.3	56.2±4.6	18.0±0.3	3.1±0.2	3.9±0.3
H1RKO	55/56	15.7±0.4	32.1±1.4	15.0±0.4	2.1±0.1	3.0±0.1
Tg-1	24/24	12.9±0.4	50.0±3.7	17.8±0.5	2.8±0.2	3.6±0.2
Tg-3	23/25	12.1±0.1	50.0±3.2	18.7±0.2	2.7±0.2	3.6±0.2
	$\chi 2 = 4.5$ $p = 0.2$	$F = 20.6$ $p < 0.0001$	18.1 < 0.0001	32.5 < 0.0001	11.7 < 0.0001	8.1 < 0.0001
			C57BL/6J = H1RKO-Tg-1 = H1RKO-Tg-3 > H1RKO			

Table 1: Clinical disease parameters in MOG_{35-55}+CFA+PTX immunized mice

Strain	Incidence	Day of onset	Cumulative disease score	Days affected	Severity index	Peak score
C57BL/6J	18/18	16.6±0.7	37.6±2.9	14.2±0.7	2.6±0.1	3.2±0.2
H1RKO	26/33	17.1±0.5	20.0±1.8	9.8±0.9	1.6±0.1	2.2±0.1
Tg-1	22/23	16.2±0.6	36.4±3.8	13.3±0.8	2.5±0.2	3.2±0.2
Tg-3	23/25	15.7±0.4	46.1±4.0	14.0±0.9	2.7±0.2	3.6±0.2
	$\chi 2 = 7.5$ $p = 0.06$	$F = 1.5$ $p = 0.051$	9.5 < 0.0001	5.1 0.0025	11.2 < 0.0001	12.2 < 0.0001
	C57BL/6J = H1RKO-Tg-1 = H1RKO-Tg-3 > H1RKO					

Table 2: Clinical disease parameters in 2x (MOG_{35-55}+CFA) immunized mice

CHAPTER 3: POLYMORPHISMS IN MURINE HISTAMINE RECEPTOR H_1 LEAD TO DIFFERENTIAL CELL SURFACE EXPRESSION AND INFLUENCE AUTOIMMUNE DISEASE PROGRESSION

Rajkumar Noubade[1], Naresha Saligrama[1], Karen Spach[1], Roxana del Rio[1], Graeme Milligan[2], Mercedes Rincon[1] and Cory Teuscher[1]

[1]Departments of Medicine and Pathology, University of Vermont, Burlington, VT 05405;

[2]Molecular Pharmacology Group, Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow G12 8QQ Scotland, UK

Address correspondence to: Dr. Cory Teuscher
Immunobiology Program
C317 Given Medical Building
University of Vermont
Burlington, VT 05405
(802) 656-3270
C.Teuscher@uvm.edu

Text pages: 27
Figures: 6
Tables: 1

Running title: H_1R polymorphisms influence EAE

Key words: Histamine receptor H_1, polymorphisms, EAE/MS, GPCR, trafficking, Autoimmunity

Acknowledgments: Supported by the National Institutes of Health Grants AI45666, AI051454 and NS36526.

ABSTRACT

Structural polymorphisms (L263P, M313V and S331P) in the third intracellular loop of the murine histamine receptor H_1 (H_1R) are candidates for *Bphs*, a shared autoimmune disease locus in experimental allergic encephalomyelitis (EAE) and experimental allergic orchitis. The P-V-P haplotype is associated with increased disease susceptibility (H_1R^S) whereas the L-M-S haplotype is associated with less severe disease (H_1R^R). Here we show that selective reexpression of the H_1R^S allele in T cells fully complements EAE susceptibility and the production of disease associated cytokines while selective reexpression of the H_1R^R allele does not. Mechanistically, we show that the two H_1R alleles exhibit differential cell surface expression and altered intracellular trafficking, with the H_1R^R allele being retained within the endoplasmic reticulum (ER). Moreover, we show that all three residues (L-M-S) comprising the H_1R^R haplotype are required for altered expression. These data are the first to demonstrate that structural polymorphisms influencing cell surface expression of a G-protein coupled receptor in T cells regulates immune functions and autoimmune disease susceptibility.

INTRODUCTION

Multiple sclerosis (MS) is the major demyelinating disease of the central nervous system (CNS) in humans, affecting more than 2.5 million people worldwide (Greenstein, 2007). Both environmental and genetic factors contribute to the immunopathologic etiology of the disease. A genetic component in disease susceptibility is supported by the 20-30% concordance rate among monozygotic twins and 3-5% for dizygotic twins. Compared to the general population, MS is 20-40 times more common in first degree relatives and there is no excess risk in adopted relatives of patients with MS (Hafler et al., 2005). Evidence of an environmental etiology in MS comes primarily from migration studies and geographic distribution data. Migration studies indicate that individuals moving from high-risk areas before puberty tend to adopt the lower risk of the native population and vice versa (Kantarci and Wingerchuk, 2006). Thus, susceptibility to MS is likely the result of environmental triggers acting on a susceptible genetic background at the population level.

Experimental allergic encephalomyelitis (EAE), the primary animal model of MS, is also a genetically determined inflammatory disease of the CNS (Gold et al., 2000). EAE can be actively induced in genetically susceptible animals by immunization with either whole spinal cord homogenate or encephalitogenic proteins/peptides and adjuvants (Kuchroo et al., 2002). EAE, like MS, is a complex polygenic disease (Andersson and Karlsson, 2004), with multiple genes exerting a modest effect, thus making it difficult to study the contribution of individual loci to overall disease pathogenesis. However, reduction of complex disease states into intermediate or subphenotypes that are

under the control of a single locus has the potential to facilitate mechanistic studies and gene identification (Andersson and Karlsson, 2004). One such phenotype associated with EAE is *Bordetella pertussis* toxin-induced histamine sensitization, which is controlled by the single autosomal dominant locus known as *Bphs* (Ma et al., 2002). Previously, we identified *Hrh1*/H_1R as the gene underlying *Bphs* (Ma et al., 2002) and as a shared autoimmune disease susceptibility gene in EAE (Linthicum and Frelinger, 1982) and experimental allergic orchitis (EAO) (Teuscher, 1985). H_1R is a seven-transmembrane spanning, G protein coupled receptor (GPCR). Generally, ligation of H_1R with histamine is believed to couple to second messenger signaling pathways via the activation of the heterotrimeric $G\alpha_{q/11}$ family of G proteins and leads to a variety of signaling cascades depending on the cell type involved (Parsons and Ganellin, 2006).

Compared to wild-type (WT) mice, H_1R deficient (H1RKO) mice exhibit significantly reduced EAE susceptibility (Ma et al., 2002). As a disease susceptibility gene, *Hrh1*/H_1R can exert its effect in multiple cell types involved in the disease process including endothelial cells, antigen presenting cells and T cells. Moreover, H_1R may function at critical check points during both the induction and effector phases of the disease. In this regard, we recently demonstrated that selective reexpression of the H_1R^S allele in T cells is sufficient to complement EAE in H1RKO mice and that H_1R signals are important during priming of naïve T cells rather than during the effector phase of the disease (Noubade et al., 2007).

Hrh1/H_1R-susceptible ($Hrh1^S$/H_1R^S) and –resistant ($Hrh1^R$/H_1R^R) alleles differ by three amino acids in their coding sequences (Ma et al., 2002). The H_1R^R haplotype possesses a L^{263}, M^{313} and S^{331} whereas the H_1R^S haplotype is characterized by P^{263},

V^{313} and P^{331} (Ma et al., 2002). The mechanism whereby these polymorphic residues influence EAE susceptibility is unknown but it was hypothesized to be the result of differential coupling to second messenger signaling pathways, because the three residues reside within the third intracytoplasmic domain associated with $G\alpha_{q/11}$ activation (Tan et al., 2004). Here we show that, unlike the H_1R^S allele (Noubade et al., 2007), expression of the H_1R^R allele in T cells does not complement EAE in H1RKO mice and that the polymorphic residues of the H_1R^R allele affect intracellular trafficking and retention in the ER rather than the inherent capacity to signal. Moreover, we show that all three residues (L-M-S) comprising the H_1R^R haplotype are required for altered cell surface expression. These data are the first to demonstrate that structural polymorphisms influencing differential cell surface expression of a GPCR in T cells can regulate immune functions and susceptibility to autoimmune disease.

RESULTS

Expression of H_1R^R does not complement EAE in H_1R deficient mice

Using transgenic complementation, we recently showed that expression of the H_1R^S allele only in T cells of H1RKO mice was sufficient to restore EAE severity to WT levels in these mice (Noubade et al., 2007). To understand if the H_1R^R allele would also complement EAE in H1RKO mice, we generated transgenic mice expressing the N-terminus hemagglutinin (HA)-tagged H_1R^R allele under the control of the distal *lck* promoter, which drives expression in peripheral T cells (Wildin et al., 1991). The transgenic founders were generated directly on the C57BL/6J background and were crossed to H1RKO mice to obtain H1RKO mice expressing the H_1R^R allele selectively in T cells. The expression of the transgene in CD4 T cells was assessed by RT-PCR using transgene-specific primers (Fig. 1A) and by real time RT-PCR using primers that recognize H_1R (Fig. 1B). The two established lines of H_1R^R (H1RKO-Tg^R1 and H1RKO-Tg^R2) expressed the transgene mRNA at levels comparable to one of the H_1R^S allele transgenic mice (H1RKO-Tg^S) that we reported previously (Noubade et al., 2007).

We then examined the susceptibility of these transgenic mice to myelin oligodendrocyte glycoprotein peptide 35-55 (MOG_{35-55}) induced EAE. We used two protocols to induce disease, one using MOG_{35-55} plus complete Freund's adjuvant (CFA) and pertussis toxin (PTX) (MOG_{35-55}-CFA plus PTX) (Fig. 1C) and the other using two injections of MOG_{35-55} plus CFA (2× MOG_{35-55}-CFA) (Fig. 1D), which does not use PTX as an ancillary adjuvant. Regression analysis revealed that the clinical disease courses elicited by both induction protocols fit a Sigmoidal curve and that the clinical

course of disease in two independent lines of H1RKO–Tg^R mice was not different from that in H1RKO mice. However, as reported previously (Noubade et al., 2007), the clinical course of EAE in H1RKO-Tg^S mice was significantly more severe than that of H1RKO mice and was equivalent to the disease course observed in WT mice. These results indicate that, unlike the H_1R^S allele, expression of the H_1R^R allele by H1RKO T cells does not complement EAE susceptibility.

An analysis of EAE-associated clinical quantitative trait variables from the two transgenic cohorts revealed that the mean day of onset (DO), cumulative disease score (CDS), overall severity index (SI) and the peak score (PS) were significantly different among the strains immunized with either MOG_{35-55}-CFA plus PTX or 2× MOG_{35-55}-CFA (Table 1). *Post hoc* multiple comparisons of each trait variable revealed that H1RKO-Tg^S mice were equivalent to WT mice while H1RKO-Tg^R mice were equivalent to H1RKO mice. Furthermore, for each trait, H1RKO-Tg^S and WT mice were significantly greater than H1RKO-Tg^R and H1RKO mice.

We next analyzed the *ex vivo* MOG_{35-55} specific proliferative response of spleen and draining lymph node (DLN) cells from mice immunized with 2× MOG_{35-55}-CFA. Significant differences in proliferative responses were not detected among WT, H1RKO, H1RKO-Tg^S and H1RKO-Tg^R mice (data not shown). Since MOG_{35-55}-stimulated splenocytes from immunized-H1RKO mice exhibit an immune deviation from Th1 to Th2 response in *ex vivo* recall assays (Ma et al., 2002), we analyzed cytokine production by MOG_{35-55}-stimulated spleen and DLN cells from mice immunized with both EAE-induction protocols. With the classical MOG_{35-55}-CFA plus PTX protocol, as we observed previously (Noubade et al., 2007), antigen-stimulated spleen and DLN cells

from H1RKO-Tg^S mice produced significantly greater amounts of IFN-γ compared to H1RKO mice and at levels comparable to WT mice (Fig. 2A). In contrast, the levels of IFN-γ produced by antigen-stimulated spleen and DLN cells from the two lines of H1RKO-Tg^R mice were equivalent to those produced by H1RKO mice. Similarly, antigen-stimulated spleen and DLN cells from H1RKO-Tg^S mice produced IL-4 at levels comparable to WT mice while those from H1RKO-Tg^R mice were similar to H1RKO mice (Fig. 2B). Similar results for IFN-γ (Fig. 2D) and IL-4 (Fig. 2E) were observed for 2× MOG_{35-55}-CFA immunized mice.

Because IL-17 is considered to be an important effector cytokine in EAE (Furuzawa-Carballeda et al., 2007), we examined IL-17 production by spleen and DLN cells following *ex vivo* stimulation with MOG_{35-55}. IL-17 production by WT, H1RKO, H1RKO-Tg^S and H1RKO-Tg^R mice immunized with MOG_{35-55}-CFA and PTX was not significantly different (Fig. 2C) among strains. In contrast, IL-17 production by MOG_{35-55} stimulated spleen and DLN cells from animals immunized with 2× MOG_{35-55}-CFA differed significantly among the strains (Fig. 2F). Compared to WT mice, H1RKO mice produced significantly less IL-17, indicating that H_1R signaling regulates IL-17 production by T cells. Moreover, production of IL-17 by H1RKO-Tg^S mice was not significantly different from WT mice and IL-17 production by H1RKO-Tg^R mice was not significantly different from H1RKO mice (Fig. 2F). Taken together, like EAE, H_1R^R expression in H1RKO T cells does not complement cytokine production by these cells.

H_1R alleles activate $G\alpha_q$ and $G\alpha_{11}$ equally well *in vitro*

The above results suggest that the H_1R^R allele is not functional relative to the H_1R^S allele. To understand the mechanism by which the polymorphic residues of the

H_1R^S and H_1R^R alleles influence H_1R function, we examined the predicted structural location for the three residues within H_1R. The three polymorphic residues reside within the third intracytoplasmic loop of H_1R (Fig 3A), which is the region frequently associated with recruitment and activation of downstream G proteins (Tan et al., 2004). We, therefore, examined whether the polymorphic residues distinguishing the H_1R^S and H_1R^R alleles might result in significant alterations in G protein activation. Since H_1R is normally coupled to $G_{\alpha q}$ and/or $G_{\alpha 11}$ proteins, we generated fusion proteins of the two H_1R alleles with both $G_{\alpha q}$ and $G_{\alpha 11}$ by linking in-frame the N-terminus of $G_{\alpha q/11}$ with the C-terminal tail of H_1R^R or H_1R^S.

HEK293 cells were transfected with the H_1R^S-$G\alpha_{q/11}$ or H_1R^R-$G\alpha_{q/11}$ fusion proteins, lysed and membrane fractions prepared from these cells. These were used initially to measure the levels of expression of each construct via the specific binding of the H_1R antagonist [^{3}H]mepyramine. There were no differences in the levels of specific binding of [^{3}H]mepyramine between the various constructs, indicating that the polymorphisms did not alter total protein expression. Also, the binding affinity of [^{3}H]mepyramine was not different between the two alleles (Fig 3B). To study their differential capacity to activate $G\alpha_q$ and $G\alpha_{11}$, membrane amounts containing exactly the same number of copies of each construct were employed in [^{35}S]GTPγS binding assays. A maximally effective concentration of histamine stimulated binding of [^{35}S]GTPγS equally to $G\alpha_q$ or $G\alpha_{11}$ when each G protein was linked to either the H_1R^S or H_1R^R variants (Fig. 3C, Fig. 3D). The dose-response curves to histamine indicated that the potency of histamine is equivalent for each receptor variant (data not shown). These data indicate that the H_1R^S and H_1R^R alleles can activate these G proteins equally well and

that the phenotypic difference associated with the H_1R alleles is not inherently a function of differential capability to activate $G\alpha_q$ or $G\alpha_{11}$.

H_1R alleles are differentially expressed on the cell surface

Specific mutations in the signaling domain of several GPCRs (e.g. vasopressin V2 receptor, rhodopsin) can interfere with their cell surface expression and are associated with disease (Tao, 2006). To determine if the polymorphisms in H_1R influence cell surface expression of the receptor, HA-H_1R^S or HA-H_1R^R expression vectors were used to transfect HEK293T cells. The expression of these receptors at the cell surface was then examined by Flow cytometric analysis using an anti-HA mAb. HA-H_1R^S was expressed at higher levels than HA-H_1R^R (Fig. 4A). The number of H_1R^S-positive cells (Fig. 4B) and the mean florescence intensity of H_1R^S were considerably higher than those of H_1R^R, (Fig. 4C) indicating that the two H_1R alleles are differentially expressed on the cell surface. We observed similar results when the H_1R^S and H_1R^R constructs were transfected into 721.221 B cells (data not shown).

In parallel, we examined the cell surface expression of H_1R^S and H_1R^R by confocal microscopy using anti-HA mAb in cells stained prior to permeabilization. The results confirmed higher expression of H_1R^S on the surface than H_1R^R (Fig. 4D). However, Western blot analysis of H_1R^S and H_1R^R expression in lysates of transfected HEK293T cells showed no difference in the amount of total protein present (Fig. 4E). Taken together, these data indicate that the polymorphic residues associated with the H_1R^S and H_1R^R haplotypes result in differential translocation of the receptor to the cell surface.

H_1R^R is retained in the endoplasmic reticulum

The Western blot results described above (Fig. 4E) suggest that the H_1R^S and H_1R^R alleles are expressed at similar levels but that the H_1R^R allele is largely retained in intracellular compartments instead of being trafficked to the cell surface. To investigate this possibility, HEK293T cells were transfected with HA-H_1R^S or HA-H_1R^R constructs. After 24 h cells were fixed, permeabilized, stained with anti-HA mAb and observed by confocal microscopy. A predominantly plasma membrane staining pattern was observed for the H_1R^S allele (Fig. 5A). In contrast, a large fraction of the H_1R^R allele appeared to localize intracellularly (Fig. 5B) indicating that H_1R^R is retained in the intracellular compartments and fails to traffic efficiently to the cell surface. The network-like intracellular distribution of H_1R^R throughout the cell (Fig. 5B, right panel) resembled that of endoplasmic reticulum (ER). Therefore, to determine if the H_1R^R allele is retained in this compartment, we transiently co-transfected HEK293T cells with H_1R^S or H_1R^R constructs and a plasmid expressing the dsRed fluorescent protein that targets the ER. Co-localization of the two proteins was examined by confocal microscopy following staining the cells for HA-H_1R. The majority of H_1R^R was again expressed intracellularly and co-localized with the dsRed protein, while minimal colocalization of H_1R^S with the ER-targeted dsRed protein was observed (Fig. 5B). Using LSM5 image browser software, we quantified the number of pixels that express both dsRed protein and HA-H_1R in multiple cells that were imaged under exactly the same settings. The results showed a significant difference in the co-localization of the H_1R^S and H_1R^R alleles in ER (Fig. 5C), suggesting that the H_1R^R L-M-S haplotype leads to its sequestration and retention in ER.

Retention of H_1R^R in the ER requires the L-M-S haplotype

To understand which of the three amino acids comprising the H_1R^R L-M-S haplotype is responsible for the observed differential cell surface expression of the allele we generated single H_1R^S mutants, replacing each of the H_1R^S haplotype associated residues with the corresponding H_1R^R allele (P263L, V312M, and P330S), by site directed mutagenesis. HEK293T cells were transfected with H_1R^S, H_1R^R and each of the three H_1R^S mutant constructs. Cells were stained with anti-HA mAb, without permeabilization, and cell surface expression of H_1R analyzed by Flow- cytometry. Each of the single H_1R^S mutants was expressed at higher levels on the cell surface than the H_1R^R allele (Fig. 6A) with the levels comparable to those observed with the H_1R^S allele. This indicates that the presence of a single H_1R^R polymorphism is not sufficient to induce its intracellular retention. We also generated double mutants of the H_1R^S allele wherein we replaced two residues of the H_1R^S haplotype with the corresponding residues of the H_1R^R allele (P263L and V312M, P263L and P330S, V312M and P330S). Similar to the single H_1R^S mutants, the double H_1R^S mutants were expressed on the cell surface at levels comparable to the H_1R^S and at significantly higher levels than the H_1R^R allele (Fig. 6B). We observed similar results in 721.221 B cells following transient transfection with H_1R^S, H_1R^S mutants and H_1R^R constructs (data not shown). Furthermore, when HEK293T cells were co-transfected with double H_1R^S mutants and the dsRed plasmid, each of the mutants showed a typical plasma membrane expression pattern with very little co-localization with the ER-targeted dsRed protein (Fig. 6C). Quantification of the number of pixels expressing dsRed- protein and HA-H_1R confirmed that each of the double H_1R^S mutants behaved like H_1R^S and only H_1R^R was retained in ER (Fig. 6D),

confirming the flow cytometry data that all the polymorphic residues are required for differential cell surface expression of the H_1R alleles. Taken together, these data indicate that all three residues of the H_1R^R L-M-S haplotype are required for its intracellular sequestration. Interestingly, we sequenced the H_1R alleles from more than 100 different inbred laboratory and wild-derived mouse strains and did not identify any recombinant haplotypes (Table 2), suggesting that the two alleles are evolutionarily conserved and have been selected for functionally.

DISCUSSION

To date, *Hrh1*/H_1R is the only murine EAE and EAO susceptibility gene that has been positionally cloned (Ma et al., 2002). In this study, using transgenic mouse models, we show that polymorphic variants in H_1R regulate cytokine production by T cells thereby influencing susceptibility to EAE. Furthermore, using HEK293T cells, we show that the polymorphisms in H_1R affect its functions by modulating cell surface expression rather than inherently altering the capacity of the receptor to generate intracellular signals.

Hrh1/H_1R has long been implicated in EAE susceptibility (Linthicum and Frelinger, 1982; Ma et al., 2002). As H_1R is widely expressed (Parsons and Ganellin, 2006), this suggested that it might act in different cell types and at multiple checkpoints. We recently showed, however, that H_1R expression in T cells is sufficient to complement EAE severity in H1RKO mice. In this study, we show that the polymorphic residues of the H_1R^R allele interfere with its ability to complement EAE in H1RKO mice. This is in accordance with genetic complementation studies in F1 hybrids between H1RKO and strains of mice expressing the H_1R^S or H_1R^R alleles. Susceptibility to histamine sensitivity could be restored in F1 hybrids of H1RKO and SJL/J, 129X1/SvJ or C57BL/6J that express H_1R^S allele but not in F1 hybrids between H1RKO and C3H/HeJ or CBA/J mice that express H_1R^R (Ma et al., 2002).

Hrh1/H_1R also controls delayed type hypersensitivity (DTH) responses when PTX is used as an adjuvant. The DTH response is mediated by CD4 T cells that produce large amounts of IFN-γ (Sewell et al., 1987; Sewell et al., 1984; Sewell et al., 1983). Using C3H.*Bphs*^{S} congenic mice expressing the H_1R^S allele from SJL/J mice on the

resistant C3H/HeJ background, Gao et al., (Gao et al., 2003) showed that polymorphisms in H_1R regulate ovalbumin-specific DTH response elicited in mice immunized with ovalbumin in CFA and PTX, indicating that the polymorphisms in H_1R regulate IFNγ production by CD4 T cells. This study confirms the role of H_1R polymorphisms in regulating IFN-γ production by these cells. Further, the complementation of IFN-γ production by splenocytes immunized using the 2× $MOG35_{-55}$ model suggests that H_1R regulation of IFNγ production by T cells does not require PTX.

Recently, IL-17-producing Th17 CD4 T cells have been considered more pathogenic in EAE (Furuzawa-Carballeda et al., 2007). We show here, for the first time, that H_1R signaling regulates IL-17 production and that H_1R polymorphisms influence IL-17 production by T cells. However, it is noteworthy that we did not observe differences in IL-17 production between WT and H1RKO mice immunized with MOG_{35-55}-CFA plus PTX, nor in Th17 cells differentiated *in vitro* in the presence of excessive amounts of IL-6. PTX promotes the generation of Th17 cells, by inducing IL-6 production (Chen et al., 2007). Thus, it is possible that immunization with PTX (*in vivo*) or addition of exogenous IL-6 (*in vitro*) enables CD4 T cells to overcome the absence of H_1R signals required for the optimal IL-6 production and generation of Th17 cells. Even though we observed significant differences in IL-17 production by spleen and DLN cells from transgenic mice selectively expressing either H_1R^S or H_1R^R in T cells, we believe, based on *in vitro* differentiation data, that the H_1R regulation of IL-6 and IL-17 is independent of H_1R signals in T cells. In this regard, compared to WT macrophages H1RKO macrophages produce significantly less IL-6 (unpublished data) and treatment of lung parenchymal macrophages with H_1R blockers results in decreased IL-6 production (Triggiani et

al., 2001). Further studies are being carried out to elucidate the role of H_1R in the generation of Th17 CD4 T cells.

GPCRs, in spite of the diversity of their polypeptide sequences, as a family retain enough structural information to allow them to be properly folded in the ER and adopt their highly conserved seven transmembrane confirmation (Spiegel and Weinstein, 2004). Several studies have identified critical residues and motifs important in many of the functions of GPCRs including ligand binding, G protein coupling, internalization, downregulation and intracellular trafficking (Duvernay et al., 2005). However, the three polymorphic residues distinguishing the H_1R^S and H_1R^R alleles are located in the third intracytoplasmic loop and do not constitute any known motif. Even though the exact PXXP motif is not present, it is worth noting that two of the three polymorphic residues associated with the H_1R^S haplotype are prolines, and that proline rich-motifs are known to mediate protein-protein interactions with Src homology SH3 domains (Sparks et al., 1996). In this regard, polymorphic residues containing polyproline motifs in the third intracytoplasmic loop of the dopamine D4 receptor and β1-adrenergic receptor have been shown to interact with multiple SH3 domain-containing proteins (Oldenhof et al., 1998) and affect the trafficking of these receptors. However, at this point, we do not have any evidence to suggest that H_1R interacts with any of the known SH3 domain-containing proteins or that such interactions differ between H_1R^S and H_1R^R alleles. Future studies will address this issue.

GPCRs interact with numerous proteins that play a role in their cellular trafficking (Tan et al., 2004). H_1R has an unusually long third intracytoplasmic loop, suggesting that the polymorphic residues may result in improper folding of the receptor to a non-

native conformation in ER, which is then recognized by the quality control machinery of molecular chaperones and excluded from ER export. Several chaperone proteins [such as Nina (Schneuwly et al., 1989; Shieh et al., 1989), ODR-4 (Dwyer et al., 1998; Gimelbrant et al., 2001) and a variety of receptor activity modifying proteins (RAMPs) (Christopoulos et al., 2003; McLatchie et al., 1998)] that support the trafficking of a range of GPCRs to their target site have been identified. Therefore, it is possible that polymorphic residue-induced misfolding of H_1R^R could hinder its interaction with an essential chaperone thereby affecting its trafficking.

Proper cell surface expression of GPCRs is required to access the requisite ligands and signal transduction machinery (Tan et al., 2004). The functional importance of proper GPCR localization is emphasized by several human diseases that result from receptor mutation and mislocalization, including X-linked nephogenic diabetes, retinitis pigmentosa and hypogonadotrophic hypogonadism, which result from intracellular accumulation of mutant V2 vasopressin receptor, rhodopsin and gonadotropin releasing hormone receptor, respectively (Tao, 2006). In fact, mutations that lead to intracellular accumulation comprise the largest class of mutations in GPCRs that result in human diseases (Tan et al., 2004). Accordingly, our results are the first to demonstrate that structural polymorphisms influencing differential trafficking and cell surface expression of a GPCR in T cells can regulate immune functions and susceptibility to autoimmune disease.

MATERIALS AND METHODS

Mice

C57BL/6J mice were purchased from the Jackson Laboratory (Bar Harbor, ME). B6.129P-*Hrh1*^{tm1Wat} (H1RKO) (Banu and Watanabe, 1999) mice were maintained in the animal facility at the University of Vermont (Burlington, VT). The experimental procedures used in this study were approved by the Animal Care and Use Committee of the University of Vermont.

For transgenic mouse generation, the HA-H_1R^S or HA-H_1R^R constructs were made by deleting the methionine of the *Bphs*-susceptible H_1R allele from SJL/J and *Bphs*-resistant C3H/HeJ mice, respectively (Ma et al., 2002), and adding an HA tag at the N-terminus using TOPO cloning vector (Invitrogen, Carlsbad, CA). The HA-H_1R was then subcloned downstream of the distal *lck* promoter (Wildin et al., 1991). The linear DNA fragment containing the distal *lck* promoter, the HA-H_1R 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 by DNA slot blot testing using a *Bam*HI–*Sac*I 0.5 kb fragment from the *hGH* gene as a probe. Two founders were generated for both the H_1R^S and H_1R^R alleles and each was crossed to H1RKO mice to establish transgenic mouse lines on the H1RKO background (H1RKO-Tg^S1 and H1RKO-Tg^S2 and H1RKO-Tg^R1 and H1RKO-Tg^R2 mice). Mice from the H1RKO-Tg^S1 line expressed the transgene at comparable levels to the two lines expressing the $H1R^R$ allele, so it was used in all the experiments in this study.

Cytokine production

For cytokine analysis spleen and lymph nodes were obtained from mice immunized ten days earlier with either MOG_{35-55}-CFA plus PTX or 2× MOG_{35-55}-CFA model, single cell suspensions prepared at a concentration of 1×10^6 cells/ml in RPMI medium and stimulated with 50 μg/ml of MOG_{35-55}. Cell culture supernatants were recovered at 72 h and cytokine levels measured by ELISA using anti-IFN-γ, anti-IL-4 and anti-IL17 mAbs and their corresponding biotinylated mAbs (BD Pharmingen, San Diego, CA). TNF-α ELISA kit was from (BD Pharmingen, San Diego, CA).

Proliferation Assays

Mice were immunized with the 2× MOG_{35-55}-CFA protocol: single cell suspensions were prepared at 2.5×10^5 cells/well in RPMI medium and stimulated in a 96 well plate with different concentrations (0, 2, 10 and 50 μg/ml) of MOG_{35-55} for 72 h and proliferation was determined by [^{3}H]-thymidine incorporation during the last 18 h of culture.

Cell surface expression studies

The pEGZ-HA vector plasmid was a generous gift from Dr. Ingolf Berberich (University of Wurzburg, Wurzburg, Germany). Two restriction sites, *BamHI* and *EcoRI* were inserted into H_1R^S or H_1R^R cDNA by PCR and cloned such that the second codon is in-frame with the HA tag of pEGZ generating an HA-H_1R fusion protein. pEGZ is a bicistronic system with IRES-EGFP. EGFP served as a marker for transfected cells.

HEK293T cells were plated at 1.25×10^6 cells/plate and cultured in DMEM-F12 containing 10% FBS. When the cells were about 50-80% confluent, they were transfected with 5 μg of pEGZ-HA-H_1R^S, pEGZ-HA- H_1R^R or the empty pEGZ vector using

calcium phosphate method. After 16-24 h, cells were scraped off the plate by rigorous pipetting with 1% Calf serum in PBS and stained with anti-HA mAb conjugated to PE (Miltenyi Biotech, Auburn, CA) according to the manufacturer's guidelines. Cells were analyzed by Flow cytometry using FACSAria instrument (BD Pharmingen, San Diego, CA) and the data were further analyzed using FlowJo flow cytometry analysis software (Tree star Inc, Ashland, OR).

Confocal microscopy

HEK293T cells were transfected with pEGA-HA-H_1R^S, pEGZ-HA-H_1R^R or empty pEGZ control vector (5 μg total DNA) using the calcium phosphate method. Cells were fixed, permeabilized and stained using an anti-HA mAb (Cell Signaling Technologies, Danvers, MA) followed by an incubation with Alexa-568 anti-mouse antibody (Molecular Probes, Eugene, Oregon). TOPRO-3 nuclear stain (Molecular Probes, Eugene, Oregon) was used as a nuclear marker. For non-permeabilized cells, the transfected HEK293T cells were stained with the anti-HA mAb and were then fixed. Cells were examined by confocal microscopy using Zeiss LSM 510 META Confocal Laser Scanning Imaging System (Carl Ziess Microimaging Inc, Thronwood, NY).

Cell lysates and Western blotting

Whole-cell lysates were prepared from HEK293T cells transfected with various pEGZ constructs in Triton lysis buffer and were then separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose membranes as described previously (Noubade et al., 2007). Anti-HA mAb (Abcam Inc. Cambridge, MA) was used as primary antibody. Anti-actin (Santa Cruz Biotechnology, Santa Cruz, CA) was used as a loading control.

[^{3}H]mepyramine binding studies

[^{3}H]mepyramine binding studies were conducted as described (Bakker et al., 2004) and were used to measure expression levels of H_1R variants and the H_1R^S-$G\alpha_{q/11}$ and H_1R^R-$G\alpha_{q/11}$ fusion proteins.

[^{35}S]GTPγS binding Assay

[^{35}S]GTPγS binding experiments to assess the capacity of H_1R variants to cause activation of $G\alpha_{q/11}$ were initiated by the addition of cell membranes containing 50 fmols of H_1R variant constructs to assay buffer (20mM HEPES (pH 7.4), 3mM $MgCl_2$, 100mM NaCl, 1μM GDP, 0.2mM ascorbic acid, and 100nCi [^{35}S]GTPγS) containing 100μM histamine. Non-specific binding was determined in the above condition with the addition of 100μM GTPγS. Reactions were incubated for 15 min at 30^0 C and were terminated by the addition of 500μl of ice-cold buffer containing 20mM HEPES (pH 7.4), 3mM $MgCl_2$, 100mM NaCl and 0.2mM ascorbic acid. The samples were centrifuged at 16,000 × g for 10 min at 4^0 C. The resulting pellets were re-suspended in solubilization buffer (100mM Tris, 200mM NaCl, 1mM EDTA, and 1.25% Nonidet P-40) plus 0.2% SDS. Samples were precleared with Pansorbin for 1 h, followed by immunoprecipitation with a C-terminal anti- $G\alpha_q/G_{\alpha 11}$ antiserum (Mitchell et al., 1991). Finally, the immunocomplexes were washed with solubilization buffer and bound [^{35}S]GTPγS was estimated by liquid scintillation-spectrometry.

Site directed mutagenesis

pEGZ-HA-H_1R^S was used as template to generate single H_1R^S mutants with each of the polymorphic residues replaced with the corresponding residue of the H_1R^R allele using the Quickchange (Strategene) site directed mutagenesis kit, according to the manufacturer's guidelines. The forward primers used for the mutagenesis were: for P263L 5'- GGGGGTCCAGAAGAGG**CCG**TCAAGAGACCCTACTGG-3', for V312M 5'- CATGCAGACACAGCC**TGT**GCCTGAGGGAGATGCCAGG-3', for P330S 5'- CCAGACCTTGAGCC**AGC**CCAAAATGGATGAGCAGAGC-3'. The reverse primers were the complementary sequences of these primers. The altered nucleotides are shown in bold and underlined. The mutants were sequence confirmed and were used as template for the generation of different combinations of double H_1R^S mutants.

Conventional and Quantitative Real-Time Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from CD4 T cells using RNeasy RNA isolation reagent (Qiagen, Valencia, CA) as recommended by the manufacturer. cDNA generated from 1 μg total RNA was used in conventional and quantitative real-time RT-PCR as described earlier (11).

Induction and Evaluation of EAE

Mice were immunized for the induction of EAE using either the MOG_{35-55}-complete Freund's adjuvant (CFA) double-inoculation (2× MOG_{35-55}-CFA) (Butterfield et al., 1998) or the MOG_{35-55}-CFA plus PTX single-inoculation (MOG_{35-55}-CFA plus PTX) protocols (Teuscher et al., 2006b). For the 2× MOG_{35-55}-CFA induction protocol mice are injected subcutaneously with an emulsion of 100 μg of MOG_{35-55} and an equal

volume of CFA containing 200 μg of *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Detroit, MI) in the posterior right and left flank; one week later all mice were similarly injected at two sites on the right and left flank anterior of the initial injection sites. Animals immunized using the MOG_{35-55}-CFA plus PTX single-inoculation protocol received an emulsion of 200 μg MOG_{35-55} and equal volume of CFA containing 200 μg of *Mycobacterium tuberculosis* H37RA by subcutaneous injections distributed equally in the posterior right and left flank and scruff of the neck. Immediately thereafter, each animal received 200 ng PTX (List Biological Laboratories, Campbell, CA) by intravenous injection. Mice were scored daily starting at day 5 post-injection as previously described (Teuscher et al., 2006b). Clinical quantitative trait variables including disease incidence and mean day of onset (DO), cumulative disease score (CDS), number of days affected (DA), overall severity index (SI) and the peak score (PS) were generated as previously described (Butterfield et al., 1998).

Statistical analysis

Statistical analyses, as detailed in the figure legends, were performed using GraphPad Prism 4 software (GraphPad software Inc, San Diego, CA). A P value of 0.05 or less was considered significant.

REFERENCES

Andersson, A., and Karlsson, J. (2004). Genetics of experimental autoimmune encephalomyelitis in the mouse. Arch Immunol Ther Exp (Warsz) *52*, 316-325.

Bakker, R.A., Dees, G., Carrillo, J.J., Booth, R.G., Lopez-Gimenez, J.F., Milligan, G., Strange, P.G., and Leurs, R. (2004). Domain swapping in the human histamine H1 receptor. The Journal of pharmacology and experimental therapeutics *311*, 131-138.

Banu, Y., and Watanabe, T. (1999). Augmentation of antigen receptor-mediated responses by histamine H1 receptor signaling. J Exp Med *189*, 673-682.

Butterfield, R.J., Sudweeks, J.D., Blankenhorn, E.P., Korngold, R., Marini, J.C., Todd, J.A., Roper, R.J., and Teuscher, C. (1998). New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. J Immunol *161*, 1860-1867.

Chen, X., Howard, O.M., and Oppenheim, J.J. (2007). Pertussis toxin by inducing IL-6 promotes the generation of IL-17-producing CD4 cells. J Immunol *178*, 6123-6129.

Christopoulos, A., Christopoulos, G., Morfis, M., Udawela, M., Laburthe, M., Couvineau, A., Kuwasako, K., Tilakaratne, N., and Sexton, P.M. (2003). Novel receptor partners and function of receptor activity-modifying proteins. J Biol Chem *278*, 3293-3297.

Duvernay, M.T., Filipeanu, C.M., and Wu, G. (2005). The regulatory mechanisms of export trafficking of G protein-coupled receptors. Cell Signal *17*, 1457-1465.

Dwyer, N.D., Troemel, E.R., Sengupta, P., and Bargmann, C.I. (1998). Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell *93*, 455-466.

Furuzawa-Carballeda, J., Vargas-Rojas, M.I., and Cabral, A.R. (2007). Autoimmune inflammation from the Th17 perspective. Autoimmun Rev *6*, 169-175.

Gao, J.F., Call, S.B., Fillmore, P.D., Watanabe, T., Meeker, N.D., and Teuscher, C. (2003). Analysis of the role of Bphs/Hrh1 in the genetic control of responsiveness to pertussis toxin. Infect Immun *71*, 1281-1287.

Gimelbrant, A.A., Haley, S.L., and McClintock, T.S. (2001). Olfactory receptor trafficking involves conserved regulatory steps. J Biol Chem *276*, 7285-7290.

Gold, R., Hartung, H.P., and Toyka, K.V. (2000). Animal models for autoimmune demyelinating disorders of the nervous system. Mol Med Today *6*, 88-91.

Greenstein, J.I. (2007). Current concepts of the cellular and molecular pathophysiology of multiple sclerosis. Dev Neurobiol *67*, 1248-1265.

Hafler, D.A., Slavik, J.M., Anderson, D.E., O'Connor, K.C., De Jager, P., and Baecher-Allan, C. (2005). Multiple sclerosis. Immunol Rev *204*, 208-231.

Kantarci, O., and Wingerchuk, D. (2006). Epidemiology and natural history of multiple sclerosis: new insights. Curr Opin Neurol *19*, 248-254.

Kuchroo, V.K., Anderson, A.C., Waldner, H., Munder, M., Bettelli, E., and Nicholson, L.B. (2002). T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. Annu Rev Immunol *20*, 101-123.

Linthicum, D.S., and Frelinger, J.A. (1982). Acute autoimmune encephalomyelitis in mice. II. Susceptibility is controlled by the combination of H-2 and histamine sensitization genes. J Exp Med *156*, 31-40.

Ma, R.Z., Gao, J., Meeker, N.D., Fillmore, P.D., Tung, K.S., Watanabe, T., Zachary, J.F., Offner, H., Blankenhorn, E.P., and Teuscher, C. (2002). Identification of Bphs, an autoimmune disease locus, as histamine receptor H1. Science *297*, 620-623.

McLatchie, L.M., Fraser, N.J., Main, M.J., Wise, A., Brown, J., Thompson, N., Solari, R., Lee, M.G., and Foord, S.M. (1998). RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature *393*, 333-339.

Mitchell, F.M., Mullaney, I., Godfrey, P.P., Arkinstall, S.J., Wakelam, M.J., and Milligan, G. (1991). Widespread distribution of Gq alpha/G11 alpha detected immunologically by an antipeptide antiserum directed against the predicted C-terminal decapeptide. FEBS letters *287*, 171-174.

Noubade, R., Milligan, G., Zachary, J.F., Blankenhorn, E.P., Del Rio, R., Rincon, M., and Teuscher, C. (2007). Histamine receptor H(1) is required for TCR-mediated p38 MAPK activation and optimal IFN-gamma production in mice. J Clin Invest *117*, 3507-3518.

Oldenhof, J., Vickery, R., Anafi, M., Oak, J., Ray, A., Schoots, O., Pawson, T., von Zastrow, M., and Van Tol, H.H. (1998). SH3 binding domains in the dopamine D4 receptor. Biochemistry *37*, 15726-15736.

Parsons, M.E., and Ganellin, C.R. (2006). Histamine and its receptors. Br J Pharmacol *147 Suppl 1*, S127-135.

Schneuwly, S., Shortridge, R.D., Larrivee, D.C., Ono, T., Ozaki, M., and Pak, W.L. (1989). Drosophila ninaA gene encodes an eye-specific cyclophilin (cyclosporine A binding protein). Proc Natl Acad Sci U S A *86*, 5390-5394.

Sewell, W.A., de Moerloose, P.A., Hamilton, J.A., Schrader, J.W., Mackay, I.R., and Vadas, M.A. (1987). Potentiation of delayed-type hypersensitivity by pertussigen or cyclophosphamide with release of different lymphokines. Immunology *61*, 483-488.

Sewell, W.A., Munoz, J.J., Scollay, R., and Vadas, M.A. (1984). Studies on the mechanism of the enhancement of delayed-type hypersensitivity by pertussigen. J Immunol *133*, 1716-1722.

Sewell, W.A., Munoz, J.J., and Vadas, M.A. (1983). Enhancement of the intensity, persistence, and passive transfer of delayed-type hypersensitivity lesions by pertussigen in mice. J Exp Med *157*, 2087-2096.

Shieh, B.H., Stamnes, M.A., Seavello, S., Harris, G.L., and Zuker, C.S. (1989). The ninaA gene required for visual transduction in Drosophila encodes a homologue of cyclosporin A-binding protein. Nature *338*, 67-70.

Sparks, A.B., Hoffman, N.G., McConnell, S.J., Fowlkes, D.M., and Kay, B.K. (1996). Cloning of ligand targets: systematic isolation of SH3 domain-containing proteins. Nat Biotechnol *14*, 741-744.

Spiegel, A.M., and Weinstein, L.S. (2004). Inherited diseases involving g proteins and g protein-coupled receptors. Annu Rev Med *55*, 27-39.

Tan, C.M., Brady, A.E., Nickols, H.H., Wang, Q., and Limbird, L.E. (2004). Membrane trafficking of G protein-coupled receptors. Annu Rev Pharmacol Toxicol *44*, 559-609.

Tao, Y.X. (2006). Inactivating mutations of G protein-coupled receptors and diseases: structure-function insights and therapeutic implications. Pharmacol Ther *111*, 949-973.

Teuscher, C. (1985). Experimental allergic orchitis in mice. II. Association of disease susceptibility to the locus controlling Bordeteall pertussis-induced sensitivity to histamine Immunogenetics *22*, 417-425.

Teuscher, C., Noubade, R., Spach, K., McElvany, B., Bunn, J.Y., Fillmore, P.D., Zachary, J.F., and Blankenhorn, E.P. (2006). Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A *103*, 8024-8029.

Triggiani, M., Gentile, M., Secondo, A., Granata, F., Oriente, A., Taglialatela, M., Annunziato, L., and Marone, G. (2001). Histamine induces exocytosis and IL-6 production from human lung macrophages through interaction with H1 receptors. J Immunol *166*, 4083-4091.

Wildin, R.S., Garvin, A.M., Pawar, S., Lewis, D.B., Abraham, K.M., Forbush, K.A., Ziegler, S.F., Allen, J.M., and Perlmutter, R.M. (1991). Developmental regulation of lck gene expression in T lymphocytes. J Exp Med *173*, 383-393.

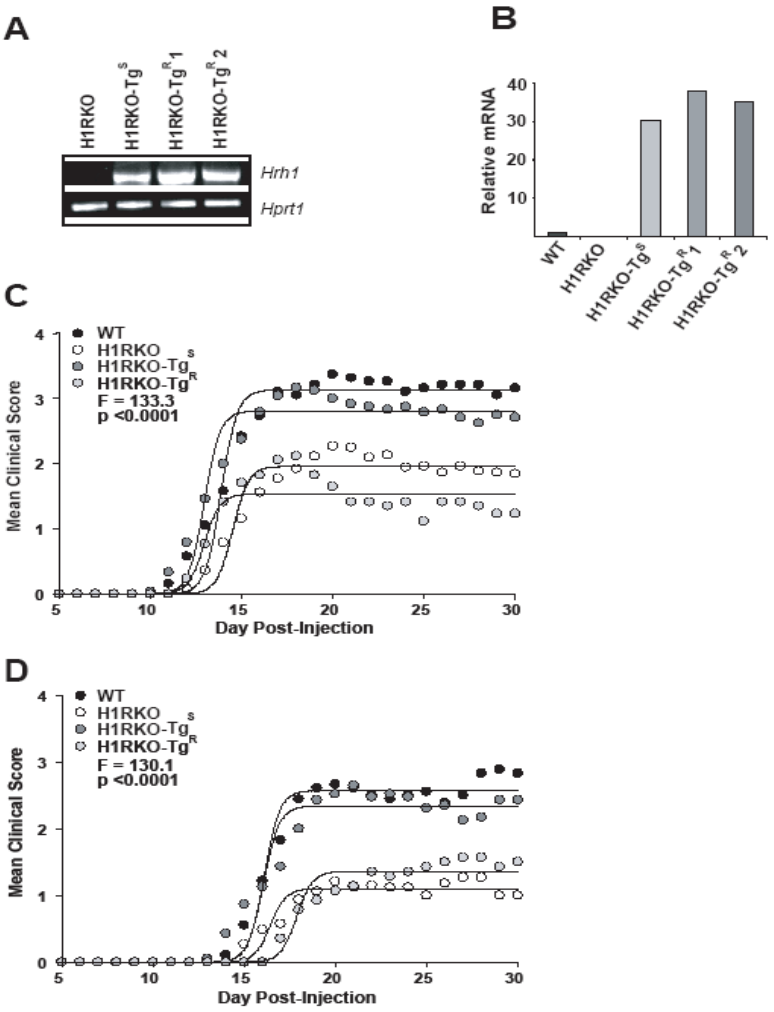


Figure 1. Transgenic expression of H_1R^R in H1RKO T cells fails to complement EAE in H1RKO mice.

H_1R transgene expression was analyzed by (**A**) RT-PCR and (**B**) quantitative RT-PCR of H_1R mRNA expression from CD4 T cells from H1RKO mice and the transgenic mice expressing H_1R^S or H_1R^R that were crossed with H1RKO mice (H1RKO-Tg^S, H1RKO-Tg^R1 and H1RKO-Tg^R2). H1RKO-TgR1 and H1RKO-TgR2 represent two independent lines. (**C**) Clinical EAE in WT (n = 19), H1RKO (n = 56), H1RKO-Tg^S (n = 24), and H1RKO-Tg^R (n = 25) mice that were immunized with MOG_{35-55}+CFA+PTX. Mice were scored daily starting at D5. Regression analysis revealed that the disease course elicited fits a Sigmoidal curve and that the clinical disease course of the animals was significantly different among the strains ($F = 66.1$; $p < 0.0001$) and that WT ($F = 132.1$; $p < 0.0001$) and H1RKO-Tg^S ($F = 127.5$; $p < 0.0001$) mice were significantly different from H1RKO-Tg^R and H1RKO mice. (**D**) WT (n = 18), H1RKO (n = 33), H1RKO-Tg^S (n = 23), and H1RKO-Tg^R (n = 25) mice were immunized 2× with MOG_{35-55}+CFA. EAE severity was significantly different among the strains ($F = 8.9$; $p < 0.0001$) with WT ($F = 226.9$; $p < 0.0001$), and H1RKO-Tg^S ($F = 134.0$; $p < 0.0001$) mice being significantly different from H1RKO-Tg^R ($F = 215.8$; $p < 0.0001$) H1RKO mice.

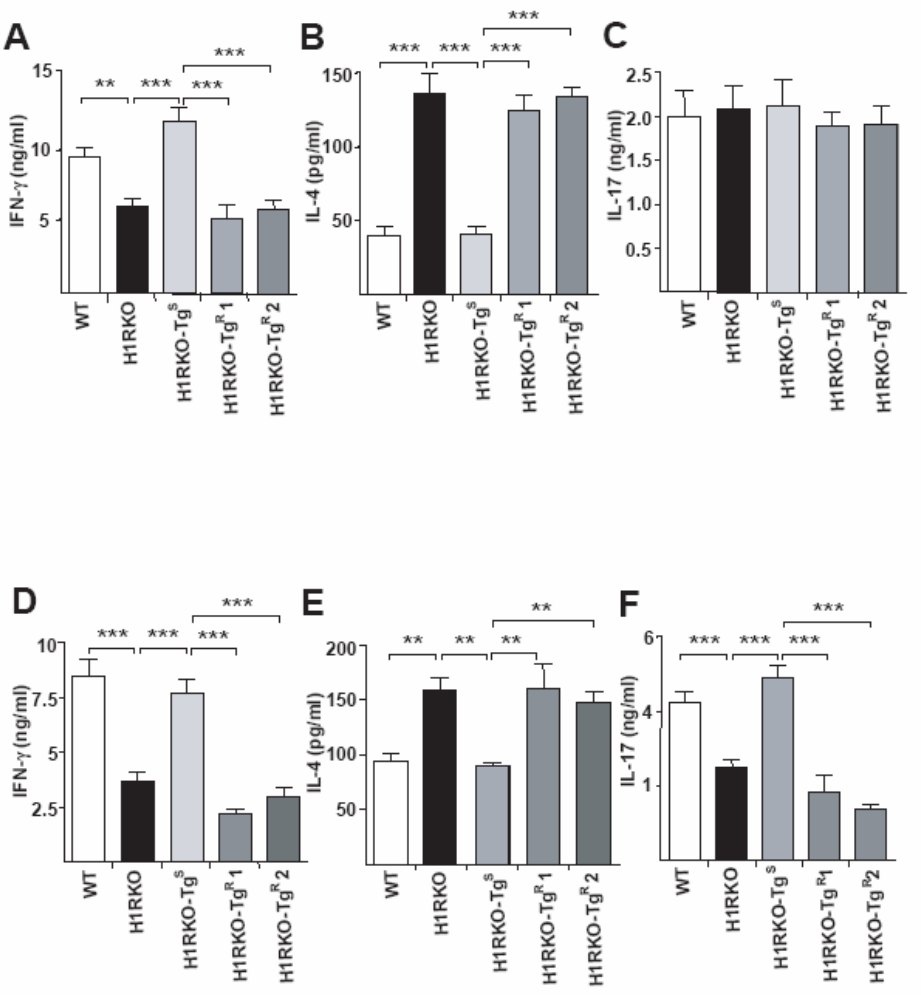


Figure 2. Transgenic expression of H_1R^R in H1RKO T cells fails to complement cytokine production by H1RKO mice.

(**A-C**) Spleen and draining lymph node (DLN) cells isolated from MOG35-55 plus CFA plus PTX-immunized WT, H1RKO, H1RKO-Tg^S and H1RKO-Tg^R mice 10 days post immunization and stimulated with the indicated 50mg/ml of MOG_{35-55} for 72h. Supernatants were collected and analyzed for the production of IFN-g (**A**), IL-4 (**B)** and IL-17 (**C**). Significance of differences in cytokine production were assessed by one-way ANOVA ($F = 14.89$; $p < 0.001$ for IFNγ, $F = 28.93$; $p < 0.001$ for IL-4, $p > 0.5$ for IL-17) followed by Benfeorroni's post-hoc comparisons. Except for IL-17, WT = H1RKO-Tg^S > H1RKO-Tg^R1 = H1RKO-Tg^R2 = H1RKO mice. (**D-F**) Spleen and DLN cells from 2x MOG_{35-55} plus CFA immunized mice were collected on day 10 post immunization and were activated with 50μg/ml of MOG_{35-55} for 72h, supernatants were collected and analyzed for IFNγ (**D**), IL-4 (**E**) and IL-17 (**F**) by ELISA in triplicate. Significance of differences in cytokine production were assessed by one-way ANOVA ($F = 28.92$; $p < 0.0001$ for IFNγ, $F = 3.766$; $p < 0.0001$ for IL-4, $F = 10.29$; $p < 0.0001$ for IL-17) followed by Benfeorroni's post-hoc comparisons. For all the cytokines tested WT = H1RKO-Tg^S > H1RKO-Tg^R1 = H1RKO-Tg^R2 = H1RKO mice.

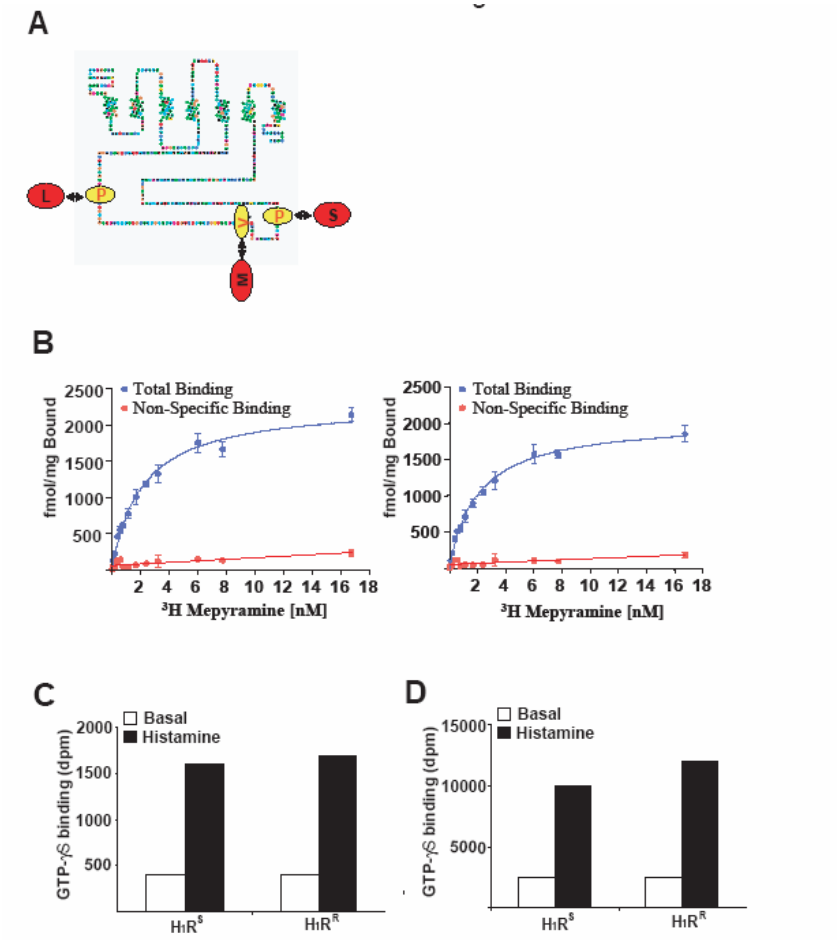


Figure 3. H_1R^S and H_1R^R activate $G\alpha_{q/11}$ G proteins equally well.

(A) The amino acid sequence of the mouse histamine H_1 receptor is displayed with differences between the H_1R^R allele (red) and the H_1R^S allele (yellow) highlighted. Each of the sites of variation is within the long, third intracellular loop. (**B**) Saturation [^{3}H]mepyramine binding studies were performed on membranes of HEK293T cells transfected to express a H_1R-$G\alpha_q$ fusion protein (H_1R^R-left panel, H_1R^S-right panel. Non-specific binding (shown in red) was determined in the same manner but with the additional presence of 1μM mianserin. These studies provided quantitation of construct expression levels. (**C & D**) Membranes containing 50 fmol of H_1R^S or H_1R^R linked to either $G\alpha_q$ (**C**) or $G\alpha_{11}$ (**D**) were used in [^{35}S]GTPγS binding studies conducted in the absence (basal, open bars) or presence (histamine, filled bars) of 100μM histamine to assess the capability of the two variants to activate the G proteins. H_1R^S and H_1R^R were equi-effective in causing activation of each G protein. Representative data are shown.

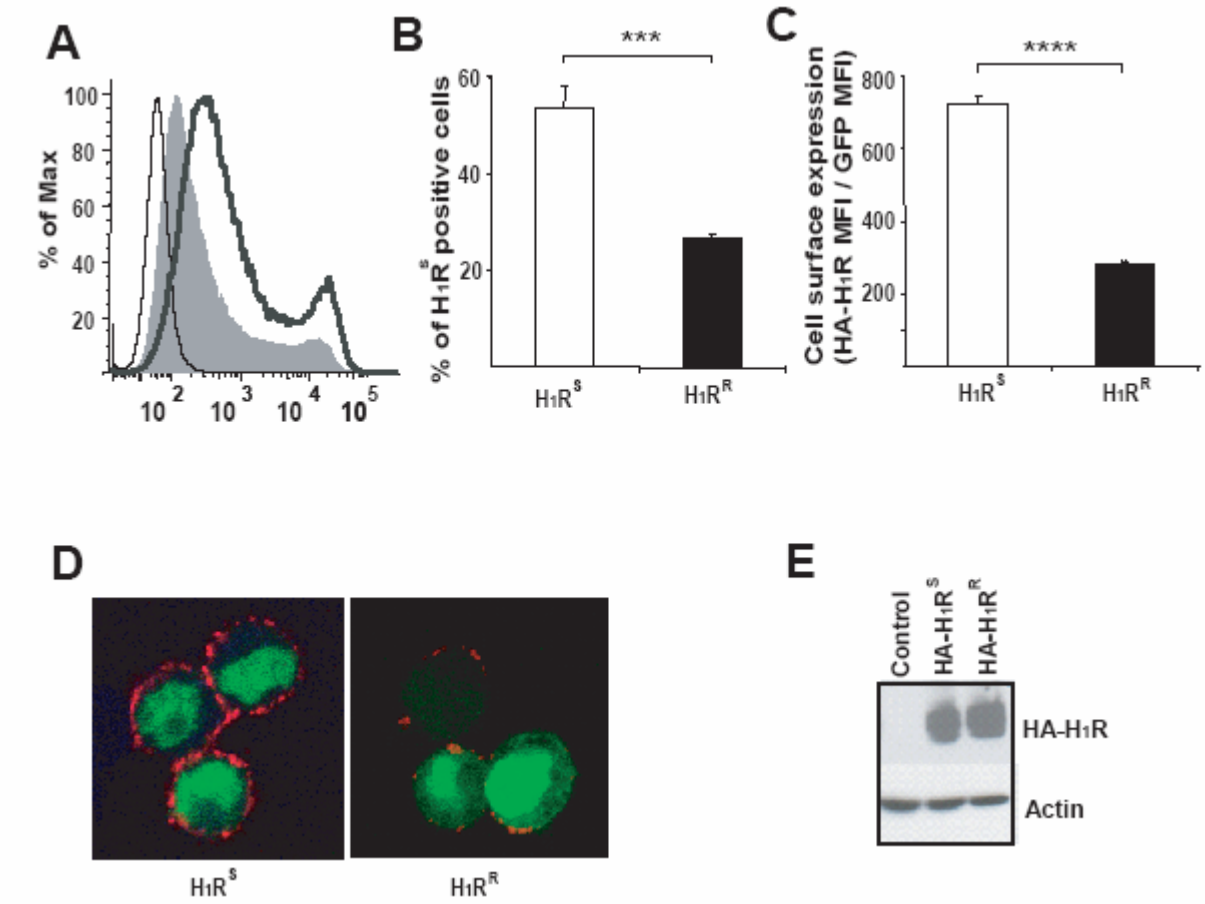


Figure 4. H_1R^S and H_1R^R are differentially expressed on the cell surface.

(**A**) HEK293T cells were transfected with empty pEGZ, pEGZ-HA-H_1R^S or pEGZ-HA-H_1R^R plasmids in triplicate cultures. Cells were collected 16-24h later without trypisinization, stained with anti-HA mAb and analyzed by Flow cytometry. Thin line represents cells transfected with empty pEGZ, thick line represents cells transfected with HA-H_1R^S and the filled area represents cells transfected with HA-H_1R^R. (**B & C**) HEK293T cells were analyzed as in (**A**) and the percentage (**B**) and the mean florescence intensity of anti-HA on H_1R^S positive cells (**C**) were determined (n=3). (**D**) HEK293T cells transfected with HA-H_1R^S or HA-H_1R^R plasmids and 24 h later cells were stained with anti-HA mAb (red) without permeabilization. Cells were visualized by confocal microscopy. GFP (green) is shown as a marker of transfected cells. (**E**). HEK293T cells were transfected as in (**A**), whole cell lysates prepared and analyzed by Western blotting using anti-HA mAb. Actin is shown as loading control.

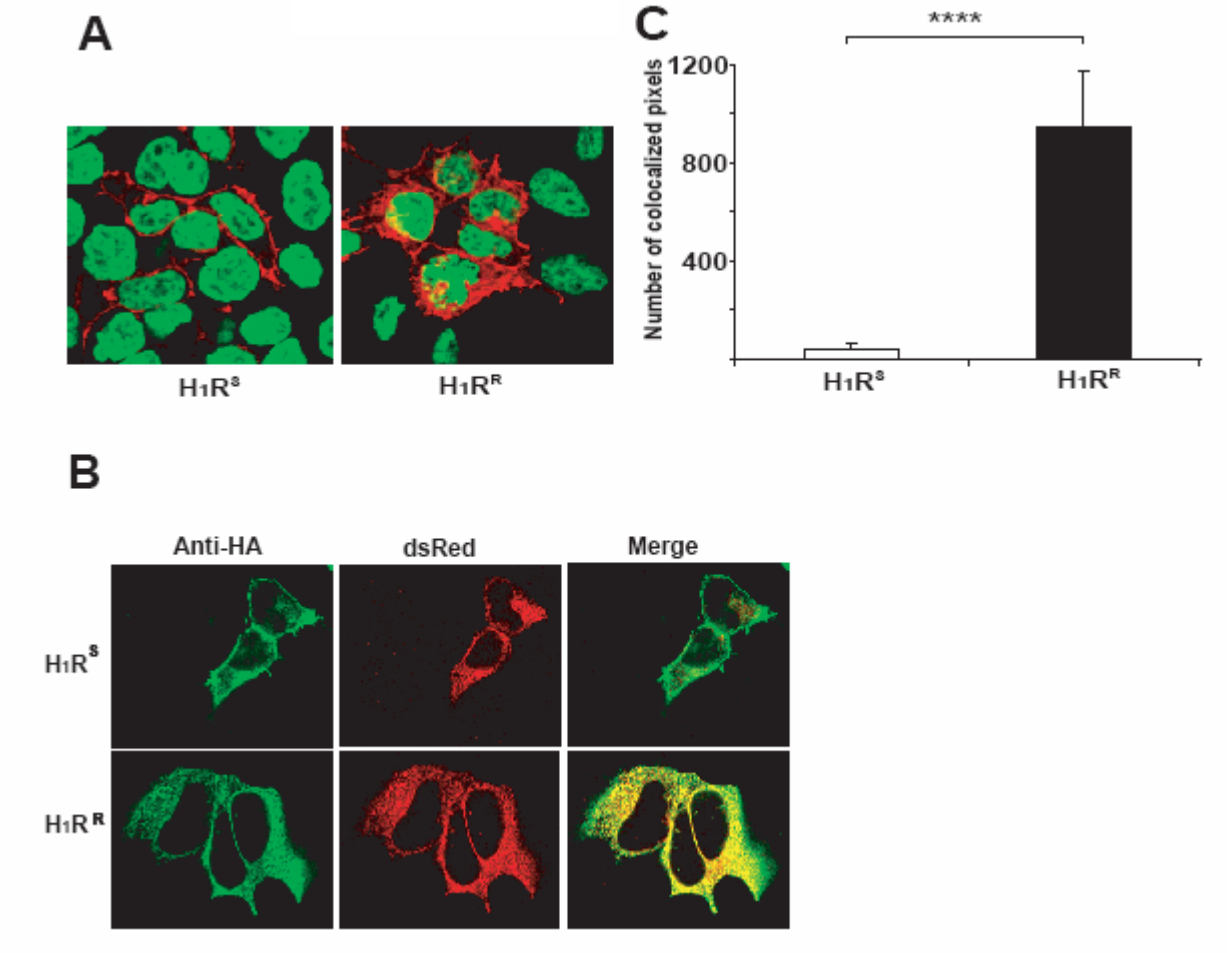


Figure 5. H_1R^R is retained in endoplasmic reticulum.

(A) HEK293T cells were transfected with HA-H_1R^S or HA-H_1R^R plasmids. 24h later, cells were fixed, permeabilized, stained with anti-HA mAb (red) and TOPRO-3 nuclear stain (green) and visualized by confocal microscopy. (C) HEK293T cells were co-transfected with pdsRed plasmid that express ER targeted florescent dsRed protein (red) and HA-H_1R^S or HA-H_1R^R. 24h later cells were fixed, permeabilized, stained with anti-HA mAb (green) and the co-localization of HA-H_1R with dsRed was visualized by confocal microscopy. Yellow color represents the co-localization of red and green colors. (D) Quantification of HA-H_1R colocalization with dsRed protein. Using Zeiss LSM 510 META Confocal imaging software the number of pixels expressing both colors were determined in a number of cells (n=36) and the data are presented as the average number of pixels that co-express dsRed and HA-H_1R. Error bars indicate SEM.

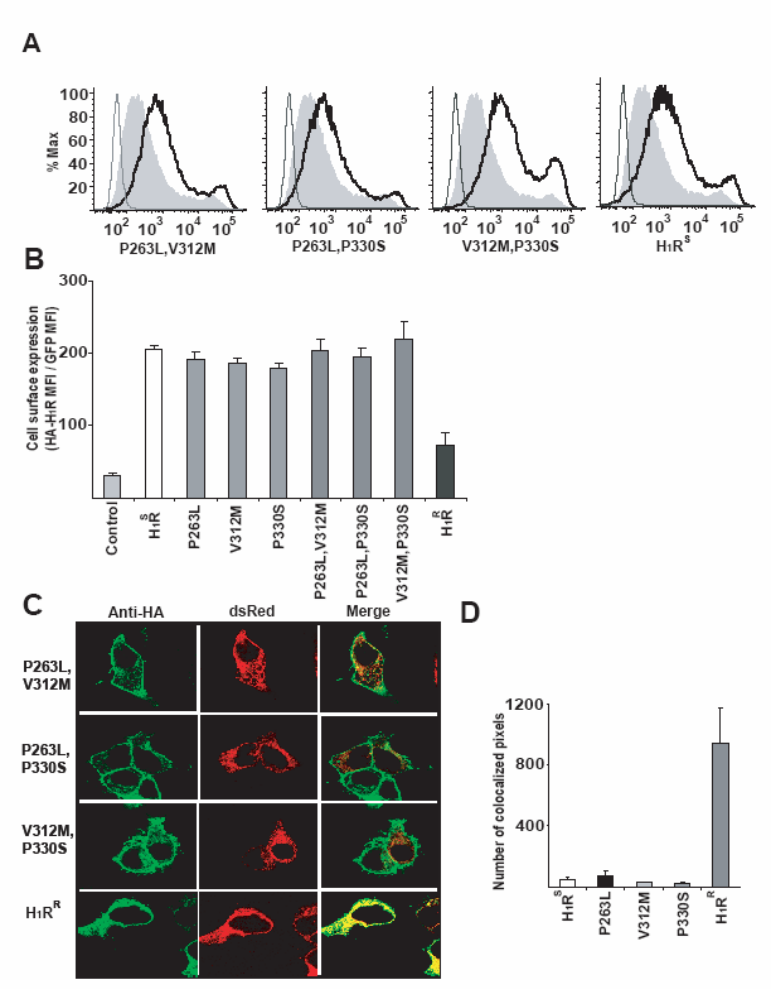


Figure 6. ER retention of H_1R^R requires all of its three polymorphic residues.

(**A**)HEK293T cells were transfected with empty control, HA-H_1R^S, single HA-H_1R^S mutants or HA-H1R^R plasmids. Cells were collected 16-24 h later without trypisinization, stained with anti-HA mAb and analyzed by flow cytometry. Thin line represents cells transfected with empty pEGZ, thick line represents cells transfected with HA-H_1R^S and the filled area represents cells transfected with HA-H_1R^R. (**B**) HEK293T cells were analyzed as in (**A**) and the mean florescence intensity of anti-HA on H_1R^S positive cells was determined. The data presented is the average of triplicate transfections. (**C**) HEK293T cells were co-transfected with pdsRed plasmid that express ER targeted dsRed protein (red) and HA-H_1R^S, mutants of HA-H_1R^S or HA-H_1R^R. 24h later, cells were fixed, permeabilized, stained with anti-HA mAb (green) and the co-localization of HA-H_1R with dsRed (red) was visualized by confocal microscopy. Yellow color represents the co-localization of red and green colors. (**D**) Quantification of HA-H_1R colocalization with dsRed protein. Using Zeiss LSM 510 META Confocal imaging software the number of pixels expressing both the colors was determined in a number of cells ($n \geq 16$) and the data is presented as the average of number of pixels that co-express dsRed and HA-H_1R. Error bars indicate SEM.

(A)

Strain	Incidence	Day of onset	Cumulative disease score	Severity index	Peak score
C57BL/6J	19/19	13.1±0.3	56.2±4.6	3.1±0.2	3.9±0.3
H1RKO	55/56	15.7±0.4	32.1±1.4	2.1±0.1	3.0±0.1
H1RKO-Tg^S	24/24	12.9±0.4	50.0±3.7	2.8±0.2	3.6±0.2
H1RKO-Tg^R	16/17	13.3±0.3	50.0±3.2	2.7±0.2	3.6±0.2
	$\chi 2 = 2.5$ $p = 0.5$	$F = 13.5$ $p < 0.0001$	21.2 < 0.0001	19.2 < 0.0001	14.4 < 0.0001
	C57BL/6J = H1RKO-Tg^S ≠ H1RKO-Tg^R = H1RKO				

(B)

Strain	Incidence	Day of onset	Cumulative disease score	Severity index	Peak score
C57BL/6J	18/18	16.6±0.7	37.6±2.9	2.6±0.1	3.2±0.2
H1RKO	26/33	17.1±0.5	20.0±1.8	1.6±0.1	2.2±0.1
H1RKO-Tg^S	22/23	16.2±0.6	36.4±3.8	2.5±0.2	3.2±0.2
H1RKO-Tg^R	13/14	18.7±0.6	18.6±3.1	1.6±0.2	1.9±0.2
	$\chi 2 = 7.4$ $p = 0.06$	$F = 2.8$ $p = 0.05$	11.6 < 0.0001	15.2 < 0.0001	14.0 < 0.0001
	C57BL/6J = H1RKO-Tg^S ≠ H1RKO-Tg^R = H1RKO				

Table 1. Clinical disease traits following imuunization of mice with (A) MOG_{35-55}+CFA + PTX and (B) 2x (MOG_{35-55}+CFA)

H_1R^S	
129 / SvJ	LEWES/EiJ
129S1/SvImJ	LG/J
129T2/SvEmsJ	LP/J
A/J	Ma/MyJ
A/WySnJ	MOR/RkJ
ALR/LtJ	NOD/LtJ
ALS/LtJ	NON/LtJ
B10.S/DvTe	NOR/LtJ
BALB / cByJ	NZO/HlLtJ
BALB/ cJ	P/J
BDP/J	PANCEVO/EiJ
BPH/2J	PERA/EiJ
BPL/1J	PERC/EiJ
BPN/3J	PL/J
BTBR T+ tf/J	RBF/DnJ
BXSB/MpJ	RIIIS/J
C57BL/10J	SB/LeJ
C57BL/10SnJ	SEA/GnJ
C57BL/6ByJ	SEC/1ReJ
C57BL/6J	SENCARA/PtJ
C57BLKS/J	SENCARB/PtJ
C57BR/cdJ	SENCARC/PtJ
C57L/J	SJL/J
C58/J	SJL/Bm
CALB/RkJ	SM/J
CE/J	SPRET/EiJ
DBA/1J	ST/bj
DBA/2J	SWR/ J
DDY/JclSidSeyFrkJ	SWXL-4/TyJ
EL/SuzSeyFrkJ	TIRANO/EiJ
FVB/NCr	WSB/EiJ
IS/CamRkJ	YBR/EiJ
KK/HIJ	ZALENDE/EiJ

H_1R^R
AKR/J
BPL/1J
C3H/HeJ
CASA/RkJ
CAST/EiJ
CBA/J
CZECHI/EiJ
CZECHI/EiJ
I/LnJ
JF1/Ms
MOLC/RkJ
MOLD/RkJ
MOLF/Eij
MRL/MpJ
MSM/Ms
PWK/PhJ
RF/J
SF/CamEiJ
SKIVE/EiJ

Table 2. The P-V-P and L-M-S haplotypes of H_1R are evolutionarily conserved in mice.

CHAPTER 4: CONCLUSIONS AND FUTURE DIRECTIONS

Complex diseases such as MS and EAE are governed by multiple susceptibility genes and the risk conferred by polymorphisms in their alleles. Identifying such "risk alleles" and gaining mechanistic understanding of their contribution in the disease development will yield newer targets for therapeutic intervention in ameliorating these diseases. Even though more than 40 QTLs (Becanovic et al., 2006) in EAE and polymorphisms in many of their candidate genes, such as IL-2 (Encinas et al., 1999), MCP-1 and MCP-5 (Butterfield et al., 1999), and Ncf-1 (Becanovic et al., 2006) have been identified, no study has systematically elaborated the pathways of their mechanisms. To date, only one QTL, originally identified as *Idd5.1* and encompassing the gene coding for costimulatory molecule ICOS-1, has been shown to modulate EAE. This is through the differential expression of ICOS-1 in activated CD4 T cells, with higher expression in the resistant NOD/J mice than the congenic mice expressing ICOS-1 from the susceptible C57BL/6J mice (Greve et al., 2004). Our laboratory had previously identified polymorphisms in *Hrh1*/H_1R and shown that it is a susceptibility gene in EAE (Ma et al., 2002). In this study, the role of these polymorphisms was delineated and shows that they affect H_1R functions by differential cell surface expression. Further, the molecular pathways, through which H_1R contributes to the pathogenesis of EAE were identified and demonstrate that H_1R influences EAE by regulating proinflammatory cytokine production by T cells.

H_1R is one of the widely expressed GPCRs and is present on multiple cells types involved in EAE pathogenesis (Parsons and Ganellin, 2006). This study demonstrates that H_1R expression in T cells is important and sufficient to influence the

susceptibility to EAE. Re-expressing the susceptible allele of H_1R (H_1R^S) in T cells of H1RKO mice complemented EAE severity and disease-associated cytokine production by these mice, but the resistant allele of H_1R (H_1R^R) failed to do so. This clearly show that the H_1R polymorphisms affect its ability to influence EAE pathogenesis and suggests that H_1R^R is not a functional receptor.

A functional GPCR such as H_1R, once synthesized, adopts a "native" structure that enables the receptor to pass through the quality control machinery of the endoplasmic reticulum (ER) and traffics to its target site, the cell surface (Spiegel and Weinstein, 2004). In transfected 293T cells, the polymorphisms in H_1R regulated the trafficking of the receptor with H_1R^R failing to reach its final compartment and being retained in ER. In contrast H_1R^S was efficiently trafficked and expressed on the cell surface. Thus, polymorphisms in H_1R^R join the group of a number of examples in which polymorphisms and mutations in GPCRs lead to their mislocalization and in many cases are associated with pathologies.

This study provides evidence that the polymorphisms in H_1R are evolutionarily conserved as haplotype blocks among several inbred strains of mice. The natural evolutionary history of the common house mouse suggests that, about a million years ago, its ancestors evolved into at least three well-developed subspecies that dominated different parts of the world (Wade and Daly, 2005). These include *M. m. domesticus* in Western Europe, *M. m. musculus* in Russia, Northern China and Eastern Europe and *M. m. castaneus* in West Asia, Southeast Asia and Southern China (Beck et al., 2000; Paigen, 2003). However, it is still unclear if the three subspecies diverged simultaneously or *M. m. musculus* and *M. m. castaneus* diverged from a common ancestor shortly after

the divergence of *M. m. domesticus* (Yang et al., 2007). Several thousand years ago, the Asian *M. m. musculus* and *M. m. castaneus* made their way to the Japanese Islands forming a new subspecies known as *M. m. molossinus* (Paigen, 2003). Because of their close cohabitation, hundreds of years ago, humans noted spontaneously arising coat colors in mice in Europe, China and Japan and domesticated several varieties as pets. Europeans even imported what were considered fancier coat colors from Asian regions and bred them with local mice to create few pools of mice. Some of these fancy mice were prized and even traded throughout Europe. With the rediscovery of Mendel's laws of inheritance, in the early twentieth century, geneticists such as Cuenot, Castle and Little started studying the discrete inheritance of the coat colors in mice. They received their animals from a large mice-breeding farm owned by Abbie Lathrop, whose colonies originated from European and Asian fancy mice. Castle and Little recognized the value of homozygous mice and established inbred strains of mice by brother x sister matings. DBA (having the coat color allele, dilute, brown and non-agouti) was the first inbred mouse strain developed in 1909. Since then, more than 450 inbred strains of mice have been established (Beck et al., 2000; Paigen, 2003). Therefore, most of the inbred laboratory mouse strains originated from a mixed, restricted number of founders. Based on this, the genomes of the common inbred mice strains were proposed to represent a mosaic of regions originating from different subspecies. *M. m. domesticus* is the major contributor while *M. m. musculus* and *M. m castaneus* are the minor contributors to the genetic background of these mice (Wade and Daly, 2005). This hypothesis was strengthened by the observations that the mitochondrial DNA in many inbred strains of mice was derived from *M. m. domesticus* while the Y-chromosome was derived from *M.*

m. musuculus (Bishop et al., 1985; Ferris et al., 1982). Further, it was noted that the musculus-type Y-chromosome originated from *M. m. molossinus* males (Nagamine et al., 1992). However, strains with Y-chromosome from *M. m. domesticus* have also been identified (Yang et al., 2007). Recently, the fine structure of this mosaic variation of the mouse genome is described (Wade et al., 2002; Yang et al., 2007). Strain-to-strain comparisons of single nucleotide polymorphisms (SNPs) revealed a long interspersed regions of high and low sequence identities. The segments genome with extremely high SNP variation is indicative of different subspecies origin and spanned only one-third of the genome. The segments of the genome covered with extremely low variation represent similar subspecies (*M. m. domesticus*) origin and spanned two thirds of the genome (Yang et al., 2007). However, another study reported a predominant introgression of *M. m. domesticus* segments at exceptionally high levels (86 to 96%) in the genomes of common laboratory strains while M. m. musculus contributed about 1-8 % and M. m. castaneus contributed only 1-2 % (Yang et al., 2007). Further, high-density sequence based studies identified three distinct genetic variation patterns indicative of the evolutionary origin; large monomorphic haplotypes representing a common ancestor, large polymorphic blocks representing recombination of two ancestor genomes, and large fragmented haplotype blocks representing greater complexity and multiple ancestor origin (Frazer et al., 2004; Sakai et al., 2005; Zhang et al., 2005). This study herein found that the H_1R alleles are conserved among several inbred strains of mice, including the wild-derived inbred strains, suggesting that the two haplotypes have evolutionarily co-evolved over a long period of time and reflect evolutionarily conserved functional differences. Future studies are necessary to ascertain the subspecies origin of the H_1R

haplotype block and examine if the haplotype block encompasses even a larger region of chromosome 6.

Traditionally, histamine was considered to be a mediator of the effector phase of EAE rather than the priming of autoreactive T cells (Bebo et al., 1996; Linthicum, 1982). This study exhibits that *Hrh1*/H_1R is expressed in unstimulated CD4 T cells but downregulated upon activation. Complementation of H_1R in naïve, but not in activated, H1RKO CD4 T was able to restore the ability of these cells to produce IFN-γ to the levels of WT CD4 T cells. These observations, along with the findings that mast cells exert their effect outside the CNS in EAE (Tanzola et al., 2003), suggests that histamine interaction with H_1R is important during the initial induction and priming of the naïve antigen-specific CD4 T cells in EAE pathogenesis.

H_1R has been previously implicated in the regulation of IFN-γ production (Banu and Watanabe, 1999; Bryce et al., 2006; Ma et al., 2002). In this study, it is evidenced that H_1R regulates IFN-γ by modulating activation of p38MAP kinase and T-bet. Further, it was found that histamine interaction with H_1R is required for p38 MAP kinase activation in TCR-stimulated CD4 T cells. However, how H1R activates p38 MAP kinase is unknown. p38 MAP kinase is normally activated through the upstream MAPKK, MKK3 and MKK6 (and MKK4 in response to some stimuli) (Kyriakis and Avruch, 2001). It has been documented that GADD45 proteins interact with MEKK4, an upstream kinase of MKK3 and MKK6 and thus activate p38 MAP kinase (Takekawa and Saito, 1998). Whether H_1R associates with GADD45 members and activates p38 MAP kinase needs to be investigated.

Overall, the results presented herein demonstrate the importance of

histamine and its interaction with H_1R as a significant immunomodulatory factor in EAE. *Hrh1*/H_1R is a shared susceptibility gene in EAE and experimental allergic orchitis (Ma et al., 2002). A shared genetic basis among different autoimmune diseases has been proposed and non-MHC candidate loci of several autoimmune or inflammatory disease are present as clusters in humans and in animal models (Becker et al., 1998). This suggests common susceptibility genes or tightly linked loci in multi-gene families. Thus, studying the role of H_1R in other autoimmune diseases may shed a light on its role as a common autoimmune modifier. Further, even though polymorphisms in human H_1R have not yet been reported, a syntenic region has been identified as a major risk factor in MS patients. Administration of antihistaminics in MS patients stabilized the disease progression (Dimitriadou et al., 2000). A retrospective epidemiological study found that the use of H_1R blockers reduced the risk of MS (Alonso et al., 2006). Together, all these observations suggest that pharmacological targeting of the H_1R may be useful early in the treatment of MS and other autoimmune inflammatory diseases.

APPNEDIX A: WEIBEL PALADE BODIES NEGATIVELY REGULATE BLOOD BRAIN BARRIER PERMEABILITY

AND BRAIN INFLAMMATION IN EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

Rajkumar Noubade[*], Roxana del Rio[*], Benjamin McElvany[*], James F. Zachary[†], Jason M. Millward[‡], Denisa D. Wagner[§], Elizabeth P. Blankenhorn[**], Cory Teuscher[*¶]

[*]Departments of Medicine and Pathology, University of Vermont, Burlington, VT 05405;

[†]Department of Pathobiology, University of Illinois at Urbana-Champaign, Urbana, IL 61802;

[‡]Montreal Neurological Institute, McGill University, Montreal, Qc H3A 2B4

[§]The CBR Institute for Biomedical Research and Department of Pathology, Harvard Medical School, Boston, MA 02115;

[**]Department of Microbiology and Immunology, Drexel University College of Medicine, Philadelphia, PA 19129;

[¶]To whom correspondence should be addressed at: C317 Given Medical Building, University of Vermont, Burlington, VT 05405.

E-mail: C.Teuscher@uvm.edu

Manuscript information:
Pages: 21
Figures: 8
Word and character counts
Abstract: 217
Characters: 37032

Abbreviations: BBB, blood brain barrier; CFA, complete Freund's adjuvant; CNS, central nervous system; EAE, experimental allergic encephalomyelitis; HA, histamine; VWF, von-Willebrand factor; VWFKO, von Willebrand factor knockout; WPB, Weibel-Palade bodies; MS, multiple sclerosis; MOG_{35-55}, myelin oligodendrocyte glycoprotein peptide-(35-55); PTX, pertussis toxin; SC, spinal cord; WT, wild-type.

Abstract

Weibel-Palade bodies (WPB) within endothelial cells (EC) are secretory granules that release von Willebrand Factor (VWF), P-selectin, chemokines and other stored molecules following exposure to histamine (HA). Mice with a disrupted VWF gene (VWFKO) have EC that are deficient in WPB. These mice were used to evaluate the role of this organelle in *Bordetella pertussis* toxin induced hypersensitivity to HA (Bphs), an intermediate phenotype associated with susceptibility to experimental allergic encephalomyelitis (EAE), the principal autoimmune model of multiple sclerosis. No significant differences in susceptibility to Bphs between wild-type and VWFKO mice were detected at three days; however, in VWFKO mice HA sensitivity persists significantly longer. Correspondingly, the onset of EAE was earlier, disease was more severe and blood brain barrier (BBB) permeability significantly increased in VWFKO mice compared to wild-type mice. Moreover, inflammation was selectively increased in the brains and not in the spinal cords of VWFKO mice compared to wild-type mice. Early increases in BBB permeability in VWFKO mice were not due to increased encephalitogenic T-cell activity because BBB permeability was significantly greater in adjuvant treated VWFKO mice compared to littermate mice immunized with encephalitogenic peptide plus adjuvants. Taken together, these data indicate that VWF and/or WPBs negatively regulate BBB permeability changes and autoimmune inflammatory lesion formation within the brain elicited by peripheral inflammatory stimuli.

Introduction

Pertussis toxin (PTX) is a major virulence factor of *Bordetella pertussis*, the causative agent of whooping cough (Bordet and Gengou, 1906). The holotoxin is a hexameric protein that conforms to the A/B model of bacterial exotoxins (Rappouli and Pizza, 1991). The A-subunit is an ADP-ribosyl transferase which affects signal transduction by ribosylation of the α-subunit of trimeric Gi/o proteins, and the β-oligomer of PTX binds cell surface receptors on a variety of mammalian cells (Kaslow and Burns, 1992). Intoxication with PTX elicits an array of physiological responses *in vivo* including increased blood brain barrier (BBB) permeability and sensitization of the vascular endothelium to vasoactive agents such as histamine (HA) (Locht, 1999; Munoz, 1985). Inbred strains of mice differ in susceptibility to challenge with vasoactive agents following sensitization with PTX in that genetically susceptible strains succumb to hypotensive and hypovolemic shock while resistant strains do not (Wardlaw, 1970). Additionally, the genetic control of susceptibility to lethal shock is agent specific (Gao et al., 2003). For example, PTX-induced vascular endothelial sensitization, controlled by *Bphs* (*Bordetella pertussis* induced HA sensitization), is detected by HA challenge, but not by serotonin challenge. *Bphs* is an autosomal dominant locus that we recently identified as the histamine H_1 receptor (*Hrh1*/H1R) (Kantarci et al., 2002). Importantly, susceptibility to experimental allergic encephalomyelitis (EAE), the principal autoimmune model of multiple sclerosis (MS) (Musio et al., 2006), is also controlled by *Bphs/Hrh1* (Kantarci et al., 2002; Linthicum, 1982), underscoring the role of genetic factors in regulating BBB permeability and susceptibility to inflammatory demyelinating diseases of the CNS.

The mechanism whereby PTX sensitizes the vascular endothelium to HA is unknown, but it is consistent with a two-step process: an induction phase, characterized by a 2- to 3-day latent period following intoxication, and an effector phase, manifest by rapid onset of lethal shock that usually occurs within ten minutes of HA challenge (Bergman and Munoz, 1968),(Munoz et al., 1981). Bphs is also characterized by a protracted period of sensitivity that persists upwards of 30 days (Munoz et al., 1981). The fact that sensitization of the vascular endothelium continues well beyond the likely half-life of the toxin *in vivo* suggests that the induction phase may be associated with the synthesis and storage of additional vasoactive factors within endothelial cells (ECs) that are released by exposure to HA during the effector phase. In this regard, it is known that following inflammation preformed KC (IL-8 homologue), eotaxin-3, von Willebrand Factor (VWF), P-selectin, CD63/lamp3, angiopoietin 2, endothelin-1, endothelin converting enzyme, tissue-type plasminogen activator (t-PA), factor XIIa and/or α1,3-fucosyltransferase VI can be stored in EC Weibel Palade bodies (WPBs) (Rondaij et al., 2006), and that HA is a secretagogue for the release of these agents (Hattori et al., 1989). Under a hypothetical two-step model, lethal shock would be due to the direct vasodilatory activity of HA combined with the effects of the stored products released from WPBs. In the absence of exposure to PTX, the ECs must be able to compensate for the effects of subsequently administered HA because most mice do not succumb following HA challenge alone. In contrast, EC would not be able to compensate for the increase in synergistic second messenger signaling arising from exposure to both HA and PTX-induced stored vasoactive factors. In this study, mice with a disrupted VWF gene (VWFKO) and a consequent deficiency in WPBs (Denis et al., 1998) were used to

directly test this hypothesis and to evaluate the role of WPBs in regulating BBB permeability and susceptibility to EAE. We report that, contrary to this model, WPBs suppress Bphs and adjuvant-induced alterations in BBB function associated with actively induced EAE.

Results

Bphs in VWFKO mice

The role of WPBs in Bphs was evaluated by i.v. sensitization of B6 and VWFKO mice with 200 ng PTX on day 0. Three days later HA sensitivity was assessed in a dose response fashion by i.v. challenge with HA and deaths were recorded at 30 min. A significant difference in the LD_{50} values between the two strains was not detected (B6 = 1.65 ± 0.05 mg/kg *vs*. VWFKO = 1.41 ± 0.08 mg/kg; $F = 0.55$, $p = 0.46$), indicating that neither VWF nor WPBs are required for Bphs susceptibility. Given the role of WPBs in vascular function, we nevertheless assessed their effect on the persistence of HA sensitivity. Compared to B6 mice, HA sensitivity persistent longer in VWFKO mice at all challenge doses studied ($F = 38.25$; $p < 0.0001$) (Fig. 1). The half-life of sensitization was 64.8 days in VWFKO mice compared 34.4 days in B6 mice at 100 mg/kg HA challenge. Similarly, the sensitization half-lives were 66.0 and 63.3 days in VWFKO mice at 50 mg/kg and 25mg/kg of HA respectively while the corresponding sensitization half-lives in WT mice were 34.2 and 30.7 days. Taken together, these data demonstrate that WPBs and/or VWF ordinarily act to shorten the longevity of HA sensitivity elicited by *in vivo* intoxication with PTX.

EAE in VWFKO mice

Because VWFKO mice exhibited significantly prolonged sensitivity to HA, and because *Bphs* is an EAE susceptibility gene (Kantarci et al., 2002), we studied the role of WPBs in regulating EAE induced by immunization with MOG_{35-55}+CFA+PTX. Compared to B6 mice, VWFKO mice developed significantly more severe clinical signs of EAE (Fig. 2). Clinical signs of EAE in VWFKO mice were notably enhanced

during the acute-early phase (D7 through D18 postimmunization) compared to the chronic late-phase of the disease (day 20-30) (Polanczyk et al., 2004). The mean day of disease onset in VWFKO mice was 14.1 ± 2.0 *vs*. 16.4 ± 3.2 ($p = 0.01$) in B6 mice. The acute-early phase cumulative disease score in VWFKO mice was 22.6 ± 11.1 *vs.* 13.3 ± 11.9 ($p = 0.008$) in B6 mice.

Histological analysis of CNS samples obtained during the acute-early phase of the disease revealed that VWFKO mice exhibited significantly greater pathology in the brain than did B6 mice (Fig. 3A-3E). VWFKO mice had a higher overall pathology index with significantly greater lesion scores, more severe demyelination, more severe suppuration, and more extensive mononuclear cell infiltrates compared to B6 mice. In contrast, however, no difference in the overall severity of the lesions between VWFKO and B6 mice was observed in the spinal cord (Fig. 3F). These results demonstrate that the absence of WPB and/or VWF selectively promotes lesion formation in the brain, compared to the spinal cord-dominant disease seen in mice with intact VWF and WPBs.

We examined a number of T cell parameters in B6 and VWFKO mice following sensitization with MOG_{35-55}+CFA+PTX in order to evaluate the encephalitogenic T cell response elicited in each strain. No difference in the proliferative response of spleen cells to MOG_{35-55} at day 10 p.i. was observed between B6 and VWFKO mice (Fig. 4A). Similarly, no significant differences in cytokine and chemokine expression following *ex vivo* restimulation with MOG_{35-55}, were detected between B6 and VWFKO animals (Fig. 4B, C). Taken together, these results indicate that the more severe acute-early phase disease seen in VWFKO mice is unlikely to be due to a direct effect of the absence of VWF or WPBs on T cell effector responses.

Increased BBB permeability in VWFKO mice during EAE

To delineate the mechanisms underlying the more severe acute-early phase of EAE in VWFKO mice, we analyzed EC function by measuring BBB permeability. A BBB permeability index (BBB-PI) was determined by measuring the traverse of systemically injected FITC-labelled bovine serum albumin (BSA) into the cerebrospinal fluid of EAE-induced mice at 8, 10 and 12 days p.i. (Fig. 5). BBB-PIs were not significantly different between unmanipulated B6 and VWFKO mice; however, BBB-PIs were significantly elevated in both mouse strains ($p = 0.01$ for B6 and $p < 0.001$ for VWFKO) following immunization with MOG_{35-55}+CFA+PTX. Moreover, the test of the main effect of group (mouse strain) showed a significant difference ($p = 0.02$) between the two strains over time, with the BBB-PI being greater in VWFKO mice compared to B6 mice.

The significant difference in clinical signs and brain pathology between B6 and VWFKO mice during the acute-early phase of the disease, despite the absence of detectable differences in encephalitogenic T cell effector responses, point to a potential role for WPBs in regulating the interface between the circulation and the brain. We therefore compared the integrity of the BBB in B6 and VWFKO mice at various time points after injection with MOG_{35-55}+CFA+PTX or CFA+PTX. Immunization with CFA+PTX alone lead to increased BBB permeability in B6 mice (Fig. 6A) to an extent that was not different from that seen following immunization with MOG_{35-55}+CFA+PTX ($p = 0.45$). The change in BBB permeability over time was also not significant in B6 mice (Fig. 6B) ($p = 0.08$). Similarly, immunization with CFA+PTX alone significantly increased the BBB permeability in VWFKO mice but again, there was no difference in

the BBB-PIs between the animals immunized with or without the encephalitogen ($p = 0.47$). This indicates that in both B6 and VWFKO mice, antigen-specific encephalitogenic T cells are not responsible for eliciting increased BBB permeability across the time points studied. Because the BBB-PIs between the animals immunized with or without MOG_{35-55} were not different, they were pooled and reanalyzed. The results revealed that the BBB-PI in VWFKO mice was significantly greater than the BBB-PI in B6 mice ($p = 0.001$) (Fig. 6C) when CFA and PTX are used as adjuvants, and that this difference is encephalitogen-independent.

To understand which component of the adjuvants (CFA or PTX) is responsible for increased BBB permeability, we determined the BBB-PIs in B6 and VWFKO mice injected with either CFA or PTX alone. Immunization with CFA alone increased BBB permeability in both B6 and VWFKO mice compared to unimmunized mice ($p < 0.001$ for both B6 and VWFKO) (Fig. 7A). The BBB-PI for CFA-immunized mice was significantly greater in VWFKO than in B6 mice ($p = 0.03$). Interestingly, the effect of CFA on BBB permeability was greater on day 8 and decreased over time, reaching almost basal levels both in B6 and VWFKO mice, indicating that ECs can overcome the CFA-induced inflammatory signals that compromise BBB integrity. Importantly, the decrease in CFA-induced BBB permeability over time was significant ($p < 0.0001$), but the rate of change was not different between strains.

PTX alone also independently increased BBB permeability in both B6 and VWFKO mice compared to untreated mice ($p = 0.0001$ for both B6 and VWFKO) (Fig. 7B). However, the effect of PTX on BBB permeability was different than that of CFA. BBB permeability changes induced by PTX did not vary over time ($p = 0.67$) and

remained elevated at each of the time points examined. However, as with CFA, the BBB-PI elicited by *in vivo* intoxication with PTX alone was significantly greater in VWFKO than in B6 mice ($p = 0.02$). These data suggest that BBB compromise and repair by ECs differs depending on the peripheral inflammatory stimulus. Therefore in order to assess if the ECs responses elicited independently by CFA and PTX are capable of cross regulating each other when administered simultaneously (CFA+PTX), we compared the BBB-PIs from animals receiving CFA and PTX alone with those from animals that received CFA+PTX. When CFA+PTX were injected at the same time, the BBB-PIs in both B6 (Fig. 8A) and VWFKO (Fig. 8B) were significantly lower compared to the BBB-PIs elicited by these agents separately. Although the CFA-induced increase in BBB permeability was greater at day 8 and decreased to basal levels by day 12 (conditions under which PTX-induced changes remain relatively constant), when the two agents were used together, the vascular permeability was, in contrast, lowest on day 8 with the BBB-PIs increasing to the PTX levels by day 12. These data support the concept that changes in BBB permeability elicited by CFA and PTX alone occur via different pathways and that the two pathways cross regulate each other with the overall differences in BBB permeability reflecting their integration over time. Moreover, these data indicate that WPBs and/or VWF protect against the vascular permeability changes induced by these inflammatory agents.

Discussion

In this study we show that the absence of WPBs and/or VWF leads to increased BBB permeability and, in appropriately immunized mice, concomitantly more severe EAE. The disruption of BBB integrity is due to adjuvants alone; and although the mechanism(s) by which CFA and PTX act to increase BBB permeability is unknown, these processes are independent of encephalitogenic T cell responses. Moreover, our study indicates that ECs respond differently to CFA and PTX with the outcome of simultaneous exposure being the integration of the different pathways over time. This is in agreement with previous studies examining the effects of inflammatory pain elicited by formalin, CFA and λ-carrageenan on BBB permeability (Brooks et al., 2005; Brooks et al., 2006). BBB permeability changes elicited by these stimuli were associated with unique alterations in the temporal expression of tight junctional proteins as well as disruption of the interaction between tight junctional complexes and the cytoskeleton. It is unclear what the mechanism(s) for tight junctional alterations following peripheral inflammation might be, but both CFA and PTX are known to lead to increased levels of IL-1β, TNF-α, and/or IL-6 within the periphery and CNS rapidly after exposure (Armstrong et al., 2003; Donnelly et al., 2001; Loscher et al., 2000; Raghavendra et al., 2004; Samad et al., 2001). Within the CNS the increase in IL-1β expression is associated with widespread changes in neuronal activity, including Cox-2 expression in CNS neurons leading to elevated prostaglandin E2 levels in the CSF (Ek et al., 2001). Consequently, alterations in BBB permeability might be subject to modulation via both peripheral and centrally mediated responses to inflammatory stimuli. In fact, we recently demonstrated that neurogenic control of BBB permeability is negatively

regulated by central histamine H_3 receptor signaling (Teuscher et al., 2007).

Alterations in BBB permeability can occur in a variety of different situations (Brooks et al., 2005), some of which relate directly to MS. There is an increasing body of evidence in both EAE (Tonra, 2002; Tonra et al., 2001) and MS (Mathews et al., 1993; Minagar et al., 2006) that subtle, progressive alterations in BBB integrity precede the formation of inflammatory lesions. These changes are detected in all MS disease subtypes, suggesting that a common abnormality in BBB function exists in the normal-appearing white matter of MS patients and may be a predisposing factor in initiating and propagating new inflammatory foci. Importantly, in our studies the WPB-related changes in BBB permeability also do not derive from T cell activity, indicating that WPBs play an important role in regulating BBB permeability in response to peripheral inflammatory stimuli. In this setting, understanding the mechanism of Bphs as a genetic model of BBB dysregulation is important.

The effector phase of Bphs is characterized by death due to hypotensive and hypovolemic shock within minutes of HA challenge (Bergman and Munoz, 1968). This time frame suggests that the effector phase may be associated with a sudden and rapid release of preformed factors generated during the sensitization phase rather than the induction of new gene expression by HA receptor signaling. Since inflammatory signals such as PTX induce the synthesis and storage of the vasoactive factors in the WPBs (Wolff et al., 1998) (Utgaard et al., 1998); (Utgaard et al., 1998) and because HA is a secretagogue for the release of these agents (Hattori et al., 1989), the shock following PTX sensitization and HA challenge could be due to the combined direct vasodilatory effects of HA and autocrine activity of the released stored products from WPBs.

However, our results in VWFKO mice demonstrate that the stored vasoactive factors in WPBs are not responsible for the sensitization and that VWF and/or other WPB components are instead protective in this model.

This observation was surprising given the fact that WPBs store several factors that are good candidates for the observed trait, the sudden release of which could change endothelial function in an autocrine fashion. WPBs are dynamic granules with very random movements in resting cells (Romani de Wit et al., 2003). WPBs are released from ECs in response to a large number of secretagogues, which can be divided into 2 distinct groups: those that act by elevating intracellular calcium levels such as thrombin and HA and those that act by raising cAMP levels such as epinephrine and vasopressin (Rondaij et al., 2006). Calcium raising agonists induce a periphery–directed movement of WPBs while cAMP-mediated agonists induce a transport-directed redistribution towards the center of the cell leading to a star-like cluster at the perinuclear region (Vischer et al., 2000). WPB clustering is believed to prevent excessive release of WPB constituents, and may also lead to a selective exclusion of subsets of WPBs from exocytosis. Clustering is induced by thrombin stimulation in human aortic EC (Vinogradova et al., 2000) but not in HUVECs (Rondaij et al., 2006). In HUVECs, PTX can inhibit both VWF and tissue plasminogen activator (tPA) release from WPBs in response to thrombin, most likely due to inhibition of calcium influx as well as inhibit HA-induced calcium release (van den Eijnden-Schrauwen et al., 1997). PTX causes ADP ribosylation of Gi/o family of G proteins that are negative regulators of Gαs protein and its subsequent activation of adenylate cyclase. Through this mechanism, PTX treatment leads to increased intracellular cAMP concentration (Wettschureck and Offermanns,

2005),(Mittra and Bourreau, 2006), (Sugden et al., 2004; Zawilska et al., 2004). Hence, it is possible that the suppression of Bphs observed in mice that have VWF and WPBs could be due to PTX-induced, cAMP-mediated clustering of WPBs and sequestration of their contents in B6 mice and not in VWFKO mice.

Several studies have provided evidence in support of the existence of different subsets of WPBs that apart from VWF do not contain the same set of additional constituents (Fiedler et al., 2004; Oynebraten et al., 2004; Utgaard et al., 1998; Wolff et al., 1998). It has also been reported that different stimuli induce different WPB responses, depending on the physiological need. In the case of vascular damage, thrombin induces a rapid, local response leading to exocytosis of the WPBs while epinephrine induces a gradual release of WPBs (Vischer et al., 2000). Moreover, epinephrine induces the exocytosis of only the peripheral WPBs whereas thrombin stimulates the exocytosis of peripheral, as well as central WPBs (Vischer et al., 2000). This difference in release pattern and the fact that different stimuli, such as thrombin and epinephrine, induce WPB exocytosis via distinct mechanisms enables the cell to regulate the exocytosis of WPBs, and possibly the release of specific WPB constituents, in such a way that it meets the patho-physiological requirements induced by distinct triggers (Burgoyne and Morgan, 2003). Therefore in an inflammatory disease as complex as EAE, stored factors in WPBs may regulate a fine balance between pro-inflammatory and anti-inflammatory responses while in animals that lack WPBs this regulation is lost, which may explain increased susceptibility of VWFKO mice to EAE. In addition, VWF is an adhesion molecule for leukocytes (Pendu et al., 2006) that may be involved in their recruitment (Wagner DD, unpublished data). VWF has also been reported to mediate clearance of metastatic tumor

cells in lungs through a mechanism still not known (Pendu et al., 2006). Therefore, it is possible that VWF may be promoting the clearance of pathogenic autoimmune cells or proinflammatory debris in the CNS of B6 mice leading to lesser CNS inflammation than in VWF deficient mice.

The mechanism of BBB disruption in MS and EAE is unknown. Currently it is believed that neuroantigen-specific T cells within the systemic circulation interacting with ECs bring about the changes that lead to the formation of inflammatory foci and promote BBB permeability (Wingerchuk et al., 2001). Our results, however, indicate that disruption of the BBB in active EAE is independent of the encephalitogenic T cell responses and that this is caused by the interaction of EC with peripheral inflammatory stimuli such as CFA and PTX, and may also include neuropathic and inflammatory pain (Brooks et al., 2005; Inoue, 2006), which can be a major component of most forms of MS including benign disease (Glad et al., 2006). Overall, our study demonstrates that the interaction of EC with environmental agents other than those that lead to pathogenic T cell responses also influence the development of autoimmune disease by modifying BBB permeability. Taken together our findings underscore the potential importance of co-infection and/or non-autoimmune related gene-by-environment interactions in the etiology of MS.

Materials and Methods

Animals. B6.129S2-*Vwf*^{tm1Wgr} (VWFKO) mice (Denis et al., 1998) were maintained in the vivarium of the Given Medical Building at the University of Vermont (Burlington, VT). C57BL/6J (B6) mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Animals were fed RMH 3000 Lab Diet Rodent Chow (Ralston-Purina, St. Louis, MO) and tap water ad libitum and maintained in accordance with the Animal Welfare Act and the Public Health Service Policy on the Humane Care and Use of Laboratory Animals. The experimental procedures used in this study were approved by the Animal Care and Use Committee of the University of Vermont.

Pertussis toxin *in vivo* intoxication. Mice were injected intravenously (i.v.) with purified PTX (List Biological Laboratories, Inc.) in 0.025 M Tris buffer containing 0.5 M NaCl and 0.017% Triton X-100, pH 7.6. Control animals received carrier.

Histamine sensitivity testing. HA sensitivity was determined by the i.v. injection of amounts of HA (mg/kg dry weight free base) suspended in PBS. Deaths were recorded at 30 min post challenge. The results are expressed as the number of deaths over the number of animals studied.

Induction and evaluation of EAE. EAE was induced as previously described (Teuscher et al., 2006a). Briefly, mice were injected subcutaneously in the flanks and neck with 0.1 ml of an emulsion containing 200 μg of myelin oligodendrocyte glycoprotein 35-55 (MOG_{35-55}) (Beckman Institute, Palo Alto, CA) in saline and an equal volume of complete Freund's adjuvant (CFA) containing 200 μg of *Mycobacterium tuberculosis* H37RA (Difco Laboratories, Detroit, MI). On the day of immunization, each mouse received 200 ng of PTX (List Biological Laboratories Inc., Campbell,

CA) by i.v. injection. The mice were assessed daily for clinical signs of EAE using the following scale: 0, normal; 1, limp tail or mild hind limb weakness; 2, moderate hind limb weakness or mild ataxia; 3, moderately severe hind limb weakness; 4, severe hind limb weakness or mild forelimb weakness or moderate ataxia; 5, paraplegia with no more than moderate forelimb weakness; 6, paraplegia with severe forelimb weakness or severe ataxia or moribund condition

Brains and spinal cords (SC) were dissected from calvaria and vertebral columns, respectively, and fixed by immersion in 10% phosphate-buffered formalin (pH 7.2). Following adequate fixation, brain and SC were trimmed and representative transverse section embedded in paraffin, sectioned at 5 μm, and mounted on glass slides. Sections were stained with hematoxylin and eosin for routine evaluation and Luxol fast blue-periodic acid Schiff for demyelination. Sections from representative areas of the brain and SC were scored in a semi-quantitative fashion for the various histopathologic parameters, as previously described (Teuscher et al., 2006a).

Blood brain barrier permeability determinations. Blood brain barrier (BBB) permeability was assessed as previously described (Tang et al., 1996). Briefly, a 50 μg/g dose of FITC-labeled BSA (Sigma, St. Louis, MO) was injected i.v. into B6 and VWFKO mice on day 8, 10 or 12 post immunization with CFA+PTX+MOG_{35-55}, CFA+PTX, CFA or PTX. Cerebrospinal fluid and blood were collected after 4 h. Both CSF and plasma samples, prepared by centrifugation at 3000 rpm for 15 min, were diluted in PBS, and the fluorescence intensity was measured with a microplate fluorescence reader (Flx-800-I, Bio-Tek Instruments Inc, Winooski, VT) using the software KC-4, with an excitation wavelength of 485 nm and an emission wavelength of

528 nm. The BBB permeability index is expressed as the ratio of the fluorescence intensity of the CSF divided by the fluorescence intensity of the plasma.

Statistical analysis. Statistical analyses, as indicated in the figure legends, were performed using GraphPad Prism 4 software (GraphPad software Inc, San Diego, CA).

Acknowledgements

This work was supported by the National Institutes of Health (grants NS36526, AI45105, AI41747, and AI45666) and the National Multiple Sclerosis Society (grant RG-3575).

References

Armstrong, M.E., Loscher, C.E., Lynch, M.A., and Mills, K.H. (2003). IL-1beta-dependent neurological effects of the whole cell pertussis vaccine: a role for IL-1-associated signalling components in vaccine reactogenicity. J Neuroimmunol *136*, 25-33.

Bordet, J., and Gengou, O. (1906). Le microbe de la coqueluche, Vol 20 (Ann Inst Pasteur).

Brooks, T.A., Ocheltree, S.M., Seelbach, M.J., Charles, R.A., Nametz, N., Egleton, R.D., and Davis, T.P. (2006). Biphasic cytoarchitecture and functional changes in the BBB induced by chronic inflammatory pain. Brain Res *1120*, 172-182.

Burgoyne, R.D., and Morgan, A. (2003). Secretory granule exocytosis. Physiol Rev *83*, 581-632.

Denis, C., Methia, N., Frenette, P.S., Rayburn, H., Ullman-Cullere, M., Hynes, R.O., and Wagner, D.D. (1998). A mouse model of severe von Willebrand disease: defects in hemostasis and thrombosis. Proc Natl Acad Sci U S A *95*, 9524-9529.

Donnelly, S., Loscher, C.E., Lynch, M.A., and Mills, K.H. (2001). Whole-cell but not acellular pertussis vaccines induce convulsive activity in mice: evidence of a role for toxin-induced interleukin-1beta in a new murine model for analysis of neuronal side effects of vaccination. Infect Immun *69*, 4217-4223.

Ek, M., Engblom, D., Saha, S., Blomqvist, A., Jakobsson, P.J., and Ericsson-Dahlstrand, A. (2001). Inflammatory response: pathway across the blood-brain barrier. Nature *410*, 430-431.

Fiedler, U., Scharpfenecker, M., Koidl, S., Hegen, A., Grunow, V., Schmidt, J.M., Kriz, W., Thurston, G., and Augustin, H.G. (2004). The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. Blood *103*, 4150-4156.

Gao, J.F., Call, S.B., Fillmore, P.D., Watanabe, T., Meeker, N.D., and Teuscher, C. (2003). Analysis of the role of Bphs/Hrh1 in the genetic control of responsiveness to pertussis toxin. Infect Immun *71*, 1281-1287.

Glad, S., Nyland, H., and Myhr, K.M. (2006). Benign multiple sclerosis. Acta Neurol Scand Suppl *183*, 55-57.

Hattori, R., Hamilton, K.K., Fugate, R.D., McEver, R.P., and Sims, P.J. (1989). Stimulated secretion of endothelial von Willebrand factor is accompanied by rapid redistribution to the cell surface of the intracellular granule membrane protein GMP-140. J Biol Chem *264*, 7768-7771.

Hawkins, B.T., and Davis, T.P. (2005). The blood-brain barrier/neurovascular unit in

health and disease. Pharmacol Rev *57*, 173-185.

Inoue, K. (2006). ATP receptors of microglia involved in pain. Novartis Found Symp *276*, 263-272; discussion 273-281.

Kaslow, H.R., and Burns, D.L. (1992). Pertussis toxin and target eukaryotic cells: binding, entry, and activation. Faseb J *6*, 2684-2690.

Linthicum, D.S., and Frelinger, J.A. (1982). Acute autoimmune encephalomyelitis in mice. II. Susceptibility is controlled by the combination of H-2 and histamine sensitization genes. J Exp Med *156*, 31-40.

Locht, C. (1999). Molecular aspects of Bordetella pertussis pathogenesis. Int Microbiol *2*, 137-144.

Loscher, C.E., Donnelly, S., Lynch, M.A., and Mills, K.H. (2000a). Induction of inflammatory cytokines in the brain following respiratory infection with Bordetella pertussis. J Neuroimmunol *102*, 172-181.

Loscher, C.E., Donnelly, S., Mills, K.H., and Lynch, M.A. (2000b). Interleukin-1beta-dependent changes in the hippocampus following parenteral immunization with a whole cell pertussis vaccine. J Neuroimmunol *111*, 68-76.

Ma, R.Z., Gao, J., Meeker, N.D., Fillmore, P.D., Tung, K.S., Watanabe, T., Zachary, J.F., Offner, H., Blankenhorn, E.P., and Teuscher, C. (2002). Identification of Bphs, an autoimmune disease locus, as histamine receptor H1. Science *297*, 620-623.

Mathews, P.M., Andermann, F., Silver, K., Karpati, G., and Arnold, D.L. (1993). Proton MR spectroscopic characterization of differences in regional brain metabolic abnormalities in mitochondrial encephalomyopathies. Neurology *43*, 2484-2490.

Minagar, A., Jy, W., Jimenez, J.J., and Alexander, J.S. (2006). Multiple sclerosis as a vascular disease. Neurol Res *28*, 230-235.

Mittra, S., and Bourreau, J.P. (2006). Gs and Gi coupling of adrenomedullin in adult rat ventricular myocytes. Am J Physiol Heart Circ Physiol *290*, H1842-1847.

Munoz, J., and Bergman, R.K. (1968). Histamine-sensitizing factors from microbial agents, with special reference to Bordetella pertussis. Bacteriol Rev *32*, 103-126.

Munoz, J.J. (1985). Biological activities of pertussigen (pertussis toxin) (Orlando, FL: Academic Press).

Munoz, J.J., Arai, H., Bergman, R.K., and Sadowski, P.L. (1981). Biological activities of crystalline pertussigen from Bordetella pertussis. Infect Immun *33*, 820-826.

Oynebraten, I., Bakke, O., Brandtzaeg, P., Johansen, F.E., and Haraldsen, G. (2004).

Rapid chemokine secretion from endothelial cells originates from 2 distinct compartments. Blood *104*, 314-320.

Pendu, R., Terraube, V., Christophe, O.D., Gahmberg, C.G., de Groot, P.G., Lenting, P.J., and Denis, C.V. (2006). P-selectin glycoprotein ligand 1 and beta2-integrins cooperate in the adhesion of leukocytes to von Willebrand factor. Blood *108*, 3746-3752.

Polanczyk, M., Yellayi, S., Zamora, A., Subramanian, S., Tovey, M., Vandenbark, A.A., Offner, H., Zachary, J.F., Fillmore, P.D., Blankenhorn, E.P.*, et al.* (2004). Estrogen receptor-1 (Esr1) and -2 (Esr2) regulate the severity of clinical experimental allergic encephalomyelitis in male mice. Am J Pathol *164*, 1915-1924.

Raghavendra, V., Tanga, F.Y., and DeLeo, J.A. (2004). Complete Freunds adjuvant-induced peripheral inflammation evokes glial activation and proinflammatory cytokine expression in the CNS. Eur J Neurosci *20*, 467-473.

Rappouli, R., and Pizza, M. (1991). Structure and evolutionary aspects of ADP-ribosylating toxins (London: Academic press).

Romani de Wit, T., Rondaij, M.G., Hordijk, P.L., Voorberg, J., and van Mourik, J.A. (2003). Real-time imaging of the dynamics and secretory behavior of Weibel-Palade bodies. Arterioscler Thromb Vasc Biol *23*, 755-761.

Rondaij, M.G., Bierings, R., Kragt, A., Gijzen, K.A., Sellink, E., van Mourik, J.A., Fernandez-Borja, M., and Voorberg, J. (2006a). Dynein-dynactin complex mediates protein kinase A-dependent clustering of Weibel-Palade bodies in endothelial cells. Arterioscler Thromb Vasc Biol *26*, 49-55.

Rondaij, M.G., Bierings, R., Kragt, A., van Mourik, J.A., and Voorberg, J. (2006b). Dynamics and plasticity of Weibel-Palade bodies in endothelial cells. Arterioscler Thromb Vasc Biol *26*, 1002-1007.

Samad, T.A., Moore, K.A., Sapirstein, A., Billet, S., Allchorne, A., Poole, S., Bonventre, J.V., and Woolf, C.J. (2001). Interleukin-1beta-mediated induction of Cox-2 in the CNS contributes to inflammatory pain hypersensitivity. Nature *410*, 471-475.

Steinman, L., and Zamvil, S.S. (2006). How to successfully apply animal studies in experimental allergic encephalomyelitis to research on multiple sclerosis. Ann Neurol *60*, 12-21.

Sugden, D., Davidson, K., Hough, K.A., and Teh, M.T. (2004). Melatonin, melatonin receptors and melanophores: a moving story. Pigment Cell Res *17*, 454-460.

Tang, T., Frenette, P.S., Hynes, R.O., Wagner, D.D., and Mayadas, T.N. (1996). Cytokine-induced meningitis is dramatically attenuated in mice deficient in endothelial selectins. J Clin Invest *97*, 2485-2490.

Terraube, V., Pendu, R., Baruch, D., Gebbink, M.F., Meyer, D., Lenting, P.J., and Denis, C.V. (2006). Increased metastatic potential of tumor cells in von Willebrand factor-deficient mice. J Thromb Haemost *4*, 519-526.

Teuscher, C., Noubade, R., Spach, K., McElvany, B., Bunn, J.Y., Fillmore, P.D., Zachary, J.F., and Blankenhorn, E.P. (2006). Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A *103*, 8024-8029.

Teuscher, C., Subramanian, M., Noubade, R., Gao, J.F., Offner, H., Zachary, J.F., and Blankenhorn, E.P. (2007). Central histamine H3 receptor signaling negatively regulates susceptibility to autoimmune inflammatory disease of the CNS. Proc Natl Acad Sci U S A *104*, 10146-10151.

Tonra, J.R. (2002). Cerebellar susceptibility to experimental autoimmune encephalomyelitis in SJL/J mice: potential interaction of immunology with vascular anatomy. Cerebellum *1*, 57-68.

Tonra, J.R., Reiseter, B.S., Kolbeck, R., Nagashima, K., Robertson, R., Keyt, B., and Lindsay, R.M. (2001). Comparison of the timing of acute blood-brain barrier breakdown to rabbit immunoglobulin G in the cerebellum and spinal cord of mice with experimental autoimmune encephalomyelitis. J Comp Neurol *430*, 131-144.

Utgaard, J.O., Jahnsen, F.L., Bakka, A., Brandtzaeg, P., and Haraldsen, G. (1998). Rapid secretion of prestored interleukin 8 from Weibel-Palade bodies of microvascular endothelial cells. J Exp Med *188*, 1751-1756.

van den Eijnden-Schrauwen, Y., Atsma, D.E., Lupu, F., de Vries, R.E., Kooistra, T., and Emeis, J.J. (1997). Involvement of calcium and G proteins in the acute release of tissue-type plasminogen activator and von Willebrand factor from cultured human endothelial cells. Arterioscler Thromb Vasc Biol *17*, 2177-2187.

Vinogradova, T.M., Roudnik, V.E., Bystrevskaya, V.B., and Smirnov, V.N. (2000). Centrosome-directed translocation of Weibel-Palade bodies is rapidly induced by thrombin, calyculin A, or cytochalasin B in human aortic endothelial cells. Cell Motil Cytoskeleton *47*, 141-153.

Vischer, U.M., Barth, H., and Wollheim, C.B. (2000). Regulated von Willebrand factor secretion is associated with agonist-specific patterns of cytoskeletal remodeling in cultured endothelial cells. Arterioscler Thromb Vasc Biol *20*, 883-891.

Wardlaw, A.C. (1970). Inheritance of responsiveness to pertussis HSF in mice. Int Arch Allergy Appl Immunol *38*, 573-589.

Wettschureck, N., and Offermanns, S. (2005). Mammalian G proteins and their cell type specific functions. Physiol Rev *85*, 1159-1204.

Wingerchuk, D.M., Lucchinetti, C.F., and Noseworthy, J.H. (2001). Multiple sclerosis: current pathophysiological concepts. Lab Invest *81*, 263-281.

Wolff, B., Burns, A.R., Middleton, J., and Rot, A. (1998). Endothelial cell "memory" of inflammatory stimulation: human venular endothelial cells store interleukin 8 in Weibel-Palade bodies. J Exp Med *188*, 1757-1762.

Zawilska, J.B., Rosiak, J., and Nowak, J.Z. (2004). Pertussis toxin-sensitive G protein modulates the ability of histamine to stimulate cAMP production in the chick pineal gland. Pol J Pharmacol *56*, 407-413.

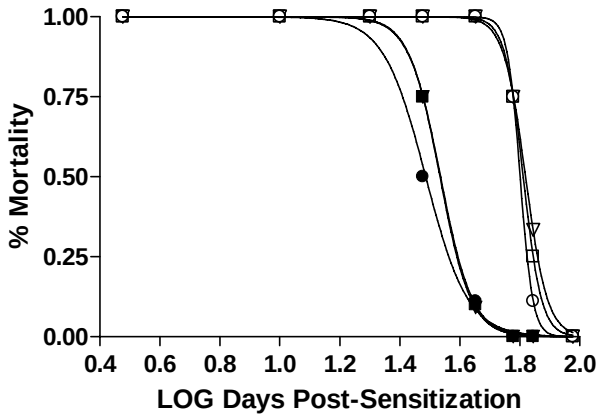


Figure 1. Assessment of Bphs in B6 and VWFKO.

Mice were sensitized with 200 ng purified PTX by i.v. injection on day 0. Mice were challenged with HA (mg dry weight free base) by i.v. injection on the indicated day and deaths were recorded at 30 min post challenge. The results are expressed as the number of animals dead over the number of animals studied (% mortality).

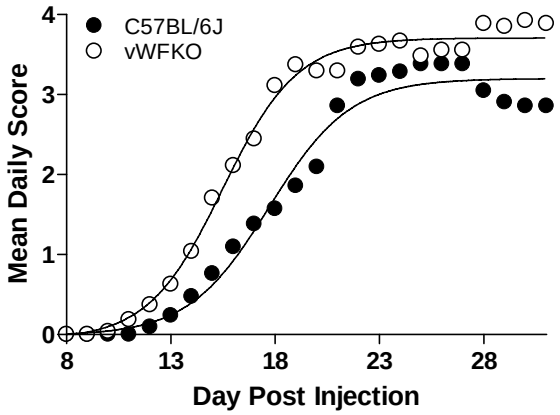


Figure 2. Early onset and severe clinical course of EAE in VWFKO mice.

EAE was induced in B6 (n = 18) and VWFKO (n = 27) mice by immunization with MOG_{35-55}+CFA+PTX. Regression analysis (Teuscher et al., 2006a) indicates that the disease course in both strains fits a variable slope sigmoidal curve and is significantly different between the two strains ($F = 32.5$; $p < 0.0001$).

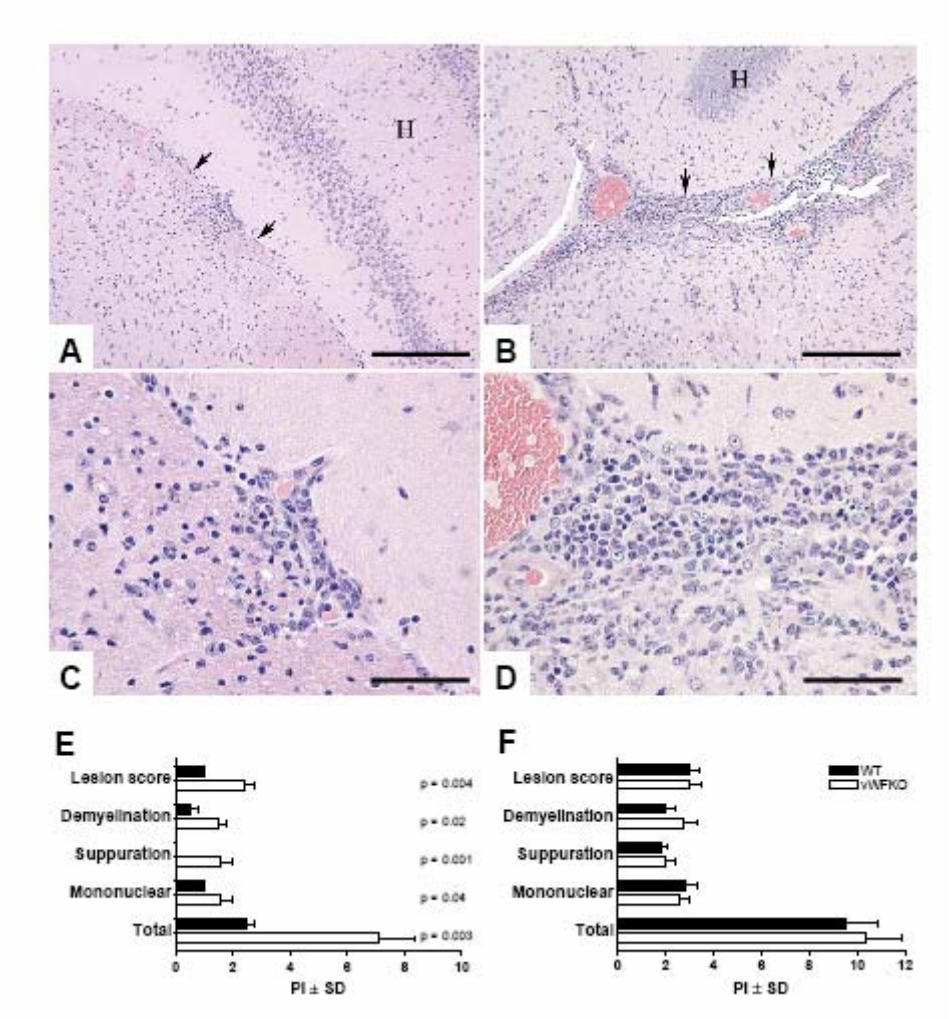


Figure 3. Severe histopathological EAE in VWFKO brain.

Comparison of the histopathologic lesions caused by EAE in the brains of B6 (**A, C**) and VWFKO (**B, D**) strains of mice. Note the minimal inflammatory response around capillaries and post capillary venules in the interface area between the brainstem and hippocampal formation (H) in the B6 brain than the marked inflammatory response in VWFKO brain. The inflammatory cells consisted of an admixture of lymphocytes and monocytes with occasional neutrophils and rare eosinophils. There was no primary demyelination in these areas of inflammation in B6 while minimal to mild primary demyelination was observed in VWFKO brain. H&E stain, scale bar = 200 μm. (**B and D**) higher magnification of B6 and VWFKO mouse brain, respectively. H&E stain, scale bar = 200 μm. Quantification of lesion severity in B6 and VWFKO mice revealed that the lesions in the brains (**E**) but not in SC (**F**) of MOG_{35-55}+CFA+PTX immunized VWFKO mice are more severe compared to WT controls. Significance of differences was determined using the Student's t-test.

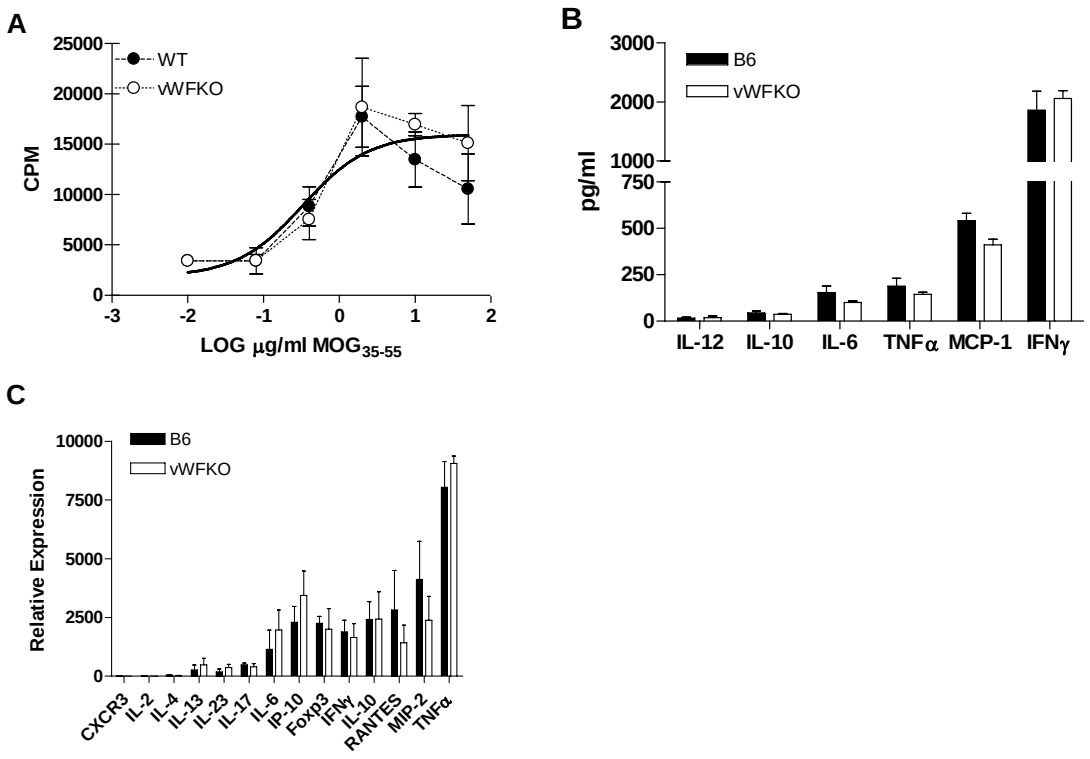


Figure 4. Normal T cell responses in EAE induced B6 and VWFKO mice.

(**A**) B6 and VWFKO CD4 T cells have equivalent *ex vivo* proliferative responses to MOG_{35-55} ten days following immunization with MOG_{35-55}+CFA+PTX. Mean CPM ± SD were calculated from triplicate wells. (**B**) Production and/or (**C**) expression of cytokines/chemokines by MOG_{35-55} stimulated splenocytes does not differ between B6 and VWFKO mice immunized with MOG_{35-55}+CFA+PTX ten days earlier. Cytokine production was determined by ELISA or cytometric bead assay and the expression were determined by real time RT-PCR. Significance of differences between B6 and VWFKO CD4 T-cells responses was determined using the Student's t-test with a p-value of 0.05 as the significance threshold.

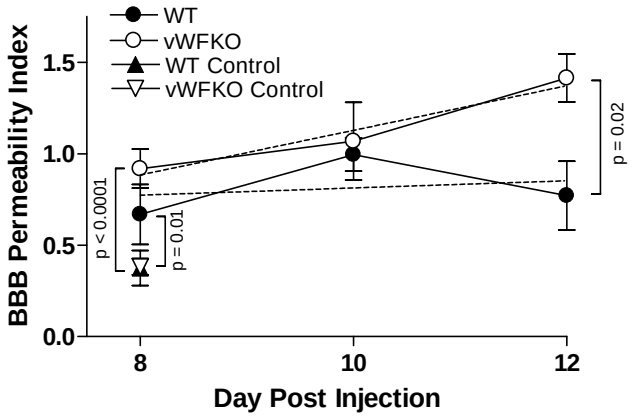


Figure 5. VWFKO mice exhibit increased BBB permeability compared to B6 mice following injection with MOG_{35-55}+CFA+PTX.

The permeability indices were calculated by determining the fluorescence in the CSF and the plasma collected 4h after i.v. injection of FITC-BSA and is the ratio of the fluorescence intensity of the CSF divided by the fluorescence intensity of the plasma. Changes in BBB permeability differ significantly over time ($F = 5.61$; $p = 0.02$).

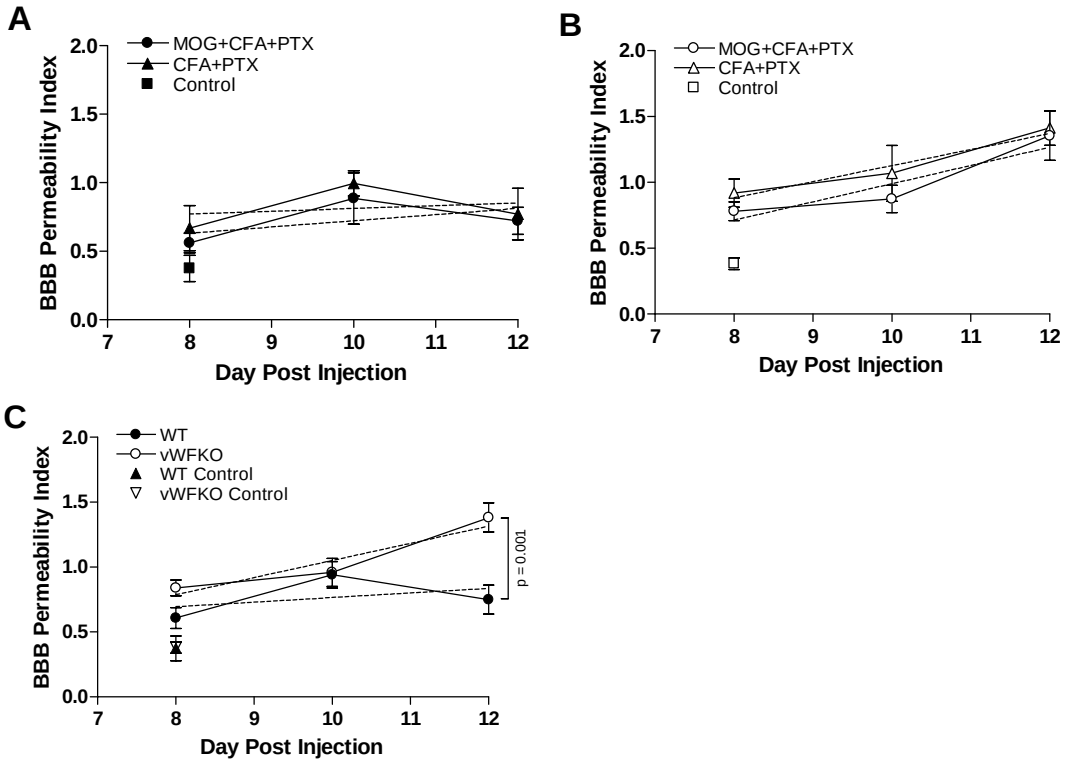


Figure 6. Increased BBB permeability in VWFKO mice is independent of encephalitogenic T cells.

BBB permeability in MOG_{35-55}+CFA+PTX and CFA+PTX immunized B6 (**A**), VWFKO mice (**B**). There was no difference in BBB permeability by treatment ($p = 0.45$) or over time ($p = 0.08$) in B6 animals while there was a significant difference in vWFKO animals over time ($p = 0.003$) but not by treatment ($p = 0.47$). Since there was no difference between MOG_{35-55}+CFA+PTX and CFA+PTX immunized animals, the data was pooled and re-analyzed (**C**). Changes in BBB permeability differ significantly over time between B6 and VWFKO mice ($p = 0.001$).

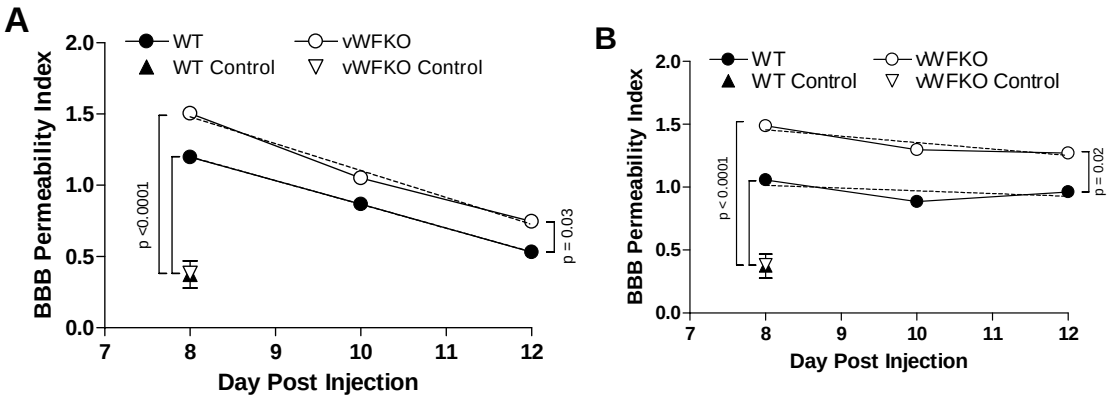


Figure 7. **BBB compromise and repair by endothelial cells differs depending on the peripheral inflammatory stimulus**.

BBB permeability in WT and VWFKO animals immunized with CFA alone (**A**), and PTX alone (**B**). Changes in BBB permeability differ significantly between WT and VWFKO mice ($p<0.05$).

Figure 8

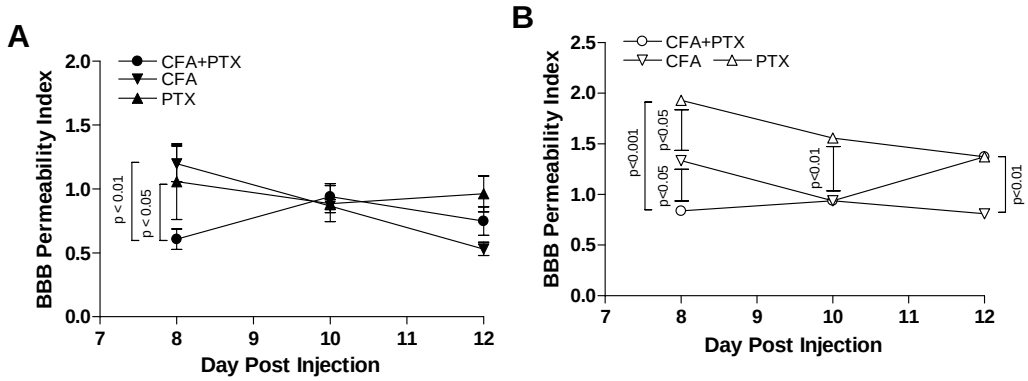


Figure 8. Comparison of BBB permeability in animals immunized with components of adjuvants either alone or in combination.

BBB indices in B6 (**A**) and VWFKO mice (**B**) immunized with CFA or PTX or both CFA+PTX. The p-values for the different interactions are shown.

COMPREHENSIVE BIBLIOGRAPHY

Abbott, N.J., Ronnback, L., and Hansson, E. (2006). Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci *7*, 41-53.

Abdul-Majid, K.B., Wefer, J., Stadelmann, C., Stefferl, A., Lassmann, H., Olsson, T., and Harris, R.A. (2003). Comparing the pathogenesis of experimental autoimmune encephalomyelitis in CD4-/- and CD8-/- DBA/1 mice defines qualitative roles of different T cell subsets. J Neuroimmunol *141*, 10-19.

Acuto, O., and Cantrell, D. (2000). T cell activation and the cytoskeleton. Annu Rev Immunol *18*, 165-184.

Agata, Y., Kawasaki, A., Nishimura, H., Ishida, Y., Tsubata, T., Yagita, H., and Honjo, T. (1996). Expression of the PD-1 antigen on the surface of stimulated mouse T and B lymphocytes. International immunology *8*, 765-772.

Agrawal, S., Anderson, P., Durbeej, M., van Rooijen, N., Ivars, F., Opdenakker, G., and Sorokin, L.M. (2006). Dystroglycan is selectively cleaved at the parenchymal basement membrane at sites of leukocyte extravasation in experimental autoimmune encephalomyelitis. J Exp Med *203*, 1007-1019.

Akdis, C.A., and Simons, F.E. (2006). Histamine receptors are hot in immunopharmacology. Eur J Pharmacol *533*, 69-76.

Alewijnse, A.E., Smit, M.J., Hoffmann, M., Verzijl, D., Timmerman, H., and Leurs, R. (1998). Constitutive activity and structural instability of the wild-type human H2 receptor. J Neurochem *71*, 799-807.

Allen, M., Sandberg-Wollheim, M., Sjogren, K., Erlich, H.A., Petterson, U., and Gyllensten, U. (1994). Association of susceptibility to multiple sclerosis in Sweden with HLA class II DRB1 and DQB1 alleles. Hum Immunol *39*, 41-48.

Alonso, A., Jick, S.S., and Hernan, M.A. (2006). Allergy, histamine 1 receptor blockers, and the risk of multiple sclerosis. Neurology *66*, 572-575.

Alter, M., Kahana, E., and Loewenson, R. (1978). Migration and risk of multiple sclerosis. Neurology *28*, 1089-1093.

Alter, M., Leibowitz, U., and Speer, J. (1966). Risk of multiple sclerosis related to age at immigration to Israel. Arch Neurol *15*, 234-237.

Andersson, A., and Karlsson, J. (2004). Genetics of experimental autoimmune encephalomyelitis in the mouse. Archivum immunologiae et therapiae experimentalis *52*, 316-325.

Ando, D.G., Clayton, J., Kono, D., Urban, J.L., and Sercarz, E.E. (1989). Encephalitogenic T cells in the B10.PL model of experimental allergic encephalomyelitis (EAE) are of the Th-1 lymphokine subtype. Cell Immunol *124*, 132-143.

Anthony, D.C., Ferguson, B., Matyzak, M.K., Miller, K.M., Esiri, M.M., and Perry, V.H. (1997). Differential matrix metalloproteinase expression in cases of multiple sclerosis and stroke. Neuropathology and applied neurobiology *23*, 406-415.

Anthony, D.C., Miller, K.M., Fearn, S., Townsend, M.J., Opdenakker, G., Wells, G.M., Clements, J.M., Chandler, S., Gearing, A.J., and Perry, V.H. (1998). Matrix metalloproteinase expression in an experimentally-induced DTH model of multiple sclerosis in the rat CNS. J Neuroimmunol *87*, 62-72.

Aoi, R., Nakashima, I., Kitamura, Y., Asai, H., and Nakano, K. (1989). Histamine synthesis by mouse T lymphocytes through induced histidine decarboxylase. Immunology *66*, 219-223.

Armstrong, M.E., Loscher, C.E., Lynch, M.A., and Mills, K.H. (2003). IL-1beta-dependent neurological effects of the whole cell pertussis vaccine: a role for IL-1-associated signalling components in vaccine reactogenicity. J Neuroimmunol *136*, 25-33.

Aronica, M.A., Mora, A.L., Mitchell, D.B., Finn, P.W., Johnson, J.E., Sheller, J.R., and Boothby, M.R. (1999). Preferential role for NF-kappa B/Rel signaling in the type 1 but not type 2 T cell-dependent immune response in vivo. J Immunol *163*, 5116-5124.

Ascherio, A., and Munger, K.L. (2007). Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. Ann Neurol *61*, 504-513.

Baker, D., Rosenwasser, O.A., O'Neill, J.K., and Turk, J.L. (1995). Genetic analysis of experimental allergic encephalomyelitis in mice. J Immunol *155*, 4046-4051.

Bakker, R.A., Dees, G., Carrillo, J.J., Booth, R.G., Lopez-Gimenez, J.F., Milligan, G., Strange, P.G., and Leurs, R. (2004). Domain swapping in the human histamine H1 receptor. J Pharmacol Exp Ther *311*, 131-138.

Bakker, R.A., Schoonus, S.B., Smit, M.J., Timmerman, H., and Leurs, R. (2001). Histamine H(1)-receptor activation of nuclear factor-kappa B: roles for G beta gamma- and G alpha(q/11)-subunits in constitutive and agonist-mediated signaling. Molecular pharmacology *60*, 1133-1142.

Bakker, R.A., Timmerman, H., and Leurs, R. (2002). Histamine receptors: specific ligands, receptor biochemistry, and signal transduction. Clin Allergy Immunol *17*, 27-64.

Banu, Y., and Watanabe, T. (1999). Augmentation of antigen receptor-mediated responses by histamine H1 receptor signaling. J Exp Med *189*, 673-682.

Baron, J.L., Madri, J.A., Ruddle, N.H., Hashim, G., and Janeway, C.A., Jr. (1993). Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. J Exp Med *177*, 57-68.

Baumann, N., and Pham-Dinh, D. (2001). Biology of oligodendrocyte and myelin in the mammalian central nervous system. Physiol Rev *81*, 871-927.

Baxter, A.G. (2007). The origin and application of experimental autoimmune encephalomyelitis. Nature reviews *7*, 904-912.

Bebo, B.F., Jr., Yong, T., Orr, E.L., and Linthicum, D.S. (1996). Hypothesis: a possible role for mast cells and their inflammatory mediators in the pathogenesis of autoimmune encephalomyelitis. J Neurosci Res *45*, 340-348.

Becanovic, K., Jagodic, M., Sheng, J.R., Dahlman, I., Aboul-Enein, F., Wallstrom, E., Olofsson, P., Holmdahl, R., Lassmann, H., and Olsson, T. (2006). Advanced intercross line mapping of Eae5 reveals Ncf-1 and CLDN4 as candidate genes for experimental autoimmune encephalomyelitis. J Immunol *176*, 6055-6064.

Becanovic, K., Jagodic, M., Wallstrom, E., and Olsson, T. (2004). Current gene-mapping strategies in experimental models of multiple sclerosis. Scand J Immunol *60*, 39-51.

Becher, B., Durell, B.G., and Noelle, R.J. (2002). Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. J Clin Invest *110*, 493-497.

Becher, B., Durell, B.G., and Noelle, R.J. (2003). IL-23 produced by CNS-resident cells controls T cell encephalitogenicity during the effector phase of experimental autoimmune encephalomyelitis. J Clin Invest *112*, 1186-1191.

Beck, J.A., Lloyd, S., Hafezparast, M., Lennon-Pierce, M., Eppig, J.T., Festing, M.F., and Fisher, E.M. (2000). Genealogies of mouse inbred strains. Nature genetics *24*, 23-25.

Becker, K.G., Simon, R.M., Bailey-Wilson, J.E., Freidlin, B., Biddison, W.E., McFarland, H.F., and Trent, J.M. (1998). Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. Proc Natl Acad Sci U S A *95*, 9979-9984.

Berenson, L.S., Yang, J., Sleckman, B.P., Murphy, T.L., and Murphy, K.M. (2006). Selective requirement of p38alpha MAPK in cytokine-dependent, but not antigen receptor-dependent, Th1 responses. J Immunol *176*, 4616-4621.

Berger, T., Rubner, P., Schautzer, F., Egg, R., Ulmer, H., Mayringer, I., Dilitz, E., Deisenhammer, F., and Reindl, M. (2003). Antimyelin antibodies as a predictor of clinically definite multiple sclerosis after a first demyelinating event. N Engl J Med *349*, 139-145.

Bergman, R.K., and Munoz, J. (1965). Circulatory Collapse In Anaphylaxis And Histamine Toxicity In Mice. J Immunol *95*, 1-8.

Bergman, R.K., and Munoz, J. (1968). Induced hypersensitivity to combinations of serotonin and histamine in a strain of mice ordinarily highly resistant to histamine sensitization. Int Arch Allergy Appl Immunol *34*, 9-17.

Bernard, C.C., Leydon, J., and Mackay, I.R. (1976). T cell necessity in the pathogenesis of experimental autoimmune encephalomyelitis in mice. Eur J Immunol *6*, 655-660.

Bernard, C.C., and Mackay, I.R. (1983). Transfer of murine experimental autoimmune encephalomyelitis and cell-mediated immunity to myelin protein is effected by Lyt-1 cells. J Neuroimmunol *4*, 61-65.

Bettelli, E., Sullivan, B., Szabo, S.J., Sobel, R.A., Glimcher, L.H., and Kuchroo, V.K. (2004). Loss of T-bet, but not STAT1, prevents the development of experimental autoimmune encephalomyelitis. J Exp Med *200*, 79-87.

Bielekova, B., Goodwin, B., Richert, N., Cortese, I., Kondo, T., Afshar, G., Gran, B., Eaton, J., Antel, J., Frank, J.A.*, et al.* (2000). Encephalitogenic potential of the myelin basic protein peptide (amino acids 83-99) in multiple sclerosis: results of a phase II clinical trial with an altered peptide ligand. Nat Med *6*, 1167-1175.

Bielekova, B., Sung, M.H., Kadom, N., Simon, R., McFarland, H., and Martin, R. (2004). Expansion and functional relevance of high-avidity myelin-specific CD4+ T cells in multiple sclerosis. J Immunol *172*, 3893-3904.

Bishop, C.E., Boursot, P., Baron, B., Bonhomme, F., and Hatat, D. (1985). Most classical Mus musculus domesticus laboratory mouse strains carry a Mus musculus musculus Y chromosome. Nature *315*, 70-72.

Black, W.J., Munoz, J.J., Peacock, M.G., Schad, P.A., Cowell, J.L., Burchall, J.J., Lim, M., Kent, A., Steinman, L., and Falkow, S. (1988). ADP-ribosyltransferase activity of pertussis toxin and immunomodulation by Bordetella pertussis. Science *240*, 656-659.

Blankenhorn, E.P., Butterfield, R.J., Rigby, R., Cort, L., Giambrone, D., McDermott, P., McEntee, K., Solowski, N., Meeker, N.D., Zachary, J.F.*, et al.* (2000). Genetic analysis of the influence of pertussis toxin on experimental allergic encephalomyelitis susceptibility: an environmental agent can override genetic checkpoints. J Immunol *164*, 3420-3425.

Boison, D., Bussow, H., D'Urso, D., Muller, H.W., and Stoffel, W. (1995). Adhesive properties of proteolipid protein are responsible for the compaction of CNS myelin sheaths. J Neurosci *15*, 5502-5513.

Booss, J., Esiri, M.M., Tourtellotte, W.W., and Mason, D.Y. (1983). Immunohistological

analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. J Neurol Sci *62*, 219-232.

Bordet, J., and Gengou, O. (1906). Le microbe de la coqueluche, Vol 20 (Ann Inst Pasteur).

Brenner, T., Brocke, S., Szafer, F., Sobel, R.A., Parkinson, J.F., Perez, D.H., and Steinman, L. (1997). Inhibition of nitric oxide synthase for treatment of experimental autoimmune encephalomyelitis. J Immunol *158*, 2940-2946.

Brenner, T., Soffer, D., Shalit, M., and Levi-Schaffer, F. (1994). Mast cells in experimental allergic encephalomyelitis: characterization, distribution in the CNS and in vitro activation by myelin basic protein and neuropeptides. J Neurol Sci *122*, 210-213.

Bretscher, P.A. (1999). A two-step, two-signal model for the primary activation of precursor helper T cells. Proc Natl Acad Sci U S A *96*, 185-190.

Brocke, S., Gaur, A., Piercy, C., Gautam, A., Gijbels, K., Fathman, C.G., and Steinman, L. (1993). Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigen. Nature *365*, 642-644.

Brooks, T.A., Hawkins, B.T., Huber, J.D., Egleton, R.D., and Davis, T.P. (2005). Chronic inflammatory pain leads to increased blood-brain barrier permeability and tight junction protein alterations. Am J Physiol Heart Circ Physiol *289*, H738-743.

Brooks, T.A., Ocheltree, S.M., Seelbach, M.J., Charles, R.A., Nametz, N., Egleton, R.D., and Davis, T.P. (2006). Biphasic cytoarchitecture and functional changes in the BBB induced by chronic inflammatory pain. Brain Res *1120*, 172-182.

Brosnan, C.F., and Tansey, F.A. (1984). Delayed onset of experimental allergic neuritis in rats treated with reserpine. J Neuropathol Exp Neurol *43*, 84-93.

Bryce, P.J., Mathias, C.B., Harrison, K.L., Watanabe, T., Geha, R.S., and Oettgen, H.C. (2006). The H1 histamine receptor regulates allergic lung responses. J Clin Invest *116*, 1624-1632.

Buckland, K.F., Williams, T.J., and Conroy, D.M. (2003). Histamine induces cytoskeletal changes in human eosinophils via the H(4) receptor. Br J Pharmacol *140*, 1117-1127.
Burgoyne, R.D., and Morgan, A. (2003). Secretory granule exocytosis. Physiol Rev *83*, 581-632.

Butcher, E.C., Williams, M., Youngman, K., Rott, L., and Briskin, M. (1999). Lymphocyte trafficking and regional immunity. Adv Immunol *72*, 209-253.

Butt, A.M., and Ransom, B.R. (1989). Visualization of oligodendrocytes and astrocytes in the intact rat optic nerve by intracellular injection of lucifer yellow and horseradish

peroxidase. Glia *2*, 470-475.

Butter, C., Baker, D., O'Neill, J.K., and Turk, J.L. (1991). Mononuclear cell trafficking and plasma protein extravasation into the CNS during chronic relapsing experimental allergic encephalomyelitis in Biozzi AB/H mice. J Neurol Sci *104*, 9-12.

Butterfield, R.J., Blankenhorn, E.P., Roper, R.J., Zachary, J.F., Doerge, R.W., Sudweeks, J., Rose, J., and Teuscher, C. (1999). Genetic analysis of disease subtypes and sexual dimorphisms in mouse experimental allergic encephalomyelitis (EAE): relapsing/remitting and monophasic remitting/nonrelapsing EAE are immunogenetically distinct. J Immunol *162*, 3096-3102.

Butterfield, R.J., Sudweeks, J.D., Blankenhorn, E.P., Korngold, R., Marini, J.C., Todd, J.A., Roper, R.J., and Teuscher, C. (1998). New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. J Immunol *161*, 1860-1867.

Cabarrocas, J., Bauer, J., Piaggio, E., Liblau, R., and Lassmann, H. (2003). Effective and selective immune surveillance of the brain by MHC class I-restricted cytotoxic T lymphocytes. Eur J Immunol *33*, 1174-1182.

Campagnoni, A.T., and Macklin, W.B. (1988). Cellular and molecular aspects of myelin protein gene expression. Molecular neurobiology *2*, 41-89.

Caron, G., Delneste, Y., Roelandts, E., Duez, C., Bonnefoy, J.Y., Pestel, J., and Jeannin, P. (2001). Histamine polarizes human dendritic cells into Th2 cell-promoting effector dendritic cells. J Immunol *167*, 3682-3686.

Chabas, D., Baranzini, S.E., Mitchell, D., Bernard, C.C., Rittling, S.R., Denhardt, D.T., Sobel, R.A., Lock, C., Karpuj, M., Pedotti, R., *et al.* (2001). The influence of the proinflammatory cytokine, osteopontin, on autoimmune demyelinating disease. Science *294*, 1731-1735.

Chang, T.T., Jabs, C., Sobel, R.A., Kuchroo, V.K., and Sharpe, A.H. (1999). Studies in B7-deficient mice reveal a critical role for B7 costimulation in both induction and effector phases of experimental autoimmune encephalomyelitis. J Exp Med *190*, 733-740.

Chen, X., Howard, O.M., and Oppenheim, J.J. (2007). Pertussis toxin by inducing IL-6 promotes the generation of IL-17-producing CD4 cells. J Immunol *178*, 6123-6129.

Chitnis, T., Najafian, N., Abdallah, K.A., Dong, V., Yagita, H., Sayegh, M.H., and Khoury, S.J. (2001). CD28-independent induction of experimental autoimmune encephalomyelitis. J Clin Invest *107*, 575-583.

Christopoulos, A., Christopoulos, G., Morfis, M., Udawela, M., Laburthe, M.,

Couvineau, A., Kuwasako, K., Tilakaratne, N., and Sexton, P.M. (2003). Novel receptor partners and function of receptor activity-modifying proteins. J Biol Chem *278*, 3293-3297.

Clements, J.M., Cossins, J.A., Wells, G.M., Corkill, D.J., Helfrich, K., Wood, L.M., Pigott, R., Stabler, G., Ward, G.A., Gearing, A.J., and Miller, K.M. (1997). Matrix metalloproteinase expression during experimental autoimmune encephalomyelitis and effects of a combined matrix metalloproteinase and tumour necrosis factor-alpha inhibitor. J Neuroimmunol *74*, 85-94.

Code, C.F., and Mitchell, R.G. (1957). Histamine, eosinophils and basophils in the blood. J Physiol *136*, 449-468.

Comabella, M., and Martin, R. (2007). Genomics in multiple sclerosis-Current state and future directions. J Neuroimmunol *187*, 1-8.

Compston, D.A., Morgan, B.P., Oleesky, D., Fifield, R., and Campbell, A.K. (1986). Cerebrospinal fluid C9 in demyelinating disease. Neurology *36*, 1503-1506.

Conlon, P., Oksenberg, J.R., Zhang, J., and Steinman, L. (1999). The immunobiology of multiple sclerosis: an autoimmune disease of the central nervous system. Neurobiology of disease *6*, 149-166.

Constantinescu, C.S., Tani, M., Ransohoff, R.M., Wysocka, M., Hilliard, B., Fujioka, T., Murphy, S., Tighe, P.J., Sarma, J.D., Trinchieri, G., and Rostami, A. (2005). Astrocytes as antigen-presenting cells: expression of IL-12/IL-23. J Neurochem *95*, 331-340.

Coo, H., and Aronson, K.J. (2004). A systematic review of several potential non-genetic risk factors for multiple sclerosis. Neuroepidemiology *23*, 1-12.

Cross, A.H., Trotter, J.L., and Lyons, J. (2001). B cells and antibodies in CNS demyelinating disease. J Neuroimmunol *112*, 1-14.

Cua, D.J., Sherlock, J., Chen, Y., Murphy, C.A., Joyce, B., Seymour, B., Lucian, L., To, W., Kwan, S., Churakova, T.*, et al.* (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature *421*, 744-748.

Dean, G. (1967). Annual incidence, prevalence, and mortality of multiple sclerosis in white South-African-born and in white immigrants to South Africa. Br Med J *2*, 724-730.

Dean, G., McLoughlin, H., Brady, R., Adelstein, A.M., and Tallett-Williams, J. (1976). Multiple sclerosis among immigrants in Greater London. Br Med J *1*, 861-864.

Dehouck, M.P., Meresse, S., Delorme, P., Fruchart, J.C., and Cecchelli, R. (1990). An easier, reproducible, and mass-production method to study the blood-brain barrier in vitro. J Neurochem *54*, 1798-1801.

Del Valle, J., and Gantz, I. (1997). Novel insights into histamine H2 receptor biology. Am J Physiol *273*, G987-996.

Denis, C., Methia, N., Frenette, P.S., Rayburn, H., Ullman-Cullere, M., Hynes, R.O., and Wagner, D.D. (1998). A mouse model of severe von Willebrand disease: defects in hemostasis and thrombosis. Proc Natl Acad Sci U S A *95*, 9524-9529.

Dietsch, G.N., and Hinrichs, D.J. (1989). The role of mast cells in the elicitation of experimental allergic encephalomyelitis. J Immunol *142*, 1476-1481.

Dimitriadou, V., Pang, X., and Theoharides, T.C. (2000). Hydroxyzine inhibits experimental allergic encephalomyelitis (EAE) and associated brain mast cell activation. Int J Immunopharmacol *22*, 673-684.

Dodeller, F., and Schulze-Koops, H. (2006). The p38 mitogen-activated protein kinase signaling cascade in CD4 T cells. Arthritis Res Ther *8*, 205.

Dogan, R.N., and Karpus, W.J. (2004). Chemokines and chemokine receptors in autoimmune encephalomyelitis as a model for central nervous system inflammatory disease regulation. Front Biosci *9*, 1500-1505.

Donnelly, S., Loscher, C.E., Lynch, M.A., and Mills, K.H. (2001). Whole-cell but not acellular pertussis vaccines induce convulsive activity in mice: evidence of a role for toxin-induced interleukin-1beta in a new murine model for analysis of neuronal side effects of vaccination. Infect Immun *69*, 4217-4223.

Dormond, O., Bezzi, M., Mariotti, A., and Ruegg, C. (2002). Prostaglandin E2 promotes integrin alpha Vbeta 3-dependent endothelial cell adhesion, rac-activation, and spreading through cAMP/PKA-dependent signaling. J Biol Chem *277*, 45838-45846.

Duvernay, M.T., Filipeanu, C.M., and Wu, G. (2005). The regulatory mechanisms of export trafficking of G protein-coupled receptors. Cell Signal *17*, 1457-1465.

Dwyer, N.D., Troemel, E.R., Sengupta, P., and Bargmann, C.I. (1998). Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell *93*, 455-466.

Ebers, G.C. (2005). A twin consensus in MS. Mult Scler *11*, 497-499.

Ebers, G.C., and Sadovnick, A.D. (1994). The role of genetic factors in multiple sclerosis susceptibility. J Neuroimmunol *54*, 1-17.

Ebers, G.C., Sadovnick, A.D., and Risch, N.J. (1995). A genetic basis for familial aggregation in multiple sclerosis. Canadian Collaborative Study Group. Nature *377*, 150-151.

Ek, M., Engblom, D., Saha, S., Blomqvist, A., Jakobsson, P.J., and Ericsson-Dahlstrand, A. (2001). Inflammatory response: pathway across the blood-brain barrier. Nature *410*, 430-431.

El Behi, M., Zephir, H., Lefranc, D., Dutoit, V., Dussart, P., Devos, P., Dessaint, J.P., Vermersch, P., and Prin, L. (2007). Changes in self-reactive IgG antibody repertoire after treatment of experimental autoimmune encephalomyelitis with anti-allergic drugs. J Neuroimmunol *182*, 80-88.

Encinas, J.A., Lees, M.B., Sobel, R.A., Symonowicz, C., Greer, J.M., Shovlin, C.L., Weiner, H.L., Seidman, C.E., Seidman, J.G., and Kuchroo, V.K. (1996). Genetic analysis of susceptibility to experimental autoimmune encephalomyelitis in a cross between SJL/J and B10.S mice. J Immunol *157*, 2186-2192.

Encinas, J.A., Lees, M.B., Sobel, R.A., Symonowicz, C., Weiner, H.L., Seidman, C.E., Seidman, J.G., and Kuchroo, V.K. (2001). Identification of genetic loci associated with paralysis, inflammation and weight loss in mouse experimental autoimmune encephalomyelitis. International immunology *13*, 257-264.

Encinas, J.A., Wicker, L.S., Peterson, L.B., Mukasa, A., Teuscher, C., Sobel, R., Weiner, H.L., Seidman, C.E., Seidman, J.G., and Kuchroo, V.K. (1999). QTL influencing autoimmune diabetes and encephalomyelitis map to a 0.15-cM region containing Il2. Nature genetics *21*, 158-160.

Endoh, M., Tabira, T., and Kunishita, T. (1986). Antibodies to proteolipid apoprotein in chronic relapsing experimental allergic encephalomyelitis. J Neurol Sci *73*, 31-38.

Engelhardt, B. (2006). Molecular mechanisms involved in T cell migration across the blood-brain barrier. J Neural Transm *113*, 477-485.

Engelhardt, B., and Ransohoff, R.M. (2005). The ins and outs of T-lymphocyte trafficking to the CNS: anatomical sites and molecular mechanisms. Trends Immunol *26*, 485-495.

Ercolini, A.M., and Miller, S.D. (2006). Mechanisms of immunopathology in murine models of central nervous system demyelinating disease. J Immunol *176*, 3293-3298.

Farley, N., Pedraza-Alva, G., Serrano-Gomez, D., Nagaleekar, V., Aronshtam, A., Krahl, T., Thornton, T., and Rincon, M. (2006). p38 mitogen-activated protein kinase mediates the Fas-induced mitochondrial death pathway in CD8+ T cells. Mol Cell Biol *26*, 2118-2129.

Ferber, I.A., Brocke, S., Taylor-Edwards, C., Ridgway, W., Dinisco, C., Steinman, L., Dalton, D., and Fathman, C.G. (1996). Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). J Immunol *156*, 5-7.

Fernald, G.H., Yeh, R.F., Hauser, S.L., Oksenberg, J.R., and Baranzini, S.E. (2005). Mapping gene activity in complex disorders: Integration of expression and genomic scans for multiple sclerosis. J Neuroimmunol *167*, 157-169.

Ferris, S.D., Sage, R.D., and Wilson, A.C. (1982). Evidence from mtDNA sequences that common laboratory strains of inbred mice are descended from a single female. Nature *295*, 163-165.

Fiedler, U., Scharpfenecker, M., Koidl, S., Hegen, A., Grunow, V., Schmidt, J.M., Kriz, W., Thurston, G., and Augustin, H.G. (2004). The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. Blood *103*, 4150-4156.

Fillmore, P.D., Blankenhorn, E.P., Zachary, J.F., and Teuscher, C. (2004). Adult gonadal hormones selectively regulate sexually dimorphic quantitative traits observed in experimental allergic encephalomyelitis. Am J Pathol *164*, 167-175.

Fillmore, P.D., Brace, M., Troutman, S.A., Blankenhorn, E.P., Diehl, S., Rincon, M., and Teuscher, C. (2003). Genetic analysis of the influence of neuroantigen-complete Freund's adjuvant emulsion structures on the sexual dimorphism and susceptibility to experimental allergic encephalomyelitis. Am J Pathol *163*, 1623-1632.

Ford, M.L., and Evavold, B.D. (2005). Specificity, magnitude, and kinetics of MOG-specific CD8+ T cell responses during experimental autoimmune encephalomyelitis. Eur J Immunol *35*, 76-85.

Frazer, K.A., Wade, C.M., Hinds, D.A., Patil, N., Cox, D.R., and Daly, M.J. (2004). Segmental phylogenetic relationships of inbred mouse strains revealed by fine-scale analysis of sequence variation across 4.6 mb of mouse genome. Genome research *14*, 1493-1500.

Fredholm, B.B., Hokfelt, T., and Milligan, G. (2007). G-protein-coupled receptors: an update. Acta Physiol (Oxf) *190*, 3-7.

Frei, K., Eugster, H.P., Bopst, M., Constantinescu, C.S., Lavi, E., and Fontana, A. (1997). Tumor necrosis factor alpha and lymphotoxin alpha are not required for induction of acute experimental autoimmune encephalomyelitis. J Exp Med *185*, 2177-2182.

Fritz, R.B., Skeen, M.J., Chou, C.H., Garcia, M., and Egorov, I.K. (1985). Major histocompatibility complex-linked control of the murine immune response to myelin basic protein. J Immunol *134*, 2328-2332.

Fruend, J., Stern, E., and Pisami, T. (1947). Isoallergic encephalomyelitis and radiculitis in guinea pigs after one injection of brain and mycobacteria in water-in-oil emulsiion. J Immunol *57*, 179-194.

Fujimoto, K., Ohta, K., Kangawa, K., Kikkawa, U., Ogino, S., and Fukui, H. (1999). Identification of protein kinase C phosphorylation sites involved in phorbol ester-induced desensitization of the histamine H1 receptor. Molecular pharmacology *55*, 735-742.

Fujinami, R.S., and Oldstone, M.B. (1985). Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. Science *230*, 1043-1045.

Fujinami, R.S., von Herrath, M.G., Christen, U., and Whitton, J.L. (2006). Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. Clin Microbiol Rev *19*, 80-94.

Furuzawa-Carballeda, J., Vargas-Rojas, M.I., and Cabral, A.R. (2007). Autoimmune inflammation from the Th17 perspective. Autoimmunity reviews *6*, 169-175.

GAMES (2001). A meta-analysis of genomic screens in multiple sclerosis. The Transatlantic Multiple Sclerosis Genetics Cooperative. Mult Scler *7*, 3-11.

GAMES (2003). A meta-analysis of while genome linkage screens in multiple sclerosis. J Neuroimmunol *143*, 39-46.

Gao, J.F., Call, S.B., Fillmore, P.D., Watanabe, T., Meeker, N.D., and Teuscher, C. (2003). Analysis of the role of Bphs/Hrh1 in the genetic control of responsiveness to pertussis toxin. Infect Immun *71*, 1281-1287.

Gao, X., Kouklis, P., Xu, N., Minshall, R.D., Sandoval, R., Vogel, S.M., and Malik, A.B. (2000). Reversibility of increased microvessel permeability in response to VE-cadherin disassembly. Am J Physiol Lung Cell Mol Physiol *279*, L1218-1225.

Gardinier, M.V., Amiguet, P., Linington, C., and Matthieu, J.M. (1992). Myelin/oligodendrocyte glycoprotein is a unique member of the immunoglobulin superfamily. J Neurosci Res *33*, 177-187.

Genain, C.P., Cannella, B., Hauser, S.L., and Raine, C.S. (1999). Identification of autoantibodies associated with myelin damage in multiple sclerosis. Nat Med *5*, 170-175.

Genain, C.P., Nguyen, M.H., Letvin, N.L., Pearl, R., Davis, R.L., Adelman, M., Lees, M.B., Linington, C., and Hauser, S.L. (1995). Antibody facilitation of multiple sclerosis-like lesions in a nonhuman primate. J Clin Invest *96*, 2966-2974.

Ghosh, A.K., Hirasawa, N., Ohtsu, H., Watanabe, T., and Ohuchi, K. (2002). Defective angiogenesis in the inflammatory granulation tissue in histidine decarboxylase-deficient mice but not in mast cell-deficient mice. J Exp Med *195*, 973-982.

Gimelbrant, A.A., Haley, S.L., and McClintock, T.S. (2001). Olfactory receptor trafficking involves conserved regulatory steps. J Biol Chem *276*, 7285-7290.

Girvin, A.M., Dal Canto, M.C., Rhee, L., Salomon, B., Sharpe, A., Bluestone, J.A., and Miller, S.D. (2000). A critical role for B7/CD28 costimulation in experimental autoimmune encephalomyelitis: a comparative study using costimulatory molecule-deficient mice and monoclonal antibody blockade. J Immunol *164*, 136-143.

Glabinski, A.R., Tani, M., Tuohy, V.K., Tuthill, R.J., and Ransohoff, R.M. (1995). Central nervous system chemokine mRNA accumulation follows initial leukocyte entry at the onset of acute murine experimental autoimmune encephalomyelitis. Brain, behavior, and immunity *9*, 315-330.

Glad, S., Nyland, H., and Myhr, K.M. (2006). Benign multiple sclerosis. Acta Neurol Scand Suppl *183*, 55-57.

Gocke, A.R., Cravens, P.D., Ben, L.H., Hussain, R.Z., Northrop, S.C., Racke, M.K., and Lovett-Racke, A.E. (2007). T-bet regulates the fate of Th1 and Th17 lymphocytes in autoimmunity. J Immunol *178*, 1341-1348.

Godiska, R., Chantry, D., Dietsch, G.N., and Gray, P.W. (1995). Chemokine expression in murine experimental allergic encephalomyelitis. J Neuroimmunol *58*, 167-176.

Gold, R., Hartung, H.P., and Toyka, K.V. (2000). Animal models for autoimmune demyelinating disorders of the nervous system. Mol Med Today *6*, 88-91.

Gold, R., Linington, C., and Lassmann, H. (2006). Understanding pathogenesis and therapy of multiple sclerosis via animal models: 70 years of merits and culprits in experimental autoimmune encephalomyelitis research. Brain *129*, 1953-1971.

Gonzalez-Scarano, F., Grossman, R.I., Galetta, S., Atlas, S.W., and Silberberg, D.H. (1987). Multiple sclerosis disease activity correlates with gadolinium-enhanced magnetic resonance imaging. Ann Neurol *21*, 300-306.

Graesser, D., Solowiej, A., Bruckner, M., Osterweil, E., Juedes, A., Davis, S., Ruddle, N.H., Engelhardt, B., and Madri, J.A. (2002). Altered vascular permeability and early onset of experimental autoimmune encephalomyelitis in PECAM-1-deficient mice. J Clin Invest *109*, 383-392.

Greenstein, J.I. (2007). Current concepts of the cellular and molecular pathophysiology of multiple sclerosis. Dev Neurobiol *67*, 1248-1265.

Gregory, S.G., Schmidt, S., Seth, P., Oksenberg, J.R., Hart, J., Prokop, A., Caillier, S.J., Ban, M., Goris, A., Barcellos, L.F., *et al.* (2007). Interleukin 7 receptor alpha chain (IL7R) shows allelic and functional association with multiple sclerosis. Nature genetics *39*, 1083-1091.

Greve, B., Vijayakrishnan, L., Kubal, A., Sobel, R.A., Peterson, L.B., Wicker, L.S., and Kuchroo, V.K. (2004). The diabetes susceptibility locus Idd5.1 on mouse chromosome 1

regulates ICOS expression and modulates murine experimental autoimmune encephalomyelitis. J Immunol *173*, 157-163.

Grigoriadis, N., and Hadjigeorgiou, G.M. (2006). Virus-mediated autoimmunity in Multiple Sclerosis. Journal of autoimmune diseases *3*, 1.

Gutcher, I., and Becher, B. (2007). APC-derived cytokines and T cell polarization in autoimmune inflammation. J Clin Invest *117*, 1119-1127.

Guyton, M.K., Wingrave, J.M., Yallapragada, A.V., Wilford, G.G., Sribnick, E.A., Matzelle, D.D., Tyor, W.R., Ray, S.K., and Banik, N.L. (2005). Upregulation of calpain correlates with increased neurodegeneration in acute experimental auto-immune encephalomyelitis. J Neurosci Res *81*, 53-61.

Hafler, D.A. (2004). Multiple sclerosis. J Clin Invest *113*, 788-794.

Hafler, D.A., Compston, A., Sawcer, S., Lander, E.S., Daly, M.J., De Jager, P.L., de Bakker, P.I., Gabriel, S.B., Mirel, D.B., Ivinson, A.J., *et al.* (2007). Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med *357*, 851-862.

Hafler, D.A., Slavik, J.M., Anderson, D.E., O'Connor, K.C., De Jager, P., and Baecher-Allan, C. (2005). Multiple sclerosis. Immunol Rev *204*, 208-231.

Haines, J.L., Ter-Minassian, M., Bazyk, A., Gusella, J.F., Kim, D.J., Terwedow, H., Pericak-Vance, M.A., Rimmler, J.B., Haynes, C.S., Roses, A.D., *et al.* (1996). A complete genomic screen for multiple sclerosis underscores a role for the major histocompatability complex. The Multiple Sclerosis Genetics Group. Nature genetics *13*, 469-471.

Haines, J.L., Terwedow, H.A., Burgess, K., Pericak-Vance, M.A., Rimmler, J.B., Martin, E.R., Oksenberg, J.R., Lincoln, R., Zhang, D.Y., Banatao, D.R., *et al.* (1998). Linkage of the MHC to familial multiple sclerosis suggests genetic heterogeneity. The Multiple Sclerosis Genetics Group. Human molecular genetics *7*, 1229-1234.

Hall, Z.W. (1992). An intrduction to molecular neurobiology (Sunderland, MA: Sinauer Associates Inc).

Hammond, S.R., English, D.R., and McLeod, J.G. (2000). The age-range of risk of developing multiple sclerosis: evidence from a migrant population in Australia. Brain *123 (Pt 5)*, 968-974.

Harrington, L.E., Hatton, R.D., Mangan, P.R., Turner, H., Murphy, T.L., Murphy, K.M., and Weaver, C.T. (2005). Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol *6*, 1123-1132.

Harrington, L.E., Mangan, P.R., and Weaver, C.T. (2006). Expanding the effector CD4

T-cell repertoire: the Th17 lineage. Current opinion in immunology *18*, 349-356.

Harris, W.E., and Fulton, J.D. (1958). Quantification of anaphylaxis in mice. Proc Soc Exp Biol Med *97*, 14-17.

Haseloff, R.F., Blasig, I.E., Bauer, H.C., and Bauer, H. (2005). In search of the astrocytic factor(s) modulating blood-brain barrier functions in brain capillary endothelial cells in vitro. Cellular and molecular neurobiology *25*, 25-39.

Hattori, R., Hamilton, K.K., Fugate, R.D., McEver, R.P., and Sims, P.J. (1989). Stimulated secretion of endothelial von Willebrand factor is accompanied by rapid redistribution to the cell surface of the intracellular granule membrane protein GMP-140. J Biol Chem *264*, 7768-7771.

Hauser, S.L., Bhan, A.K., Gilles, F., Kemp, M., Kerr, C., and Weiner, H.L. (1986). Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. Ann Neurol *19*, 578-587.

Hernan, M.A., Olek, M.J., and Ascherio, A. (1999). Geographic variation of MS incidence in two prospective studies of US women. Neurology *53*, 1711-1718.

Hickey, W.F. (2001). Basic principles of immunological surveillance of the normal central nervous system. Glia *36*, 118-124.

Hickey, W.F., Hsu, B.L., and Kimura, H. (1991). T-lymphocyte entry into the central nervous system. J Neurosci Res *28*, 254-260.

Hill, S.J. (1992). Multiple histamine receptors: properties and functional characteristics. Biochem Soc Trans *20*, 122-125.

Hill, S.J., Ganellin, C.R., Timmerman, H., Schwartz, J.C., Shankley, N.P., Young, J.M., Schunack, W., Levi, R., and Haas, H.L. (1997). International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev *49*, 253-278.

Hofstra, C.L., Desai, P.J., Thurmond, R.L., and Fung-Leung, W.P. (2003). Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. J Pharmacol Exp Ther *305*, 1212-1221.

Hohnoki, K., Inoue, A., and Koh, C.S. (1998). Elevated serum levels of IFN-gamma, IL-4 and TNF-alpha/unelevated serum levels of IL-10 in patients with demyelinating diseases during the acute stage. J Neuroimmunol *87*, 27-32.

Holmoy, T., and Vartdal, F. (2007). The immunological basis for treatment of multiple sclerosis. Scand J Immunol *66*, 374-382.

Huseby, E.S., Liggitt, D., Brabb, T., Schnabel, B., Ohlen, C., and Goverman, J. (2001). A

pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. J Exp Med *194*, 669-676.

Hutloff, A., Dittrich, A.M., Beier, K.C., Eljaschewitsch, B., Kraft, R., Anagnostopoulos, I., and Kroczek, R.A. (1999). ICOS is an inducible T-cell co-stimulator structurally and functionally related to CD28. Nature *397*, 263-266.

Ibrahim, M.Z., Reder, A.T., Lawand, R., Takash, W., and Sallouh-Khatib, S. (1996). The mast cells of the multiple sclerosis brain. J Neuroimmunol *70*, 131-138.

Iff, E.T., and Vaz, N.M. (1966). Mechanisms of anaphylaxis in the mouse. Similarity of shock induced by anaphylaxis and by mixtures of histamine and serotonin. Int Arch Allergy Appl Immunol *30*, 313-322.

Iglesias, A., Bauer, J., Litzenburger, T., Schubart, A., and Linington, C. (2001). T- and B-cell responses to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis and multiple sclerosis. Glia *36*, 220-234.

Innes, J.R. (1951). Experimental "allergic" encephalitis: attempts to produce the disease in sheep and goats. Journal of comparative pathology *61*, 241-250.

Inoue, I., Taniuchi, I., Kitamura, D., Jenkins, N.A., Gilbert, D.J., Copeland, N.G., and Watanabe, T. (1996). Characteristics of the mouse genomic histamine H1 receptor gene. Genomics *36*, 178-181.

Inoue, K. (2006). ATP receptors of microglia involved in pain. Novartis Found Symp *276*, 263-272; discussion 273-281.

Jegou, J.F., Chan, P., Schouft, M.T., Griffiths, M.R., Neal, J.W., Gasque, P., Vaudry, H., and Fontaine, M. (2007). C3d binding to the myelin oligodendrocyte glycoprotein results in an exacerbated experimental autoimmune encephalomyelitis. J Immunol *178*, 3323-3331.

Jersild, C., Fog, T., Hansen, G.S., Thomsen, M., Svejgaard, A., and Dupont, B. (1973). Histocompatibility determinants in multiple sclerosis, with special reference to clinical course. Lancet *2*, 1221-1225.

Ji, Q., and Goverman, J. (2007). Experimental autoimmune encephalomyelitis mediated by CD8+ T cells. Ann N Y Acad Sci *1103*, 157-166.

Jiang, H., Zhang, S.I., and Pernis, B. (1992). Role of CD8+ T cells in murine experimental allergic encephalomyelitis. Science *256*, 1213-1215.

Johns, T.G., and Bernard, C.C. (1999). The structure and function of myelin oligodendrocyte glycoprotein. J Neurochem *72*, 1-9.

Jones, D.C., Ding, X., Zhang, T.Y., and Daynes, R.A. (2003). Peroxisome proliferator-activated receptor alpha negatively regulates T-bet transcription through suppression of p38 mitogen-activated protein kinase activation. J Immunol *171*, 196-203.

Jongejan, A., Bruysters, M., Ballesteros, J.A., Haaksma, E., Bakker, R.A., Pardo, L., and Leurs, R. (2005). Linking agonist binding to histamine H1 receptor activation. Nat Chem Biol *1*, 98-103.

Juedes, A.E., Hjelmstrom, P., Bergman, C.M., Neild, A.L., and Ruddle, N.H. (2000). Kinetics and cellular origin of cytokines in the central nervous system: insight into mechanisms of myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis. J Immunol *164*, 419-426.

Jutel, M., Watanabe, T., Klunker, S., Akdis, M., Thomet, O.A., Malolepszy, J., Zak-Nejmark, T., Koga, R., Kobayashi, T., Blaser, K., and Akdis, C.A. (2001). Histamine regulates T-cell and antibody responses by differential expression of H1 and H2 receptors. Nature *413*, 420-425.

Kahlson, G., and Rosengren, E. (1968). New approaches to the physiology of histamine. Physiol Rev *48*, 155-196.

Kantarci, O., and Wingerchuk, D. (2006). Epidemiology and natural history of multiple sclerosis: new insights. Curr Opin Neurol *19*, 248-254.

Kantarci, O.H., de Andrade, M., and Weinshenker, B.G. (2002). Identifying disease modifying genes in multiple sclerosis. J Neuroimmunol *123*, 144-159.

Karandikar, N.J., Vanderlugt, C.L., Walunas, T.L., Miller, S.D., and Bluestone, J.A. (1996). CTLA-4: a negative regulator of autoimmune disease. J Exp Med *184*, 783-788.

Karlsson, J., Zhao, X., Lonskaya, I., Neptin, M., Holmdahl, R., and Andersson, A. (2003). Novel quantitative trait loci controlling development of experimental autoimmune encephalomyelitis and proportion of lymphocyte subpopulations. J Immunol *170*, 1019-1026.

Kaslow, H.R., and Burns, D.L. (1992). Pertussis toxin and target eukaryotic cells: binding, entry, and activation. Faseb J *6*, 2684-2690.

Keegan, M., Konig, F., McClelland, R., Bruck, W., Morales, Y., Bitsch, A., Panitch, H., Lassmann, H., Weinshenker, B., Rodriguez, M., *et al.* (2005). Relation between humoral pathological changes in multiple sclerosis and response to therapeutic plasma exchange. Lancet *366*, 579-582.

Kermode, A.G., Thompson, A.J., Tofts, P., MacManus, D.G., Kendall, B.E., Kingsley, D.P., Moseley, I.F., Rudge, P., and McDonald, W.I. (1990). Breakdown of the blood-brain barrier precedes symptoms and other MRI signs of new lesions in multiple

sclerosis. Pathogenetic and clinical implications. Brain *113 (Pt 5)*, 1477-1489.

Kieseier, B.C., Kiefer, R., Clements, J.M., Miller, K., Wells, G.M., Schweitzer, T., Gearing, A.J., and Hartung, H.P. (1998). Matrix metalloproteinase-9 and -7 are regulated in experimental autoimmune encephalomyelitis. Brain *121 (Pt 1)*, 159-166.

Kim, J.H., Kim, J.H., Park, J.A., Lee, S.W., Kim, W.J., Yu, Y.S., and Kim, K.W. (2006). Blood-neural barrier: intercellular communication at glio-vascular interface. Journal of biochemistry and molecular biology *39*, 339-345.

Kleinschek, M.A., Owyang, A.M., Joyce-Shaikh, B., Langrish, C.L., Chen, Y., Gorman, D.M., Blumenschein, W.M., McClanahan, T., Brombacher, F., Hurst, S.D., *et al.* (2007). IL-25 regulates Th17 function in autoimmune inflammation. J Exp Med *204*, 161-170.

Klugmann, M., Schwab, M.H., Puhlhofer, A., Schneider, A., Zimmermann, F., Griffiths, I.R., and Nave, K.A. (1997). Assembly of CNS myelin in the absence of proteolipid protein. Neuron *18*, 59-70.

Koh, D.R., Fung-Leung, W.P., Ho, A., Gray, D., Acha-Orbea, H., and Mak, T.W. (1992). Less mortality but more relapses in experimental allergic encephalomyelitis in CD8-/- mice. Science *256*, 1210-1213.

Komiyama, Y., Nakae, S., Matsuki, T., Nambu, A., Ishigame, H., Kakuta, S., Sudo, K., and Iwakura, Y. (2006). IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. J Immunol *177*, 566-573.

Krueger, K.M., Witte, D.G., Ireland-Denny, L., Miller, T.R., Baranowski, J.L., Buckner, S., Milicic, I., Esbenshade, T.A., and Hancock, A.A. (2005). G protein-dependent pharmacology of histamine H3 receptor ligands: evidence for heterogeneous active state receptor conformations. J Pharmacol Exp Ther *314*, 271-281.

Kubes, P., and Kanwar, S. (1994). Histamine induces leukocyte rolling in post-capillary venules. A P-selectin-mediated event. J Immunol *152*, 3570-3577.

Kubo, Y., and Nakano, K. (1999). Regulation of histamine synthesis in mouse CD4+ and CD8+ T lymphocytes. Inflamm Res *48*, 149-153.

Kuchroo, V.K., Anderson, A.C., Waldner, H., Munder, M., Bettelli, E., and Nicholson, L.B. (2002). T cell response in experimental autoimmune encephalomyelitis (EAE): role of self and cross-reactive antigens in shaping, tuning, and regulating the autopathogenic T cell repertoire. Annu Rev Immunol *20*, 101-123.

Kuchroo, V.K., Martin, C.A., Greer, J.M., Ju, S.T., Sobel, R.A., and Dorf, M.E. (1993). Cytokines and adhesion molecules contribute to the ability of myelin proteolipid protein-specific T cell clones to mediate experimental allergic encephalomyelitis. J Immunol *151*, 4371-4382.

Kursula, P. (2006). Structural properties of proteins specific to the myelin sheath. Amino Acids.

Kurtzke, J.F. (1968). Multiple sclerosis and infection from an epidemiologic aspect. Neurology *18*, 170-175.

Kurtzke, J.F. (1993). Epidemiologic evidence for multiple sclerosis as an infection. Clin Microbiol Rev *6*, 382-427.

Kurtzke, J.F., Beebe, G.W., and Norman, J.E., Jr. (1985). Epidemiology of multiple sclerosis in US veterans: III. Migration and the risk of MS. Neurology *35*, 672-678.

Kurtzke, J.F., Dean, G., and Botha, D.P. (1970). A method for estimating the age at immigration of white immigrants to South Africa, with an example of its importance. S Afr Med J *44*, 663-669.

Kurtzke, J.F., Gudmundsson, K.R., and Bergmann, S. (1982). Multiple sclerosis in Iceland: 1. Evidence of a postwar epidemic. Neurology *32*, 143-150.

Kurtzke, J.F., and Hyllested, K. (1987). MS epidemiology in Faroe Islands. Riv Neurol *57*, 77-87.

Kyriakis, J.M., and Avruch, J. (2001). Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiol Rev *81*, 807-869.

Lalive, P.H., Menge, T., Delarasse, C., Della Gaspera, B., Pham-Dinh, D., Villoslada, P., von Budingen, H.C., and Genain, C.P. (2006). Antibodies to native myelin oligodendrocyte glycoprotein are serologic markers of early inflammation in multiple sclerosis. Proc Natl Acad Sci U S A *103*, 2280-2285.

Lang, H.L., Jacobsen, H., Ikemizu, S., Andersson, C., Harlos, K., Madsen, L., Hjorth, P., Sondergaard, L., Svejgaard, A., Wucherpfennig, K., *et al.* (2002). A functional and structural basis for TCR cross-reactivity in multiple sclerosis. Nat Immunol *3*, 940-943.

Langrish, C.L., Chen, Y., Blumenschein, W.M., Mattson, J., Basham, B., Sedgwick, J.D., McClanahan, T., Kastelein, R.A., and Cua, D.J. (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med *201*, 233-240.

Laurell, A.B., and Link, H. (1972). Complement-fixing antibrain antibodies in multiple sclerosis. A preliminary report. Acta Neurol Scand *48*, 461-466.

Lebar, R., Lubetzki, C., Vincent, C., Lombrail, P., and Boutry, J.M. (1986). The M2 autoantigen of central nervous system myelin, a glycoprotein present in oligodendrocyte membrane. Clinical and experimental immunology *66*, 423-434.

Leonard, J.P., Waldburger, K.E., and Goldman, S.J. (1995). Prevention of experimental autoimmune encephalomyelitis by antibodies against interleukin 12. J Exp Med *181*, 381-386.

Leurs, R., Smit, M.J., Menge, W.M., and Timmerman, H. (1994). Pharmacological characterization of the human histamine H2 receptor stably expressed in Chinese hamster ovary cells. Br J Pharmacol *112*, 847-854.

Leurs, R., Smit, M.J., and Timmerman, H. (1995). Molecular pharmacological aspects of histamine receptors. Pharmacology & therapeutics *66*, 413-463.

Li, C., Tropak, M.B., Gerlai, R., Clapoff, S., Abramow-Newerly, W., Trapp, B., Peterson, A., and Roder, J. (1994). Myelination in the absence of myelin-associated glycoprotein. Nature *369*, 747-750.

Lindberg, R.L., De Groot, C.J., Montagne, L., Freitag, P., van der Valk, P., Kappos, L., and Leppert, D. (2001). The expression profile of matrix metalloproteinases (MMPs) and their inhibitors (TIMPs) in lesions and normal appearing white matter of multiple sclerosis. Brain *124*, 1743-1753.

Linthicum, D.S. (1982). Development of acute autoimmune encephalomyelitis in mice: factors regulating the effector phase of the disease. Immunobiology *162*, 211-220.
Linthicum, D.S., and Frelinger, J.A. (1982). Acute autoimmune encephalomyelitis in mice. II. Susceptibility is controlled by the combination of H-2 and histamine sensitization genes. J Exp Med *156*, 31-40.

Lipnik-Stangelj, M., and Carman-Krzan, M. (2004). Histamine-stimulated nerve growth factor secretion from cultured astrocyctes is blocked by protein kinase C inhibitors. Inflamm Res *53 Suppl 1*, S57-58.

Lipton, H.L., and Dal Canto, M.C. (1976). Theiler's virus-induced demyelination: prevention by immunosuppression. Science *192*, 62-64.

Lipton, M.M., and Freund, J. (1952). Encephalomyelitis in the rat following intracutaneous injection of central nervous system tissue with adjuvant. Proc Soc Exp Biol Med *81*, 260-261.

Litzenburger, T., Fassler, R., Bauer, J., Lassmann, H., Linington, C., Wekerle, H., and Iglesias, A. (1998). B lymphocytes producing demyelinating autoantibodies: development and function in gene-targeted transgenic mice. J Exp Med *188*, 169-180.

Locht, C. (1999). Molecular aspects of Bordetella pertussis pathogenesis. Int Microbiol *2*, 137-144.

Logothetis, L., Mylonas, I.A., Baloyannis, S., Pashalidou, M., Orologas, A., Zafeiropoulos, A., Kosta, V., and Theoharides, T.C. (2005). A pilot, open label, clinical

trial using hydroxyzine in multiple sclerosis. International journal of immunopathology and pharmacology *18*, 771-778.

Loscher, C.E., Donnelly, S., Lynch, M.A., and Mills, K.H. (2000). Induction of inflammatory cytokines in the brain following respiratory infection with Bordetella pertussis. J Neuroimmunol *102*, 172-181.

Lucchinetti, C.F., Bruck, W., Rodriguez, M., and Lassmann, H. (1996). Distinct patterns of multiple sclerosis pathology indicates heterogeneity on pathogenesis. Brain Pathol *6*, 259-274.

Lum, H., and Malik, A.B. (1994). Regulation of vascular endothelial barrier function. Am J Physiol *267*, L223-241.

Lumsden, C. (1949). Experimental allergic encephalomyelitis II - on the nature of the encephalitogenic agent. Brain *27*, 517-537.

Lundmark, F., Duvefelt, K., Iacobaeus, E., Kockum, I., Wallstrom, E., Khademi, M., Oturai, A., Ryder, L.P., Saarela, J., Harbo, H.F., *et al.* (2007). Variation in interleukin 7 receptor alpha chain (IL7R) influences risk of multiple sclerosis. Nature genetics *39*, 1108-1113.

Lyons, J.A., San, M., Happ, M.P., and Cross, A.H. (1999). B cells are critical to induction of experimental allergic encephalomyelitis by protein but not by a short encephalitogenic peptide. Eur J Immunol *29*, 3432-3439.

Ma, R.Z., Gao, J., Meeker, N.D., Fillmore, P.D., Tung, K.S., Watanabe, T., Zachary, J.F., Offner, H., Blankenhorn, E.P., and Teuscher, C. (2002). Identification of Bphs, an autoimmune disease locus, as histamine receptor H1. Science *297*, 620-623.

Mackay, R.P., and Myrianthopoulos, N.C. (1966). Multiple sclerosis in twins and their relatives. Arch Neurol *15*, 449-462.

MacMicking, J.D., Willenborg, D.O., Weidemann, M.J., Rockett, K.A., and Cowden, W.B. (1992). Elevated secretion of reactive nitrogen and oxygen intermediates by inflammatory leukocytes in hyperacute experimental autoimmune encephalomyelitis: enhancement by the soluble products of encephalitogenic T cells. J Exp Med *176*, 303-307.

Marrie, R.A. (2004). Environmental risk factors in multiple sclerosis aetiology. Lancet Neurol *3*, 709-718.

Marta, C.B., Oliver, A.R., Sweet, R.A., Pfeiffer, S.E., and Ruddle, N.H. (2005). Pathogenic myelin oligodendrocyte glycoprotein antibodies recognize glycosylated epitopes and perturb oligodendrocyte physiology. Proc Natl Acad Sci U S A *102*, 13992-13997.

Martin, R., and McFarland, H.F. (1995). Immunological aspects of experimental allergic encephalomyelitis and multiple sclerosis. Critical reviews in clinical laboratory sciences *32*, 121-182.

Matejuk, A., Dwyer, J., Ito, A., Bruender, Z., Vandenbark, A.A., and Offner, H. (2002). Effects of cytokine deficiency on chemokine expression in CNS of mice with EAE. J Neurosci Res *67*, 680-688.

Mathews, P.M., Andermann, F., Silver, K., Karpati, G., and Arnold, D.L. (1993). Proton MR spectroscopic characterization of differences in regional brain metabolic abnormalities in mitochondrial encephalomyopathies. Neurology *43*, 2484-2490.

Matsumoto, Y., Ohmori, K., and Fujiwara, M. (1992). Immune regulation by brain cells in the central nervous system: microglia but not astrocytes present myelin basic protein to encephalitogenic T cells under in vivo-mimicking conditions. Immunology *76*, 209-216.

Matthews, A.E., Weiss, S.R., and Paterson, Y. (2002). Murine hepatitis virus--a model for virus-induced CNS demyelination. Journal of neurovirology *8*, 76-85.

Matusevicius, D., Kivisakk, P., He, B., Kostulas, N., Ozenci, V., Fredrikson, S., and Link, H. (1999). Interleukin-17 mRNA expression in blood and CSF mononuclear cells is augmented in multiple sclerosis. Mult Scler *5*, 101-104.

Mazon Pelaez, I., Vogler, S., Strauss, U., Wernhoff, P., Pahnke, J., Brockmann, G., Moch, H., Thiesen, H.J., Rolfs, A., and Ibrahim, S.M. (2005). Identification of quantitative trait loci controlling cortical motor evoked potentials in experimental autoimmune encephalomyelitis: correlation with incidence, onset and severity of disease. Human molecular genetics *14*, 1977-1989.

Mazzoni, A., Young, H.A., Spitzer, J.H., Visintin, A., and Segal, D.M. (2001). Histamine regulates cytokine production in maturing dendritic cells, resulting in altered T cell polarization. J Clin Invest *108*, 1865-1873.

McAdam, A.J., Schweitzer, A.N., and Sharpe, A.H. (1998). The role of B7 co-stimulation in activation and differentiation of CD4+ and CD8+ T cells. Immunol Rev *165*, 231-247.

McCreath, G., Hall, I.P., and Hill, S.J. (1994). Agonist-induced desensitization of histamine H1 receptor-mediated inositol phospholipid hydrolysis in human umbilical vein endothelial cells. Br J Pharmacol *113*, 823-830.

McFarland, H.F., and Martin, R. (2007). Multiple sclerosis: a complicated picture of autoimmunity. Nat Immunol *8*, 913-919.

McLatchie, L.M., Fraser, N.J., Main, M.J., Wise, A., Brown, J., Thompson, N., Solari, R., Lee, M.G., and Foord, S.M. (1998). RAMPs regulate the transport and ligand specificity of the calcitonin-receptor-like receptor. Nature *393*, 333-339.

Meeker, N.D., Stafford, A.N., Lunceford, J.K., Avner, P., Ma, R.Z., and Teuscher, C. (1999). 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 *10*, 858-863.

Megson, A.C., Walker, E.M., and Hill, S.J. (2001). Role of protein kinase Calpha in signaling from the histamine H(1) receptor to the nucleus. Molecular pharmacology *59*, 1012-1021.

Mekori, Y.A., and Metcalfe, D.D. (2000). Mast cells in innate immunity. Immunol Rev *173*, 131-140.

Meretey, K., Falus, A., Taga, T., and Kishimoto, T. (1991). Histamine influences the expression of the interleukin-6 receptor on human lymphoid, monocytoid and hepatoma cell lines. Agents Actions *33*, 189-191.

Merrill, J.E., Kono, D.H., Clayton, J., Ando, D.G., Hinton, D.R., and Hofman, F.M. (1992). Inflammatory leukocytes and cytokines in the peptide-induced disease of experimental allergic encephalomyelitis in SJL and B10.PL mice. Proc Natl Acad Sci U S A *89*, 574-578.

Minagar, A., and Alexander, J.S. (2003). Blood-brain barrier disruption in multiple sclerosis. Mult Scler *9*, 540-549.

Minagar, A., Jy, W., Jimenez, J.J., and Alexander, J.S. (2006). Multiple sclerosis as a vascular disease. Neurol Res *28*, 230-235.

Minagar, A., Shapshak, P., Fujimura, R., Ownby, R., Heyes, M., and Eisdorfer, C. (2002). The role of macrophage/microglia and astrocytes in the pathogenesis of three neurologic disorders: HIV-associated dementia, Alzheimer disease, and multiple sclerosis. J Neurol Sci *202*, 13-23.

Mitchell, F.M., Mullaney, I., Godfrey, P.P., Arkinstall, S.J., Wakelam, M.J., and Milligan, G. (1991). Widespread distribution of Gq alpha/G11 alpha detected immunologically by an antipeptide antiserum directed against the predicted C-terminal decapeptide. FEBS letters *287*, 171-174.

Mittra, S., and Bourreau, J.P. (2006). Gs and Gi coupling of adrenomedullin in adult rat ventricular myocytes. Am J Physiol Heart Circ Physiol *290*, H1842-1847.

Miyoshi, K., Das, A.K., Fujimoto, K., Horio, S., and Fukui, H. (2006). Recent advances in molecular pharmacology of the histamine systems: regulation of histamine H1 receptor signaling by changing its expression level. J Pharmacol Sci *101*, 3-6.

Miyoshi, K., Kawakami, N., Horio, S., and Fukui, H. (2004). Homologous and heterologous phosphorylations of human histamine H1 receptor in intact cells. J

Pharmacol Sci *96*, 474-482.

Mokhtarian, F., Grob, D., and Griffin, D.E. (1989). Role of the immune response in Sindbis virus-induced paralysis of SJL/J mice. J Immunol *143*, 633-637.

Mokhtarian, F., and Swoveland, P. (1987). Predisposition to EAE induction in resistant mice by prior infection with Semliki Forest virus. J Immunol *138*, 3264-3268.

Montag, D., Giese, K.P., Bartsch, U., Martini, R., Lang, Y., Bluthmann, H., Karthigasan, J., Kirschner, D.A., Wintergerst, E.S., Nave, K.A., and et al. (1994). Mice deficient for the myelin-associated glycoprotein show subtle abnormalities in myelin. Neuron *13*, 229-246.

Morgan, B.P., Campbell, A.K., and Compston, D.A. (1984). Terminal component of complement (C9) in cerebrospinal fluid of patients with multiple sclerosis. Lancet *2*, 251-254.

Morrison, L. (1947). Disseminated encephalomyelitis experimentally produced by the use of homologous antigen. Arch. Neurol.Psyciat. *58*, 391-416.

Morse, K.L., Behan, J., Laz, T.M., West, R.E., Jr., Greenfeder, S.A., Anthes, J.C., Umland, S., Wan, Y., Hipkin, R.W., Gonsiorek, W., *et al.* (2001). Cloning and characterization of a novel human histamine receptor. J Pharmacol Exp Ther *296*, 1058-1066.

Munger, K.L., Zhang, S.M., O'Reilly, E., Hernan, M.A., Olek, M.J., Willett, W.C., and Ascherio, A. (2004). Vitamin D intake and incidence of multiple sclerosis. Neurology *62*, 60-65.

Munoz, J. (1957). Effect of H. pertussis on sensitivity of mice to serotonin. Proc Soc Exp Biol Med *95*, 328-331.

Munoz, J.J. (1963). Symposium On Relationship Of Structure Of Microorganisms To Their Immunological Properties. I. Immunological And Other Biological Activities Of Bordetella Pertussis Antigens. Bacteriol Rev *27*, 325-340.

Munoz, J.J. (1985). Biological activities of pertussigen (pertussis toxin) (Orlando, FL: Academic Press).

Munoz, J.J., Arai, H., Bergman, R.K., and Sadowski, P.L. (1981). Biological activities of crystalline pertussigen from Bordetella pertussis. Infect Immun *33*, 820-826.

Musio, S., Gallo, B., Scabeni, S., Lapilla, M., Poliani, P.L., Matarese, G., Ohtsu, H., Galli, S.J., Mantegazza, R., Steinman, L., and Pedotti, R. (2006). A key regulatory role for histamine in experimental autoimmune encephalomyelitis: disease exacerbation in histidine decarboxylase-deficient mice. J Immunol *176*, 17-26.

Nagamine, C.M., Nishioka, Y., Moriwaki, K., Boursot, P., Bonhomme, F., and Lau, Y.F. (1992). The musculus-type Y chromosome of the laboratory mouse is of Asian origin. Mamm Genome *3*, 84-91.

Neumann, H., Medana, I.M., Bauer, J., and Lassmann, H. (2002). Cytotoxic T lymphocytes in autoimmune and degenerative CNS diseases. Trends in neurosciences *25*, 313-319.

Noubade, R., Milligan, G., Zachary, J.F., Blankenhorn, E.P., Del Rio, R., Rincon, M., and Teuscher, C. (2007). Histamine receptor H(1) is required for TCR-mediated p38 MAPK activation and optimal IFN-gamma production in mice. J Clin Invest *117*, 3507-3518.

Okamura, H., Tsutsi, H., Komatsu, T., Yutsudo, M., Hakura, A., Tanimoto, T., Torigoe, K., Okura, T., Nukada, Y., Hattori, K., and et al. (1995). Cloning of a new cytokine that induces IFN-gamma production by T cells. Nature *378*, 88-91.

Okuda, Y., Sakoda, S., Bernard, C.C., Fujimura, H., Saeki, Y., Kishimoto, T., and Yanagihara, T. (1998). IL-6-deficient mice are resistant to the induction of experimental autoimmune encephalomyelitis provoked by myelin oligodendrocyte glycoprotein. International immunology *10*, 703-708.

Oldenhof, J., Vickery, R., Anafi, M., Oak, J., Ray, A., Schoots, O., Pawson, T., von Zastrow, M., and Van Tol, H.H. (1998). SH3 binding domains in the dopamine D4 receptor. Biochemistry *37*, 15726-15736.

Olitsky, P.K., Casals, J., and Tal, C. (1950). Relative susceptibility of various stocks of mice to experimental disseminated encephalomyelitis. Proc Soc Exp Biol Med *75*, 276-279.

Olitsky, P.K., and Yager, R.H. (1949). Experimental disseminated encephalomyelitis in white mice. J Exp Med *90*, 213-223.

Oliveira-dos-Santos, A.J., Ho, A., Tada, Y., Lafaille, J.J., Tonegawa, S., Mak, T.W., and Penninger, J.M. (1999). CD28 costimulation is crucial for the development of spontaneous autoimmune encephalomyelitis. J Immunol *162*, 4490-4495.

Olson, J.K., Croxford, J.L., Calenoff, M.A., Dal Canto, M.C., and Miller, S.D. (2001). A virus-induced molecular mimicry model of multiple sclerosis. J Clin Invest *108*, 311-318.

Olsson, T., Sun, J., Hillert, J., Hojeberg, B., Ekre, H.P., Andersson, G., Olerup, O., and Link, H. (1992). Increased numbers of T cells recognizing multiple myelin basic protein epitopes in multiple sclerosis. Eur J Immunol *22*, 1083-1087.

Olsson, Y. (1974). Mast cells in plaques of multiple sclerosis. Acta Neurol Scand *50*, 611-618.

Orr, E.L. (1988). Presence and distribution of nervous system-associated mast cells that may modulate experimental autoimmune encephalomyelitis. Ann N Y Acad Sci *540*, 723-726.

Oynebraten, I., Bakke, O., Brandtzaeg, P., Johansen, F.E., and Haraldsen, G. (2004). Rapid chemokine secretion from endothelial cells originates from 2 distinct compartments. Blood *104*, 314-320.

Pagenstecher, A., Stalder, A.K., Kincaid, C.L., Shapiro, S.D., and Campbell, I.L. (1998). Differential expression of matrix metalloproteinase and tissue inhibitor of matrix metalloproteinase genes in the mouse central nervous system in normal and inflammatory states. Am J Pathol *152*, 729-741.

Paigen, K. (2003). One hundred years of mouse genetics: an intellectual history. I. The classical period (1902-1980). Genetics *163*, 1-7.

Palakal, M., Bright, J., Sebastian, T., and Hartanto, S. (2007). A comparative study of cells in inflammation, EAE and MS using biomedical literature data mining. Journal of biomedical science *14*, 67-85.

Panitch, H.S., Hirsch, R.L., Haley, A.S., and Johnson, K.P. (1987). Exacerbations of multiple sclerosis in patients treated with gamma interferon. Lancet *1*, 893-895.

Paolino, E., Fainardi, E., Ruppi, P., Tola, M.R., Govoni, V., Casetta, I., Monetti, V.C., Granieri, E., and Carreras, M. (1996). A prospective study on the predictive value of CSF oligoclonal bands and MRI in acute isolated neurological syndromes for subsequent progression to multiple sclerosis. J Neurol Neurosurg Psychiatry *60*, 572-575.

Parfentjev, I.A. (1955). Anaphylaxis and histamine shock in mice. Proc Soc Exp Biol Med *89*, 297-299.

Park, H., Li, Z., Yang, X.O., Chang, S.H., Nurieva, R., Wang, Y.H., Wang, Y., Hood, L., Zhu, Z., Tian, Q., and Dong, C. (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol *6*, 1133-1141.

Parsons, M.E., and Ganellin, C.R. (2006). Histamine and its receptors. Br J Pharmacol *147 Suppl 1*, S127-135.

Pedotti, R., DeVoss, J.J., Youssef, S., Mitchell, D., Wedemeyer, J., Madanat, R., Garren, H., Fontoura, P., Tsai, M., Galli, S.J., *et al.* (2003). Multiple elements of the allergic arm of the immune response modulate autoimmune demyelination. Proc Natl Acad Sci U S A *100*, 1867-1872.

Peltonen, L. (2007). Old suspects found guilty--the first genome profile of multiple sclerosis. N Engl J Med *357*, 927-929.

Pender, M.P. (1987). Demyelination and neurological signs in experimental allergic encephalomyelitis. J Neuroimmunol *15*, 11-24.

Pendu, R., Terraube, V., Christophe, O.D., Gahmberg, C.G., de Groot, P.G., Lenting, P.J., and Denis, C.V. (2006). P-selectin glycoprotein ligand 1 and beta2-integrins cooperate in the adhesion of leukocytes to von Willebrand factor. Blood *108*, 3746-3752.

Perrin, P.J., June, C.H., Maldonado, J.H., Ratts, R.B., and Racke, M.K. (1999). Blockade of CD28 during in vitro activation of encephalitogenic T cells or after disease onset ameliorates experimental autoimmune encephalomyelitis. J Immunol *163*, 1704-1710.

Piddlesden, S.J., Lassmann, H., Zimprich, F., Morgan, B.P., and Linington, C. (1993). The demyelinating potential of antibodies to myelin oligodendrocyte glycoprotein is related to their ability to fix complement. Am J Pathol *143*, 555-564.

Polanczyk, M., Yellayi, S., Zamora, A., Subramanian, S., Tovey, M., Vandenbark, A.A., Offner, H., Zachary, J.F., Fillmore, P.D., Blankenhorn, E.P.*, et al.* (2004). Estrogen receptor-1 (Esr1) and -2 (Esr2) regulate the severity of clinical experimental allergic encephalomyelitis in male mice. Am J Pathol *164*, 1915-1924.

Porter, B.E., and Tennekoon, G. (2000). Myelin and disorders that affect the formation and maintenance of this sheath. Mental retardation and developmental disabilities research reviews *6*, 47-58.

Pouly, S., Antel, J.P., Ladiwala, U., Nalbantoglu, J., and Becher, B. (2000). Mechanisms of tissue injury in multiple sclerosis: opportunities for neuroprotective therapy. J Neural Transm Suppl, 193-203.

Powell, M.B., Mitchell, D., Lederman, J., Buckmeier, J., Zamvil, S.S., Graham, M., Ruddle, N.H., and Steinman, L. (1990). Lymphotoxin and tumor necrosis factor-alpha production by myelin basic protein-specific T cell clones correlates with encephalitogenicity. International immunology *2*, 539-544.

Privat, A., Jacque, C., Bourre, J.M., Dupouey, P., and Baumann, N. (1979). Absence of the major dense line in myelin of the mutant mouse "shiverer". Neuroscience letters *12*, 107-112.

Pype, J.L., Dupont, L.J., Mak, J.C., Barnes, P.J., and Verleden, G.M. (1998). Regulation of H1-receptor coupling and H1-receptor mRNA by histamine in bovine tracheal smooth muscle. Br J Pharmacol *123*, 984-990.

Raghavendra, V., Tanga, F.Y., and DeLeo, J.A. (2004). Complete Freunds adjuvant-induced peripheral inflammation evokes glial activation and proinflammatory cytokine expression in the CNS. Eur J Neurosci *20*, 467-473.

Ramana, C.V., Gil, M.P., Schreiber, R.D., and Stark, G.R. (2002). Stat1-dependent and -

independent pathways in IFN-gamma-dependent signaling. Trends Immunol *23*, 96-101.

Rappouli, R., and Pizza, M. (1991). Structure and evolutionary aspects of ADP-ribosylating toxins (London: Academic press).

Riley, J.F., and West, G.B. (1953). The presence of histamine in tissue mast cells. J Physiol *120*, 528-537.

Rincon, M., Enslen, H., Raingeaud, J., Recht, M., Zapton, T., Su, M.S., Penix, L.A., Davis, R.J., and Flavell, R.A. (1998). Interferon-gamma expression by Th1 effector T cells mediated by the p38 MAP kinase signaling pathway. Embo J *17*, 2817-2829.

Rios, J.C., Rubin, M., St Martin, M., Downey, R.T., Einheber, S., Rosenbluth, J., Levinson, S.R., Bhat, M., and Salzer, J.L. (2003). Paranodal interactions regulate expression of sodium channel subtypes and provide a diffusion barrier for the node of Ranvier. J Neurosci *23*, 7001-7011.

Risch, N., and Merikangas, K. (1996). The future of genetic studies of complex human diseases. Science *273*, 1516-1517.

Rivers, T., sprunt, D., and Berry, G. (1933). Observations on attempts to produce acute disseminated encephalomyelitis in monkeys. J Exp Med *58*, 39-53

Roach, A., Takahashi, N., Pravtcheva, D., Ruddle, F., and Hood, L. (1985). Chromosomal mapping of mouse myelin basic protein gene and structure and transcription of the partially deleted gene in shiverer mutant mice. Cell *42*, 149-155.

Robinson, A.J., and Dickenson, J.M. (2001). Activation of the p38 and p42/p44 mitogen-activated protein kinase families by the histamine H(1) receptor in DDT(1)MF-2 cells. Br J Pharmacol *133*, 1378-1386.

Robinson, D.S., and O'Garra, A. (2002). Further checkpoints in Th1 development. Immunity *16*, 755-758.

Romani de Wit, T., Rondaij, M.G., Hordijk, P.L., Voorberg, J., and van Mourik, J.A. (2003). Real-time imaging of the dynamics and secretory behavior of Weibel-Palade bodies. Arterioscler Thromb Vasc Biol *23*, 755-761.

Rondaij, M.G., Bierings, R., Kragt, A., Gijzen, K.A., Sellink, E., van Mourik, J.A., Fernandez-Borja, M., and Voorberg, J. (2006). Dynein-dynactin complex mediates protein kinase A-dependent clustering of Weibel-Palade bodies in endothelial cells. Arterioscler Thromb Vasc Biol *26*, 49-55.

Ruat, M., Korner, M., Garbarg, M., Gros, C., Schwartz, J.C., Tertiuk, W., and Ganellin, C.R. (1988). Characterization of histamine H1-receptor binding peptides in guinea pig brain using [125I]iodoazidophenpyramine, an irreversible specific photoaffinity probe.

Proc Natl Acad Sci U S A *85*, 2743-2747.

Ruat, M., and Schwartz, J.C. (1989). Photoaffinity labeling and electrophoretic identification of the H1-receptor: comparison of several brain regions and animal species. J Neurochem *53*, 335-339.

Ruat, M., Traiffort, E., Bouthenet, M.L., Schwartz, J.C., Hirschfeld, J., Buschauer, A., and Schunack, W. (1990). Reversible and irreversible labeling and autoradiographic localization of the cerebral histamine H2 receptor using [125I]iodinated probes. Proc Natl Acad Sci U S A *87*, 1658-1662.

Rubin, L.L., Hall, D.E., Porter, S., Barbu, K., Cannon, C., Horner, H.C., Janatpour, M., Liaw, C.W., Manning, K., Morales, J., and et al. (1991). A cell culture model of the blood-brain barrier. The Journal of cell biology *115*, 1725-1735.

Sacca, B., Fiori, S., and Moroder, L. (2003). Studies of the local conformational properties of the cell-adhesion domain of collagen type IV in synthetic heterotrimeric peptides. Biochemistry *42*, 3429-3436.

Sachs, B., Hertl, M., and Merk, H.F. (2000). Histamine receptors on lymphocytes: distribution and functional significance. Skin Pharmacol Appl Skin Physiol *13*, 313-323.

Sadovnick, A.D., Baird, P.A., and Ward, R.H. (1988). Multiple sclerosis: updated risks for relatives. Am J Med Genet *29*, 533-541.

Sakai, T., Kikkawa, Y., Miura, I., Inoue, T., Moriwaki, K., Shiroishi, T., Satta, Y., Takahata, N., and Yonekawa, H. (2005). Origins of mouse inbred strains deduced from whole-genome scanning by polymorphic microsatellite loci. Mamm Genome *16*, 11-19.

Sakhalkar, S.P., Patterson, E.B., and Khan, M.M. (2005). Involvement of histamine H1 and H2 receptors in the regulation of STAT-1 phosphorylation: inverse agonism exhibited by the receptor antagonists. International immunopharmacology *5*, 1299-1309.

Salama, A.D., Chitnis, T., Imitola, J., Ansari, M.J., Akiba, H., Tushima, F., Azuma, M., Yagita, H., Sayegh, M.H., and Khoury, S.J. (2003). Critical role of the programmed death-1 (PD-1) pathway in regulation of experimental autoimmune encephalomyelitis. J Exp Med *198*, 71-78.

Salvador, J.M., Mittelstadt, P.R., Guszczynski, T., Copeland, T.D., Yamaguchi, H., Appella, E., Fornace, A.J., Jr., and Ashwell, J.D. (2005). Alternative p38 activation pathway mediated by T cell receptor-proximal tyrosine kinases. Nat Immunol *6*, 390-395.

Salzer, J.L. (2002). Nodes of Ranvier come of age. Trends in neurosciences *25*, 2-5.

Samad, T.A., Moore, K.A., Sapirstein, A., Billet, S., Allchorne, A., Poole, S., Bonventre, J.V., and Woolf, C.J. (2001). Interleukin-1beta-mediated induction of Cox-2 in the CNS

contributes to inflammatory pain hypersensitivity. Nature *410*, 471-475.

Satoh, H., and Inui, J. (1984). Endothelial cell-dependent relaxation and contraction induced by histamine in the isolated guinea-pig pulmonary artery. Eur J Pharmacol *97*, 321-324.

Sawcer, S. (2006). A new era in the genetic analysis of multiple sclerosis. Curr Opin Neurol *19*, 237-241.

Sayed, B.A., and Brown, M.A. (2007). Mast cells as modulators of T-cell responses. Immunol Rev *217*, 53-64.

Schiffenbauer, J., Streit, W.J., Butfiloski, E., LaBow, M., Edwards, C., 3rd, and Moldawer, L.L. (2000). The induction of EAE is only partially dependent on TNF receptor signaling but requires the IL-1 type I receptor. Clinical immunology (Orlando, Fla *95*, 117-123.

Schluesener, H.J., Sobel, R.A., Linington, C., and Weiner, H.L. (1987). A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and demyelination in central nervous system autoimmune disease. J Immunol *139*, 4016-4021.

Schneider, E., Rolli-Derkinderen, M., Arock, M., and Dy, M. (2002). Trends in histamine research: new functions during immune responses and hematopoiesis. Trends Immunol *23*, 255-263.

Schneuwly, S., Shortridge, R.D., Larrivee, D.C., Ono, T., Ozaki, M., and Pak, W.L. (1989). Drosophila ninaA gene encodes an eye-specific cyclophilin (cyclosporine A binding protein). Proc Natl Acad Sci U S A *86*, 5390-5394.

Secor, V.H., Secor, W.E., Gutekunst, C.A., and Brown, M.A. (2000). Mast cells are essential for early onset and severe disease in a murine model of multiple sclerosis. J Exp Med *191*, 813-822.

Seeldrayers, P.A., Yasui, D., Weiner, H.L., and Johnson, D. (1989). Treatment of experimental allergic neuritis with nedocromil sodium. J Neuroimmunol *25*, 221-226.

Segal, B.M., Dwyer, B.K., and Shevach, E.M. (1998). An interleukin (IL)-10/IL-12 immunoregulatory circuit controls susceptibility to autoimmune disease. J Exp Med *187*, 537-546.

Selmaj, K., Raine, C.S., and Cross, A.H. (1991). Anti-tumor necrosis factor therapy abrogates autoimmune demyelination. Ann Neurol *30*, 694-700.

Sewell, W.A., de Moerloose, P.A., Hamilton, J.A., Schrader, J.W., Mackay, I.R., and Vadas, M.A. (1987). Potentiation of delayed-type hypersensitivity by pertussigen or

cyclophosphamide with release of different lymphokines. Immunology *61*, 483-488.

Sewell, W.A., Munoz, J.J., Scollay, R., and Vadas, M.A. (1984). Studies on the mechanism of the enhancement of delayed-type hypersensitivity by pertussigen. J Immunol *133*, 1716-1722.

Sewell, W.A., Munoz, J.J., and Vadas, M.A. (1983). Enhancement of the intensity, persistence, and passive transfer of delayed-type hypersensitivity lesions by pertussigen in mice. J Exp Med *157*, 2087-2096.

Sharief, M.K., and Hentges, R. (1991). Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis. N Engl J Med *325*, 467-472.

Sharpe, A.H., and Freeman, G.J. (2002). The B7-CD28 superfamily. Nature reviews *2*, 116-126.

Shaw, M.K., Lorens, J.B., Dhawan, A., DalCanto, R., Tse, H.Y., Tran, A.B., Bonpane, C., Eswaran, S.L., Brocke, S., Sarvetnick, N.*, et al.* (1997). Local delivery of interleukin 4 by retrovirus-transduced T lymphocytes ameliorates experimental autoimmune encephalomyelitis. J Exp Med *185*, 1711-1714.

Shi, F.D., Takeda, K., Akira, S., Sarvetnick, N., and Ljunggren, H.G. (2000). IL-18 directs autoreactive T cells and promotes autodestruction in the central nervous system via induction of IFN-gamma by NK cells. J Immunol *165*, 3099-3104.

Shieh, B.H., Stamnes, M.A., Seavello, S., Harris, G.L., and Zuker, C.S. (1989). The ninaA gene required for visual transduction in Drosophila encodes a homologue of cyclosporin A-binding protein. Nature *338*, 67-70.

Shiraishi, M., Hirasawa, N., Oikawa, S., Kobayashi, Y., and Ohuchi, K. (2000). Analysis of histamine-producing cells at the late phase of allergic inflammation in rats. Immunology *99*, 600-606.

Simons, F.E. (2004). Advances in H1-antihistamines. N Engl J Med *351*, 2203-2217.

Skulina, C., Schmidt, S., Dornmair, K., Babbe, H., Roers, A., Rajewsky, K., Wekerle, H., Hohlfeld, R., and Goebels, N. (2004). Multiple sclerosis: brain-infiltrating CD8+ T cells persist as clonal expansions in the cerebrospinal fluid and blood. Proc Natl Acad Sci U S A *101*, 2428-2433.

Smit, M.J., Bloemers, S.M., Leurs, R., Tertoolen, L.G., Bast, A., de Laat, S.W., and Timmerman, H. (1992). Short-term desensitization of the histamine H1 receptor in human HeLa cells: involvement of protein kinase C dependent and independent pathways. Br J Pharmacol *107*, 448-455.

Smit, M.J., Hoffmann, M., Timmerman, H., and Leurs, R. (1999). Molecular properties

and signalling pathways of the histamine H1 receptor. Clin Exp Allergy *29 Suppl 3*, 19-28.

Smit, M.J., Timmerman, H., Blauw, J., Beukers, M.W., Roovers, E., Jacobs, E.H., Hoffmann, M., and Leurs, R. (1996). The C terminal tail of the histamine H2 receptor contains positive and negative signals important for signal transduction and receptor down-regulation. J Neurochem *67*, 1791-1800.

Soderstrom, M., Link, H., Sun, J.B., Fredrikson, S., Kostulas, V., Hojeberg, B., Li, B.L., and Olsson, T. (1993). T cells recognizing multiple peptides of myelin basic protein are found in blood and enriched in cerebrospinal fluid in optic neuritis and multiple sclerosis. Scand J Immunol *37*, 355-368.

Soilu-Hanninen, M., Airas, L., Mononen, I., Heikkila, A., Viljanen, M., and Hanninen, A. (2005). 25-Hydroxyvitamin D levels in serum at the onset of multiple sclerosis. Mult Scler *11*, 266-271.

Soilu-Hanninen, M., Laaksonen, M., Laitinen, I., Eralinna, J.P., Lilius, E.M., and Mononen, I. (2007). A longitudinal study of serum 25-hydroxyvitamin D and intact PTH levels indicate the importance of vitamin D and calcium homeostasis regulation in multiple sclerosis. J Neurol Neurosurg Psychiatry.

Solly, S.K., Thomas, J.L., Monge, M., Demerens, C., Lubetzki, C., Gardinier, M.V., Matthieu, J.M., and Zalc, B. (1996). Myelin/oligodendrocyte glycoprotein (MOG) expression is associated with myelin deposition. Glia *18*, 39-48.

Sospedra, M., and Martin, R. (2005). Immunology of multiple sclerosis. Annu Rev Immunol *23*, 683-747.

Sparks, A.B., Hoffman, N.G., McConnell, S.J., Fowlkes, D.M., and Kay, B.K. (1996). Cloning of ligand targets: systematic isolation of SH3 domain-containing proteins. Nat Biotechnol *14*, 741-744.

Spiegel, A.M., and Weinstein, L.S. (2004). Inherited diseases involving g proteins and g protein-coupled receptors. Annual review of medicine *55*, 27-39.

Stanners, J., Kabouridis, P.S., McGuire, K.L., and Tsoukas, C.D. (1995). Interaction between G proteins and tyrosine kinases upon T cell receptor.CD3-mediated signaling. J Biol Chem *270*, 30635-30642.

Steffel, J., Akhmedov, A., Greutert, H., Luscher, T.F., and Tanner, F.C. (2005). Histamine induces tissue factor expression: implications for acute coronary syndromes. Circulation *112*, 341-349.

Stuve, O., Youssef, S., Slavin, A.J., King, C.L., Patarroyo, J.C., Hirschberg, D.L., Brickey, W.J., Soos, J.M., Piskurich, J.F., Chapman, H.A., and Zamvil, S.S. (2002). The

role of the MHC class II transactivator in class II expression and antigen presentation by astrocytes and in susceptibility to central nervous system autoimmune disease. J Immunol *169*, 6720-6732.

Sudweeks, J.D., Todd, J.A., Blankenhorn, E.P., Wardell, B.B., Woodward, S.R., Meeker, N.D., Estes, S.S., and Teuscher, C. (1993). 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 *90*, 3700-3704.

Sugden, D., Davidson, K., Hough, K.A., and Teh, M.T. (2004). Melatonin, melatonin receptors and melanophores: a moving story. Pigment Cell Res *17*, 454-460.

Sun, D., Whitaker, J.N., Huang, Z., Liu, D., Coleclough, C., Wekerle, H., and Raine, C.S. (2001). Myelin antigen-specific CD8+ T cells are encephalitogenic and produce severe disease in C57BL/6 mice. J Immunol *166*, 7579-7587.

Sundvall, M., Jirholt, J., Yang, H.T., Jansson, L., Engstrom, A., Pettersson, U., and Holmdahl, R. (1995). Identification of murine loci associated with susceptibility to chronic experimental autoimmune encephalomyelitis. Nature genetics *10*, 313-317.

Suryani, S., and Sutton, I. (2007). An interferon-gamma-producing Th1 subset is the major source of IL-17 in experimental autoimmune encephalitis. J Neuroimmunol *183*, 96-103.

Svensson, L., Abdul-Majid, K.B., Bauer, J., Lassmann, H., Harris, R.A., and Holmdahl, R. (2002). A comparative analysis of B cell-mediated myelin oligodendrocyte glycoprotein-experimental autoimmune encephalomyelitis pathogenesis in B cell-deficient mice reveals an effect on demyelination. Eur J Immunol *32*, 1939-1946.

Szabo, S.J., Kim, S.T., Costa, G.L., Zhang, X., Fathman, C.G., and Glimcher, L.H. (2000). A novel transcription factor, T-bet, directs Th1 lineage commitment. Cell *100*, 655-669.

Szeberenyi, J.B., Pallinger, E., Zsinko, M., Pos, Z., Rothe, G., Orso, E., Szeberenyi, S., Schmitz, G., Falus, A., and Laszlo, V. (2001). Inhibition of effects of endogenously synthesized histamine disturbs in vitro human dendritic cell differentiation. Immunol Lett *76*, 175-182.

Taguchi, Y., Tsuyama, K., Watanabe, T., Wada, H., and Kitamura, Y. (1982). Increase in histidine decarboxylase activity in skin of genetically mast-cell-deficient W/Wv mice after application of phorbol 12-myristate 13-acetate: evidence for the presence of histamine-producing cells without basophilic granules. Proc Natl Acad Sci U S A *79*, 6837-6841.

Takamatsu, S., Nakashima, I., and Nakano, K. (1996). Modulation of endotoxin-induced

histamine synthesis by cytokines in mouse bone marrow-derived macrophages. J Immunol *156*, 778-785.

Takekawa, M., and Saito, H. (1998). A family of stress-inducible GADD45-like proteins mediate activation of the stress-responsive MTK1/MEKK4 MAPKKK. Cell *95*, 521-530.

Tal, C., Laufer, A., and Behar, A.J. (1958). An experimental demyelinative disease in the Syrian hamster. British journal of experimental pathology *39*, 158-164.

Tan, C.M., Brady, A.E., Nickols, H.H., Wang, Q., and Limbird, L.E. (2004). Membrane trafficking of G protein-coupled receptors. Annu Rev Pharmacol Toxicol *44*, 559-609.

Tanaka, S., Deai, K., Konomi, A., Takahashi, K., Yamane, H., Sugimoto, Y., and Ichikawa, A. (2004). Expression of L-histidine decarboxylase in granules of elicited mouse polymorphonuclear leukocytes. Eur J Immunol *34*, 1472-1482.

Tang, T., Frenette, P.S., Hynes, R.O., Wagner, D.D., and Mayadas, T.N. (1996). Cytokine-induced meningitis is dramatically attenuated in mice deficient in endothelial selectins. J Clin Invest *97*, 2485-2490.

Tanzola, M.B., Robbie-Ryan, M., Gutekunst, C.A., and Brown, M.A. (2003). Mast cells exert effects outside the central nervous system to influence experimental allergic encephalomyelitis disease course. J Immunol *171*, 4385-4391.

Tao, Y.X. (2006). Inactivating mutations of G protein-coupled receptors and diseases: structure-function insights and therapeutic implications. Pharmacology & therapeutics *111*, 949-973.

Tejada-Simon, M.V., Zang, Y.C., Hong, J., Rivera, V.M., and Zhang, J.Z. (2003). Cross-reactivity with myelin basic protein and human herpesvirus-6 in multiple sclerosis. Ann Neurol *53*, 189-197.

Teuscher, C. (1985). Experimental allergic orchitis in mice. II. Association of disease susceptibility with the locus controlling Bordetella pertussis-induced sensitivity to histamine. Immunogenetics *22*, 417-425.

Teuscher, C., Doerge, R.W., Fillmore, P.D., and Blankenhorn, E.P. (2006a). eae36, a locus on mouse chromosome 4, controls susceptibility to experimental allergic encephalomyelitis in older mice and mice immunized in the winter. Genetics *172*, 1147-1153.

Teuscher, C., Noubade, R., Spach, K., McElvany, B., Bunn, J.Y., Fillmore, P.D., Zachary, J.F., and Blankenhorn, E.P. (2006b). Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A *103*, 8024-8029.

Teuscher, C., Subramanian, M., Noubade, R., Gao, J.F., Offner, H., Zachary, J.F., and Blankenhorn, E.P. (2007). Central histamine H3 receptor signaling negatively regulates susceptibility to autoimmune inflammatory disease of the CNS. Proc Natl Acad Sci U S A *104*, 10146-10151.

Thacker, E.L., Mirzaei, F., and Ascherio, A. (2006). Infectious mononucleosis and risk for multiple sclerosis: a meta-analysis. Ann Neurol *59*, 499-503.

Thomas, L., Paterson, P.Y., and Smithwick, B. (1950). Acute disseminated encephalomyelitis following immunization with homologous brain extracts; studies on the role of a circulating antibody in the production of the condition in dogs. J Exp Med *92*, 133-152.

Toda, N. (1987). Mechanism of histamine actions in human coronary arteries. Circ Res *61*, 280-286.

Toft-Hansen, H., Nuttall, R.K., Edwards, D.R., and Owens, T. (2004). Key metalloproteinases are expressed by specific cell types in experimental autoimmune encephalomyelitis. J Immunol *173*, 5209-5218.

Tokuda, S., Weiser, R.S., Munoz, J., and Laxson, C. (1963). Comparative studies on the anaphylactic reactivity of mice of the Swiss, A/Jax, and DBA/2 strains. J Infect Dis *112*, 77-83.

Tolhurst, D.J., and Lewis, P.R. (1992). Effect of myelination on the conduction velocity of optic nerve fibres. Ophthalmic Physiol Opt *12*, 241-243.

Tonra, J.R. (2002). Cerebellar susceptibility to experimental autoimmune encephalomyelitis in SJL/J mice: potential interaction of immunology with vascular anatomy. Cerebellum *1*, 57-68.

Tonra, J.R., Reiseter, B.S., Kolbeck, R., Nagashima, K., Robertson, R., Keyt, B., and Lindsay, R.M. (2001). Comparison of the timing of acute blood-brain barrier breakdown to rabbit immunoglobulin G in the cerebellum and spinal cord of mice with experimental autoimmune encephalomyelitis. J Comp Neurol *430*, 131-144.

Trapp, B.D., Peterson, J., Ransohoff, R.M., Rudick, R., Mork, S., and Bo, L. (1998). Axonal transection in the lesions of multiple sclerosis. N Engl J Med *338*, 278-285.

Triggiani, M., Gentile, M., Secondo, A., Granata, F., Oriente, A., Taglialatela, M., Annunziato, L., and Marone, G. (2001). Histamine induces exocytosis and IL-6 production from human lung macrophages through interaction with H1 receptors. J Immunol *166*, 4083-4091.

Triggiani, M., Petraroli, A., Loffredo, S., Frattini, A., Granata, F., Morabito, P., Staiano, R.I., Secondo, A., Annunziato, L., and Marone, G. (2007). Differentiation of monocytes

into macrophages induces the upregulation of histamine H1 receptor. J Allergy Clin Immunol *119*, 472-481.

Trotter, J., Zamvil, S.S., and Steinman, L. (1987). Comparison of antigen specificity, class II major histocompatibility complex restriction, and in vivo behavior of myelin basic protein-specific T cell lines and clones derived from (BALB/c x SJL/J) mice. J Immunol *139*, 1834-1839.

Tuomisto, L., Kilpelainen, H., and Riekkinen, P. (1983). Histamine and histamine-N-methyltransferase in the CSF of patients with multiple sclerosis. Agents Actions *13*, 255-257.

Tzakos, A.G., Troganis, A., Theodorou, V., Tselios, T., Svarnas, C., Matsoukas, J., Apostolopoulos, V., and Gerothanassis, I.P. (2005). Structure and function of the myelin proteins: current status and perspectives in relation to multiple sclerosis. Current medicinal chemistry *12*, 1569-1587.

Urich, E., Gutcher, I., Prinz, M., and Becher, B. (2006). Autoantibody-mediated demyelination depends on complement activation but not activatory Fc-receptors. Proc Natl Acad Sci U S A *103*, 18697-18702.

Utgaard, J.O., Jahnsen, F.L., Bakka, A., Brandtzaeg, P., and Haraldsen, G. (1998). Rapid secretion of prestored interleukin 8 from Weibel-Palade bodies of microvascular endothelial cells. J Exp Med *188*, 1751-1756.

Vaknin-Dembinsky, A., Balashov, K., and Weiner, H.L. (2006). 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 *176*, 7768-7774.

van den Eijnden-Schrauwen, Y., Atsma, D.E., Lupu, F., de Vries, R.E., Kooistra, T., and Emeis, J.J. (1997). Involvement of calcium and G proteins in the acute release of tissue-type plasminogen activator and von Willebrand factor from cultured human endothelial cells. Arterioscler Thromb Vasc Biol *17*, 2177-2187.

Vaz, N.M., de Souza, C.M., Maia, L.C., and Hanson, D.G. (1977). Effects of Bordetella pertussis on the sensitivity of inbred mice to vasoactive amines. Int Arch Allergy Appl Immunol *53*, 560-568.

Vinogradova, T.M., Roudnik, V.E., Bystrevskaya, V.B., and Smirnov, V.N. (2000). Centrosome-directed translocation of Weibel-Palade bodies is rapidly induced by thrombin, calyculin A, or cytochalasin B in human aortic endothelial cells. Cell Motil Cytoskeleton *47*, 141-153.

Vischer, U.M., Barth, H., and Wollheim, C.B. (2000). Regulated von Willebrand factor secretion is associated with agonist-specific patterns of cytoskeletal remodeling in cultured endothelial cells. Arterioscler Thromb Vasc Biol *20*, 883-891.

Wade, C.M., and Daly, M.J. (2005). Genetic variation in laboratory mice. Nature genetics *37*, 1175-1180.

Wade, C.M., Kulbokas, E.J., 3rd, Kirby, A.W., Zody, M.C., Mullikin, J.C., Lander, E.S., Lindblad-Toh, K., and Daly, M.J. (2002). The mosaic structure of variation in the laboratory mouse genome. Nature *420*, 574-578.

Waldner, H., Collins, M., and Kuchroo, V.K. (2004). Activation of antigen-presenting cells by microbial products breaks self tolerance and induces autoimmune disease. J Clin Invest *113*, 990-997.

Wardlaw, A.C. (1970). Inheritance of responsiveness to pertussis HSF in mice. Int Arch Allergy Appl Immunol *38*, 573-589.

Waxman, F.J., Taguiam, J.M., and Whitacre, C.C. (1984). Modification of the clinical and histopathologic expression of experimental allergic encephalomyelitis by the vasoactive amine antagonist cyproheptadine. Cell Immunol *85*, 82-93.

Waxman, S.G., and Sims, T.J. (1984). Specificity in central myelination: evidence for local regulation of myelin thickness. Brain Res *292*, 179-185.

Weinshenker, B.G. (1996). Epidemiology of multiple sclerosis. Neurol Clin *14*, 291-308.

Weinshenker, B.G., Bass, B., Rice, G.P., Noseworthy, J., Carriere, W., Baskerville, J., and Ebers, G.C. (1989). The natural history of multiple sclerosis: a geographically based study. I. Clinical course and disability. Brain *112 (Pt 1)*, 133-146.

Weiss, L., Whitmarsh, A.J., Yang, D.D., Rincon, M., Davis, R.J., and Flavell, R.A. (2000). Regulation of c-Jun NH(2)-terminal kinase (Jnk) gene expression during T cell activation. J Exp Med *191*, 139-146.

Wekerle, H., Sun, D., Oropeza-Wekerle, R.L., and Meyermann, R. (1987). Immune reactivity in the nervous system: modulation of T-lymphocyte activation by glial cells. The Journal of experimental biology *132*, 43-57.

Wettschureck, N., and Offermanns, S. (2005). Mammalian G proteins and their cell type specific functions. Physiol Rev *85*, 1159-1204.

Wilcox, C.E., Ward, A.M., Evans, A., Baker, D., Rothlein, R., and Turk, J.L. (1990). Endothelial cell expression of the intercellular adhesion molecule-1 (ICAM-1) in the central nervous system of guinea pigs during acute and chronic relapsing experimental allergic encephalomyelitis. J Neuroimmunol *30*, 43-51.

Wildbaum, G., Youssef, S., Grabie, N., and Karin, N. (1998). Neutralizing antibodies to IFN-gamma-inducing factor prevent experimental autoimmune encephalomyelitis. J Immunol *161*, 6368-6374.

Wildin, R.S., Garvin, A.M., Pawar, S., Lewis, D.B., Abraham, K.M., Forbush, K.A., Ziegler, S.F., Allen, J.M., and Perlmutter, R.M. (1991). Developmental regulation of lck gene expression in T lymphocytes. J Exp Med *173*, 383-393.

Willenborg, D.O., Fordham, S., Bernard, C.C., Cowden, W.B., and Ramshaw, I.A. (1996). IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. J Immunol *157*, 3223-3227.

Wingerchuk, D.M., Lucchinetti, C.F., and Noseworthy, J.H. (2001). Multiple sclerosis: current pathophysiological concepts. Lab Invest *81*, 263-281.

Winter, M.C., Kamath, A.M., Ries, D.R., Shasby, S.S., Chen, Y.T., and Shasby, D.M. (1999). Histamine alters cadherin-mediated sites of endothelial adhesion. Am J Physiol *277*, L988-995.

Wolf, S.D., Dittel, B.N., Hardardottir, F., and Janeway, C.A., Jr. (1996). Experimental autoimmune encephalomyelitis induction in genetically B cell-deficient mice. J Exp Med *184*, 2271-2278.

Wolff, B., Burns, A.R., Middleton, J., and Rot, A. (1998). Endothelial cell "memory" of inflammatory stimulation: human venular endothelial cells store interleukin 8 in Weibel-Palade bodies. J Exp Med *188*, 1757-1762.

Wucherpfennig, K.W., and Strominger, J.L. (1995). Molecular mimicry in T cell-mediated autoimmunity: viral peptides activate human T cell clones specific for myelin basic protein. Cell *80*, 695-705.

Yamaki, K., Thorlacius, H., Xie, X., Lindbom, L., Hedqvist, P., and Raud, J. (1998). Characteristics of histamine-induced leukocyte rolling in the undisturbed microcirculation of the rat mesentery. Br J Pharmacol *123*, 390-399.

Yamashita, M., Fukui, H., Sugama, K., Horio, Y., Ito, S., Mizuguchi, H., and Wada, H. (1991). Expression cloning of a cDNA encoding the bovine histamine H1 receptor. Proc Natl Acad Sci U S A *88*, 11515-11519.

Yang, H., Bell, T.A., Churchill, G.A., and Pardo-Manuel de Villena, F. (2007). On the subspecific origin of the laboratory mouse. Nature genetics *39*, 1100-1107.

Yednock, T.A., Cannon, C., Fritz, L.C., Sanchez-Madrid, F., Steinman, L., and Karin, N. (1992). Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature *356*, 63-66.

Yoshimura, S., Mishima, R., Miyoshi, K., Fujimoto, K., Murata, Y., Kitamura, Y., Takeda, N., and Fukui, H. (2005). Histamine H1 receptor-mediated histamine H1 receptor gene expression. Inflamm Res *54 Suppl 1*, S42-43.

Zamvil, S.S., Nelson, P.A., Mitchell, D.J., Knobler, R.L., Fritz, R.B., and Steinman, L. (1985). Encephalitogenic T cell clones specific for myelin basic protein. An unusual bias in antigen recognition. J Exp Med *162*, 2107-2124.

Zawilska, J.B., Rosiak, J., and Nowak, J.Z. (2004). Pertussis toxin-sensitive G protein modulates the ability of histamine to stimulate cAMP production in the chick pineal gland. Pol J Pharmacol *56*, 407-413.

Zhang, J., Hunter, K.W., Gandolph, M., Rowe, W.L., Finney, R.P., Kelley, J.M., Edmonson, M., and Buetow, K.H. (2005). A high-resolution multistrain haplotype analysis of laboratory mouse genome reveals three distinctive genetic variation patterns. Genome research *15*, 241-249.

Ziemssen, T., and Ziemssen, F. (2005). The role of the humoral immune system in multiple sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). Autoimmunity reviews *4*, 460-467.