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A Single Nucleotide Polymorphism in *Tyk2* Controls Susceptibility to Experimental Allergic Encephalomyelitis

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Abstract

Genes controlling immunopathologic diseases of differing etiopathology may also influence susceptibility to autoimmune disease. B10.D1-*H2^q*/SgJ mice with a 2538 G→A missense mutation in *tyrosine kinase-2* (*Tyk2*) are susceptible to *Toxoplasma gondii*, yet resistant to autoimmune arthritis, unlike the wild-type B10.Q/Ai substrain. To understand if *Tyk2* is also important in a second autoimmune model, experimental allergic encephalomyelitis (EAE) was induced in B10.D1-*H2^q*/SgJ ($Tyk2^A$) and B10.Q/Ai ($Tyk2^G$) mice with myelin oligodendrocyte glycoprotein peptide$_{79–96}$. B10.D1-*H2^q*/SgJ ($Tyk2^A$) mice were resistant to EAE while B10.Q/Ai ($Tyk2^G$) mice were susceptible and a single copy of the $Tyk2^G$ allele conferred EAE susceptibility in F_1 hybrids. Furthermore, EAE susceptibility in B10.D1-*H2^q*/SgJ ($Tyk2^A$) mice was complemented when pertussis toxin (PTX) was used to mimic the effects of environmental factors derived from infectious agents. Numerous cytokines and chemokines were increased when PTX was included in the immunization protocol. However, only RANTES, interleukin-6, and interferon-γ increased significantly with both genetic compensation and PTX complementation. These data indicate that *Tyk2* is a shared autoimmune disease susceptibility gene that can be complemented both genetically and environmentally. Single nucleotide polymorphisms like the one that distinguishes *Tyk2* alleles are of considerable significance given the potential role of gene-by-environment interactions in autoimmune disease susceptibility.

Keywords

EAE/MS; autoimmunity; neuroimmunology; protein kinases/phosphatases

INTRODUCTION

Autoimmune diseases have common features and understanding the commonalities underlying them may aid in the design of rational treatments. One approach to understanding these commonalities is to identify genes, termed shared autoimmune disease genes, whose alternately expressed alleles influence susceptibility to multiple autoimmune

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diseases. *Bphs/Hrh1*, which controls susceptibility to both experimental allergic encephalomyelitis (EAE) (1) and autoimmune orchitis (2) was the first non-antigen dependent shared autoimmune disease susceptibility gene to be identified and positionally cloned in the mouse (3). Another example of a potential shared autoimmune disease susceptibility gene is *Eae3/Idd3/Aod2* (4–6), which controls susceptibility to EAE, autoimmune insulin-dependent type I diabetes mellitus, and day 3 thymectomy induced autoimmune ovarian dysgenesis. In both mouse and humans, genetic clustering of autoimmune disease quantitative trait loci (QTL) supports the hypothesis that susceptibility to autoimmune disease may be controlled by shared genes (7). Moreover, relatives of multiple sclerosis (MS) and celiac disease patients are at increased risk of other autoimmune diseases suggesting a shared genetic susceptibility (8–11). Similarly, multiple autoimmune diseases are often observed within a single patient (12).

Tyrosine kinase 2 (*Tyk2*/Tyk2) participates in the signaling pathways of multiple cytokines in innate and acquired immunity (13,14). Tyk2 is a member of the JAK/STAT signaling pathway (15) and contributes to the signaling of IFN-α/β (16), IL-6 (17), IL-10 (18), IL-12 (19), IL-13 (20) and IL-23 (21). Depending on the ligand, cytokine receptor aggregation activates Tyk2 leading to phosphorylation of STAT 1, −3, −4, or −5 (15). The phospho-STAT dimerizes and translocates to the nucleus to promote gene transcription.

Although Tyk2 activation has been implicated in the signaling of multiple cytokines (22), $Tyk2^{tm1Shmd}$ ($Tyk2^{-/-}$) mice primarily exhibit defects in responses to IL-12 and type I IFN (14,23). B10.D1-$H2^{q}$/SgJ (B10.D1) mice are a substrain of B10.Q mice that have a naturally occurring mutation in *Tyk2*, designated $Tyk2^{A}$ (24). The 2538 G→A base substitution is predicted to result in a non-conservative amino acid substitution (E775K) within a critical APE motif of the JH2 (pseudokinase) domain of Tyk2. The JH2 domain is required for Tyk2 activation via ligand-activated cytokine receptors (16). Although $Tyk2^{A}$-specific transcripts are present at normal levels in B10.D1 mice, immunoreactive protein cannot be detected (24). B10.D1 splenocytes exhibit impaired STAT phosphorylation in response to IL-12, IL-23 and IFNα stimulation (24). In addition, neither T cells nor NK cells from B10.D1 mice produce IFNγ when stimulated with IL-12; however, this defect can be overcome by increasing the concentration of IL-12 and the incubation time or by stimulation through an IL-12-independent pathway (25,26).

Importantly, while B10.Q/Ai mice, which express a wild-type $Tyk2^{G}$ allele, are susceptible to collagen-induced arthritis (CIA) and resistant to *Toxoplasma gondii* infections, the $Tyk2^{A}$ mutation renders B10.D1 mice resistant to CIA and highly susceptible to *Toxoplasma gondii* (24–27), demonstrating that *Tyk2* is a shared immunopathology gene. Because autoimmune disease susceptibility genes can also be shared (7,28,29), we tested the hypothesis that *Tyk2* is a shared autoimmune disease gene by assessing susceptibility of B10.D1 and B10.Q/Ai mice to EAE, the principal animal model of MS. We found that *Tyk2* is a critical genetic regulator of EAE susceptibility and that the resistant $Tyk2^{A}$ allele can be compensated by one copy of the wild-type $Tyk2^{G}$ allele and by environmental factors such as pertussis toxin (PTX). These results are of particular significance given that *TYK2* polymorphisms are associated with increased risk of systemic lupus erythematosus (30,31) and that *TYK2* has recently been identified as a strong MS susceptibility gene in a genome-wide association study (32), and confirmed through independent replication (J. Oksenberg, personal communication).

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MATERIALS AND METHODS

Mice

B10.D1-*H2q*/SgJ (B10.D1) (Strain #002024) mice bearing the *Tyk2$^{rs2538\text{-}A}$* (*Tyk^{A}*) allele were purchased from The Jackson Laboratory (Bar Harbor, ME) and B10.Q/Ai (Line #4059) mice with the *Tyk2$^{rs2538\text{-}G}$* (*Tyk^{G}*) allele were purchased from Taconic Farms (Tarrytown, NY) through the National Institute of Allergy and Infectious Diseases Animal Supply Contract. Reciprocal F_1 hybrid progeny were generated and bred at the University of Vermont. Mice were housed at 25°C with 12:12h light-dark cycles and 40–60% humidity. Naïve, age-matched male and female mice were used throughout. The experimental procedures performed in this study were approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Induction and Evaluation of EAE

Mice were immunized for the induction of EAE using either the double injection or single injection protocols. For the double injection protocol mice were injected subcutaneously in the posterior right and left flank with a sonicated emulsion of 50 µg myelin oligodendrocyte glycoprotein peptide 79–96 [GKVALRIQNVRFSDEGGY] ($MOG_{79–96}$) (33) and 200 µg *Mycobacterium tuberculosis* H37Ra in CFA (Difco Laboratories, Detroit, MI) in 0.1 ml; one week later mice received the same injection on the right and left flank anterior of the original injection site (34). For the single injection protocol with PTX as an auxiliary adjuvant ($MOG_{79–96}$-CFA + PTX), mice were injected subcutaneously with 100 µg $MOG_{79–96}$ and 200 µg *M. tuberculosis* H37Ra in CFA in 0.1 ml on the posterior right and left flank and the scruff of the neck. Immediately afterward, each mouse received 200 ng PTX (List Biological Laboratories, Campbell, CA) in 0.2 ml by intravenous injection (34). EAE was evaluated daily beginning at day 5 as follows: 0, no clinical expression of disease; 1, flaccid tail without hind limb weakness; 2, hind limb weakness; 3, complete hind limb paralysis and floppy tail; 4, hind leg paralysis accompanied by a floppy tail and urinary or fecal incontinence; 5, moribund. Clinical quantitative trait variables were generated as previously described (35). Mice were considered positive for incidence if they showed any clinical signs greater than or equal to one for two or more consecutive days. The severity index is the cumulative disease score per days affected. Histological assessment of EAE neuropathology was done as previously described (35–38). Briefly, brains and spinal cords were dissected from calvarias and vertebral columns, respectively, and fixed by immersion in phosphate buffered (pH 7.2) 10% formalin. Representative areas of the brain and SC, including brainstem, cerebrum, cerebellum, and the cervical, thoracic, and lumbar segments of the SC, were selected for histopathological evaluation. EAE pathology reflects the overall severity of the lesions observed, extent and degree of myelin loss and tissue injury (swollen axon sheathes, swollen axons, and reactive gliosis), severity of the acute inflammatory response (predominantly neutrophils), and the severity of the chronic inflammatory response (lymphocytes/macrophages).

Cell culture conditions and lymphokine assays

For *ex vivo* cytokine and chemokine analysis, spleens and draining lymph nodes were obtained from mice immunized by both methods 10 days earlier for EAE as described above. Single cell suspensions at 1×10^6 cells/ml in RPMI 1640 media (Cellgro Mediatech, Inc., Manassas, VA) plus 5% FBS (HyClone, Logan, UT) were stimulated with 50 µg $MOG_{79–96}$. Cell culture supernatants were recovered at 72 hours and 23 different cytokine and chemokine levels quantified in duplicate by Bioplex multiplex cytokine assay (Becton Dickinson Bioscience, San Jose, CA), including IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17, Eotaxin, G-CSF, GM-CSF, IFN-γ, KC,MCP-1, MIP-1α, MIP-1β, RANTES, and TNF-α. To confirm the IL-17 and IFN-γ

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results, ELISAs were performed as described (34), using primary anti-IL-17A and anti-IFNγ antibodies and their corresponding biotinylated secondary antibodies (BD Biosciences-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 IFN-γ and IL-17 (R&D Systems, Inc., Minneapolis, MN) used as standards. IL-6 was confirmed using the mouse IL-6 DuoSet ELISA (R&D Systems, Inc., Minneapolis, MN).

Proliferation assays

Mice were immunized for EAE induction, and draining lymph nodes and spleens were harvested on day 10. Single cell suspensions were prepared, and 5×10^5 cells/well in RPMI 1640 media (5% FBS) were plated on standard 96-well U-bottom tissue culture plates and stimulated with 0, 2, 10 or 50 µg $MOG_{79–96}$ for 72 h at 37°C. During the last 18 h of culture, 1µCi of [^{3}H]-thymidine (PerkinElmer, Stelton, CT) was added. Cells were harvested onto glass fiber filters and thymidine uptake was determined by liquid scintillation.

Statistical analysis

Statistical analyses (two way ANOVA, Kruskal-Wallis test followed by Dunn's post hoc multiple comparisons, nonlinear regression-based curve fitting (34), and Chi-square test) were performed using GraphPad Prism version 5 (GraphPad Software, San Diego, CA). A *p* value of < 0.05 was considered significant.

RESULTS

B10.D1 mice are resistant to myelin oligodendrocyte glycoprotein peptide$_{79–96}$ ($MOG_{79–96}$)-CFA induced EAE

To determine if *Tyk2* is a shared autoimmune disease gene, EAE susceptibility was assessed in B10.D1 and B10.Q/Ai mice using the $MOG_{79–96}$-CFA double-injection protocol. B10.D1 mice were resistant (0/46) to EAE whereas B10.Q/Ai mice were susceptible (31/36; p<0.001) (Fig. 1A and Table I). On day 30, EAE pathology in the brain and spinal cord was assessed using previously defined neuropathologic trait variables (35,37). The susceptible B10.Q/Ai mice had marked EAE pathology in the spinal cord while B10.D1 had no EAE pathology (Figure 2). Neither strain showed significant EAE pathology in the brain. (B10.D1 × B10.Q/Ai) F_1 hybrids (16/18) and the reciprocal (B10.Q/Ai × B10.D1) F_1 hybrids (26/31) were both susceptible to $MOG_{79–96}$-CFA induced EAE compared to B10.D1 mice (p<0.001). The F_1 hybrids had significantly greater disease severity for each clinical disease trait compared to B10.D1 (p<0.001), and did not differ significantly from B10.Q/Ai mice (Fig. 1B and Table I). Similarly, lesion severity, monocyte/lymphocyte infiltration, and total EAE pathology score was significantly greater in the reciprocal F_1 hybrids compared to B10.D1 mice but did not differ from B10.Q/Ai mice in the spinal cord, p < 0.05 (Figure 2). Again, very little EAE pathology was noted in the brains of reciprocal F_1 hybrids. These data indicate that *Tyk2* alleles determine EAE susceptibility and that the $Tyk2^A$ allele is recessive. Therefore, EAE resistance in B10.D1 mice can be compensated genetically with a single copy of the wild-type $Tyk2^G$ allele, and since *Tyk2* is also a susceptibility gene in CIA (25), it is by definition a shared autoimmune disease susceptibility gene.

Immune responses of $Tyk2^A$ and $Tyk2^G$ mice elicited by $MOG_{79–96}$-CFA immunization

To investigate the mechanism whereby *Tyk2* alleles control T cell effector responses, the $MOG_{79–96}$-specific immune responses of B10.D1, B10.Q/Ai, and F_1 hybrid mice were

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compared ten days after $MOG_{79–96}$-CFA immunization. Although B10.D1 mice were resistant to $MOG_{79–96}$-CFA induced EAE, *ex vivo* proliferative responses did not differ between the strains (Fig. 3G). However, in *ex vivo* re-stimulation assays, B10.Q/Ai and F_1 mice produced significantly more IFNγ, IL-6, and RANTES compared to B10.D1 mice (Fig. 3). Surprisingly, IL-17 was not significantly higher in supernatants from B10.Q/Ai or F_1 cells, although a trend toward increased IL-17 production was noted when the results were grouped by strain (Fig. 3C). The three strains did not exhibit significant differences in any of the other cytokines and chemokines assayed (e.g., IL-4 and TNFα, Fig 3, and others not shown). Importantly, IL-12(p40) and IL-12(p70) were not different indicating IL-12 insufficiency is not responsible for EAE resistance in B10.D1 mice.

$Tyk2^A$-mediated resistance is abrogated by PTX

PTX is an example of an environmental factor derived from an infectious agent that influences susceptibility to EAE and is capable of overriding genetic checkpoints in this autoimmune disease (39). Therefore, we included PTX in the immunization protocol and tested the susceptibility of B10.D1 and B10.Q/Ai mice to EAE. In the $MOG_{79–96}$-CFA + PTX single injection protocol (34), mice receive the same total amount of $MOG_{79–96}$ in CFA as the double injection protocol but also receive an i.v. injection of PTX on day 0. In B10.Q/Ai mice injected with $MOG_{79–96}$-CFA + PTX (Fig. 4B), there was increased incidence and more severe disease compared to B10.Q/Ai mice injected with $MOG_{79–96}$-CFA alone (Table II). A more dramatic difference was seen in B10.D1 mice, which are much more susceptible (33/37) to EAE induced using PTX (Fig. 4A and Table II) than with $MOG_{79–96}$-CFA alone. Clearly, EAE susceptibility in B10.D1 ($Tyk2^A$) mice can be complemented by environmental factors derived from infectious agents such as PTX.

PTX complementation of EAE susceptibility correlates with increased cytokine production

The complementation of EAE susceptibility in B10.D1 ($Tyk2^A$) mice following immunization with PTX was not reflected in their antigen-specific *ex vivo* proliferative responses, which were not significantly different between immunization protocols (Fig. 5B). Inclusion of PTX in the immunization protocol, however, resulted in increased cytokine secretion by cultured cells from both strains, consistent with the increased severity of EAE induced by the PTX protocol. PTX is known to induce many cytokines that are proinflammatory and pathogenic in EAE, but PTX also induces T_H2 responses (40,41). The changes due to inclusion of PTX in the immunization of B10.D1 mice as assayed in cell culture supernatants were consistent with these published reports. For example, cytokines characteristic of T_H1 (IFNγ, IL-12, TNFα), T_H17 (IL-17, IL-6) and T_H2 (IL-4, IL-5, IL-9, and IL-13) responses were elevated compared to those elicited by immunization without PTX (Fig. 5A). In addition, members of the CC chemokine family, MIP-1α and RANTES, were elevated more than fourfold compared to those induced by $MOG_{79–96}$-CFA alone, suggesting that there is likely to be a distinct mobilization of T_H1 cells in the PTX-exposed mice (42,43). Notably, among the changes in cytokine and chemokine production elicited in B10.D1 mice by the inclusion of PTX in the immunization protocol, IFNγ, IL-6, and RANTES were also significantly elevated by genetic compensation (Fig. 3).

DISCUSSION

A naturally-occurring single nucleotide polymorphism (SNP) within the pseudokinase domain of *Tyk2* influences the immunopathologic outcomes of CIA (25) and *Toxoplasma gondii* infection in B10.Q/Ai and B10.D1 (26), making *Tyk2* a shared immunopathology gene. The data presented here showing that *Tyk2* alleles control EAE susceptibility demonstrate that *Tyk2* is also a shared autoimmune disease susceptibility gene. Specifically, B10.D1 mice which possess the $Tyk2^A$ allele are completely resistant to $MOG_{79–96}$-CFA-

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induced EAE while B10.Q/Ai substrain mice expressing the *Tyk2*^{G} allele are susceptible. Moreover, a single copy of the *Tyk2*^{G} allele fully confers EAE susceptibility in B10.D1 F_1 hybrids clearly establishing that *Tyk2* is important in controlling autoimmune disease susceptibility. When PTX was included in the immunization regime to mimic the effects of environmental agents derived from infectious agents, EAE susceptibility was also complemented thus emphasizing the contextual role that gene-by-environment interactions play in determining susceptibility to autoimmune diseases.

It is not surprising that *Tyk2* is a player in mouse autoimmunity and inflammation, due to its importance in supporting IL-12-induced IFN-γ responses (14,15,44), and its ability to down-regulate T_H2-mediated antibody production, especially IgE (45). There are differences between the requirements for *Tyk2* in mouse as compared to human immune responses. For example, human *TYK2*-deficient cell lines are completely unresponsive to type 1 IFN, and IL-6 and IL-10 signaling is severely impaired (13), whereas these phenotypes are leaky in *Tyk2*$^{-/-}$ mice, and high concentrations of IFNα can overcome the mouse *Tyk2* deficiency MHC class I expression (23). However, evidence exists that *Tyk2* shares some functions between these two species. There is a report of one patient with a mutation in *TYK2*, who had a remarkably similar immune response to that seen in B10.D1 mice, with hyper-IgE syndrome, increased susceptibility to multiple microbial pathogens, an impaired STAT-4 phosphorylation pattern, and undetectable IFNγ production in response to IL-12 (13). This supports the hypothesis that human *TYK2* is also a shared immunopathology gene. Supporting the hypothesis that TYK2 is a shared human autoimmune disease susceptibility gene, it has recently been identified as a strong MS susceptibility gene in a genome-wide association study (32), and confirmed through independent replication (J. Oksenberg, personal communication) and *TYK2* polymorphisms have also been associated with increased risk of systemic lupus erythematosus (30,31).

However, the manner by which *Tyk2* complements autoimmunity is not known. T cell proliferation induced by IL-12 is not *Tyk2* dependent (23) and likewise, no differences in antigen-specific proliferation were observed between B10.D1 and wild type B10.Q/Ai mice following immunization with $MOG_{79–96}$-CFA in this study. B10.D1 mice make IL-12 in normal abundance (26) and both IL-12 and TNFα levels were similar between B10.D1 and B10.Q/Ai and F_1 hybrid mice after induction of EAE without PTX. Therefore, differences in proliferation and/or levels of these cytokines are not likely an important mechanism by which B10.D1 mice are resistant to EAE. The *Tyk2*^{A} mutation impairs signaling through both IL-12R and IL-23R pathways (24). It is more likely therefore that their EAE resistance is due to this signaling defect, leading to an inability to up-regulate encephalitogenic levels of IFNγ (via IL-12R, reviewed in (44)) and IL-17 (via IL-23R, reviewed in (44)) or to activate T cells that make these cytokines. We observed differences between B10.D1 and B10.Q/Ai mice for both of these effector molecules, although the difference in IL-17 was only a trend. In addition, B10.D1 (*Tyk2*^{A}) mice had significantly impaired ability to produce pro-inflammatory molecules such as IL-6 and RANTES. IL-17 and IFNγ have well-documented roles in EAE (46–49), and the development of EAE is blocked by antibodies or antagonists of IL-6 and RANTES (50,51). It is important to note that the effects of the *Tyk2*^{A} allele may not be exclusively in T cells as *Tyk2* is also required in dendritic cells for IL-12, IL-23 and IFNγ production (52).

In the present study, PTX was included in the EAE induction protocol to reveal gene-by-environment interactions, and especially the effects of an environmental factor derived from an infectious agent. B10.D1 mice were susceptible to EAE only when it was induced with $MOG_{79–96}$-CFA + PTX, and they produced significantly higher levels of multiple T_H1-, T_H2-, and T_H17-type cytokines and chemokines compared to the $MOG_{79–96}$-CFA-inoculated mice (Fig. 5A). Of interest, both GM-CSF and IL-5 showed large increases with

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PTX treatment. Multiple sclerosis patients in the active phase of disease have elevated levels of GM-CSF compared to patients in remission (53) and mice lacking GM-CSF are resistant to MOG_{35-55}-induced EAE (54). Taken together, these reports and the present study suggest that GM-CSF is critically important in EAE and MS pathogenesis. A small study of MS patients and controls found patients with highly proliferating MBP-specific T cells produced higher levels of IL-5 and IL-17 and this correlated with number of MRI-identified active plaques (55). However IL-5 has also been shown to increase in MS patients treated with IFN-beta (56) or glatiramer acetate (57).

Although it is not known how PTX restores EAE susceptibility in B10.D1 mice, several possibilities exist. PTX may act intrinsically in T cells because they express PTX-sensitive $G_{i/o}$ proteins. Inhibitory $G_{i/o}$ proteins are inactivated by PTX-mediated ADP-ribosylation (58). Following direct TCR stimulation, T cells from $G_{\alpha i2}{}^{-/-}$ mice produce more IL-2, IL-4 and IFNγ than wild type mice (59). Thus, the PTX-mediated abrogation of G_i coupled inhibitory signals in T cells would increase the magnitude of the cytokine responses. This may be one mechanism by which PTX complements EAE susceptibility in B10.D1 mice. Accordingly, PTX also increases the expression of the co-stimulatory molecule CD28 on T cells (41) which would therefore potentiate the immune response.

PTX could also increase T cell cytokine responses and complement EAE susceptibility through its effects on APCs. PTX increases secretion of proinflammatory cytokines such as IL-1β (41), IL-6 (60), IL-12 (61) and TNFα (62). Increased IL-6 production by PTX-treated APCs promotes the generation of IL-17 producing T cells (60). Indeed, IL-6 and IL-17 were up-regulated by PTX in both B10.D1 (*Tyk2^A^*) and B10.Q/Ai (*Tyk2^G^*) mice. PTX also enhances the ability of the APC to activate T cells by inducing dendritic cell maturation (62) and up-regulating expression of the co-stimulatory molecules CD80 and CD86 in the spleen and spinal cord (40,41,63). PTX-induced cytokine production and T cell clonal expansion are thought to occur primarily through the CD28:CD80/86 costimulatory pathway (64). The PTX-induced changes in EAE susceptibility of B10.D1 mice cannot be attributed solely to effects on either T cells or APCs based on these experiments. Although it is tantalizing to speculate that PTX increases co-stimulatory molecules on APCs which then increases both APC maturation and T cell differentiation and clonal expansion as evidence suggests (40,63,65), leading to the cytokine differences we observed, careful bone marrow chimera experiments must be performed to conclude this. These experiments are underway to attempt to determine if the effect of PTX is directly on APC or T cells through APCs.

In B10.D1 ($Tyk2^A$) mice, IFNγ and IL-17 levels were both elevated, indicating that PTX must enhance their production via Tyk2-independent pathways, such as IL-18R-mediated IFNγ production (26). The Tyk2−/− mouse has decreased expression of Il-18R (66), so if PTX modulated production of IFN-γ via IL-18, it would also have to up-regulate the IL-18R for this to occur. The role of IL-18 is paradoxical, despite its apparently excellent candidacy. IL-18Rα-deficient mice are protected from EAE, in contrast to IL-18-deficient mice, which are susceptible (67). Further complicating matters, an alternative unidentified ligand for IL-18R on APCs responsible for Th17 pathogenicity has been postulated (68). Within the type I IFN pathway, alternate routes of STAT4 phosphorylation have also been noted (69). Additionally, PTX intoxication may produce sufficient IL-12 stimulation as to cause receptor aggregation leading to phosphorylation of Jak2 that can stand in for Tyk2. We observed high levels of active IL-12 after PTX inoculation (Fig. 5A), and it has been shown that greater exposure to IL-12 *in vitro* can overcome the effects of the $Tyk2^A$ allele as measured by STAT4 phosphorylation (25). Thus, IL-12 alone could explain the results we have observed. Because the issue of direct and indirect effects on the pathogenic T cells is further complicated by the independent roles of IL-12, IL-18, and an unknown IL-18R-

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ligand, it is unclear which pathway PTX uses to modulate IFN-γ and IL-17 in the B10.D1 mice.

The ability of environmental factors derived from infectious agents to alter autoimmune disease susceptibility controlled by a SNP highlights the contextual importance of gene-by-environment interactions in determining autoimmune disease susceptibility. There is increasing evidence that human autoimmune disease results from a complex interaction of environmental effects in genetically susceptible individuals. The 75% discordance rate for MS in monozygotic twins (70) suggests that environmental factors are important in MS. In particular, low ultraviolet light exposure (71–74) and the resulting low serum 25-$(OH)_2D_3$ levels correlate with increased MS risk (75–78). It is therefore interesting in this regard, that *in vivo* treatment of mice with 1,25-dihydroxyvitamin D_3 ameliorates EAE (79), and treatment of activated T cells *in vitro* with 1,25-dihydroxyvitamin D_3 inhibits IL-12-induced tyrosine phosphorylation of Tyk2, thereby reducing T cell responses to antigen (80). Another important finding is that the sexual dimorphism observed in MS is increasing in the last 50 years suggesting that emergent factors such as environmental estrogens could selectively promote MS in women (81). The phytoestrogen quercetin, known to reduce signs of EAE in mice, is also capable of blocking IL-12 induced phosphorylation of Tyk2 (82). In preliminary work we note that EAE susceptibility in B10.D1 ($Tyk2^A$) and B10.Q/Ai ($Tyk2^G$) mice is in fact sexually dimorphic (Blankenhorn, Spach, and Teuscher, in preparation, 2009). In the context of the present report, therefore, the mutant $Tyk2^A$ allele is a good candidate for an environmentally sensitive genetic modifier of demyelinating diseases, responding to a wide variety of environmental factors including 1,25-dihydroxyvitamin D_3, estrogenic compounds, and toxins produced by microorganisms, such as PTX.

In summary, we demonstrate that *Tyk2* is both a shared immunopathology gene and also a shared autoimmune disease susceptibility gene in mice. We further demonstrate that it can be complemented both genetically and environmentally. As such, this model provides a unique opportunity to identify additional environmental factors impacting a core genetic network underlying susceptibility to autoimmune disease.

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REFERENCES

1. Linthicum DS, Frelinger JA. Acute autoimmune encephalomyelitis in mice. II. Susceptibility is controlled by the combination of H-2 and histamine sensitization genes. J Exp Med 1982;156:31–40. [PubMed: 6806429]
2. Teuscher C, Smith SM, Goldberg EH, Shearer GM, Tung KS. Experimental allergic orchitis in mice. I. Genetic control of susceptibility and resistance to induction of autoimmune orchitis. Immunogenetics 1985;22:323–333. [PubMed: 3932196]
3. Ma RZ, Gao J, Meeker ND, Fillmore PD, Tung KS, Watanabe T, Zachary JF, Offner H, Blankenhorn EP, Teuscher C. Identification of Bphs, an autoimmune disease locus, as histamine receptor H1. Science 2002;297:620–623. [PubMed: 12142541]
4. Teuscher C, Wardell BB, Lunceford JK, Michael SD, Tung KS. Aod2, the locus controlling development of atrophy in neonatal thymectomy-induced autoimmune ovarian dysgenesis, co-localizes with Il2, Fgfb, and Idd3. J Exp Med 1996;183:631–637. [PubMed: 8627174]
5. Teuscher C, Rhein DM, Livingstone KD, Paynter RA, Doerge RW, Nicholson SM, Melvold RW. Evidence that Tmevd2 and eae3 may represent either a common locus or members of a gene

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complex controlling susceptibility to immunologically mediated demyelination in mice. J Immunol 1997;159:4930–4934. [PubMed: 9366418]

6. del Rio R, Noubade R, Subramanian M, Saligrama N, Diehl S, Rincon M, Teuscher C. SNPs upstream of the minimal promoter control IL-2 expression and are candidates for the autoimmune disease-susceptibility locus Aod2/Idd3/Eae3. Genes Immun 2008;9:115–121. [PubMed: 18200031]
7. Becker KG, Simon RM, Bailey-Wilson JE, Freidlin B, Biddison WE, McFarland HF, Trent JM. Clustering of non-major histocompatibility complex susceptibility candidate loci in human autoimmune diseases. Proc Natl Acad Sci U S A 1998;95:9979–9984. [PubMed: 9707586]
8. Hemminki K, Li X, Sundquist J, Hillert J, Sundquist K. Risk for multiple sclerosis in relatives and spouses of patients diagnosed with autoimmune and related conditions. Neurogenetics. 2008
9. Neuhausen SL, Steele L, Ryan S, Mousavi M, Pinto M, Osann KE, Flodman P, Zone JJ. Co-occurrence of celiac disease and other autoimmune diseases in celiacs and their first-degree relatives. J Autoimmun 2008;31:160–165. [PubMed: 18692362]
10. Barcellos LF, Kamdar BB, Ramsay PP, DeLoa C, Lincoln RR, Caillier S, Schmidt S, Haines JL, Pericak-Vance MA, Oksenberg JR, Hauser SL. Clustering of autoimmune diseases in families with a high-risk for multiple sclerosis: a descriptive study. Lancet Neurol 2006;5:924–931. [PubMed: 17052659]
11. Lettre G, Rioux JD. Autoimmune diseases: insights from genome-wide association studies. Hum Mol Genet 2008;17:R116–R121. [PubMed: 18852199]
12. Huber A, Menconi F, Corathers S, Jacobson EM, Tomer Y. Joint genetic susceptibility to type 1 diabetes and autoimmune thyroiditis: from epidemiology to mechanisms. Endocr Rev 2008;29:697–725. [PubMed: 18776148]
13. Minegishi Y, Saito M, Morio T, Watanabe K, Agematsu K, Tsuchiya S, Takada H, Hara T, Kawamura N, Ariga T, Kaneko H, Kondo N, Tsuge I, Yachie A, Sakiyama Y, Iwata T, Bessho F, Ohishi T, Joh K, Imai K, Kogawa K, Shinohara M, Fujieda M, Wakiguchi H, Pasic S, Abinun M, Ochs HD, Renner ED, Jansson A, Belohradsky BH, Metin A, Shimizu N, Mizutani S, Miyawaki T, Nonoyama S, Karasuyama H. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. Immunity 2006;25:745–755. [PubMed: 17088085]
14. Karaghiosoff M, Neubauer H, Lassnig C, Kovarik P, Schindler H, Pircher H, McCoy B, Bogdan C, Decker T, Brem G, Pfeffer K, Muller M. Partial impairment of cytokine responses in Tyk2-deficient mice. Immunity 2000;13:549–560. [PubMed: 11070173]
15. Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene 2002;285:1–24. [PubMed: 12039028]
16. Velazquez L, Mogensen KE, Barbieri G, Fellous M, Uze G, Pellegrini S. Distinct domains of the protein tyrosine kinase tyk2 required for binding of interferon-alpha/beta and for signal transduction. J Biol Chem 1995;270:3327–3334. [PubMed: 7531704]
17. Stahl N, Boulton TG, Farruggella T, Ip NY, Davis S, Witthuhn BA, Quelle FW, Silvennoinen O, Barbieri G, Pellegrini S, et al. Association and activation of Jak-Tyk kinases by CNTF-LIF-OSM-IL-6 beta receptor components. Science 1994;263:92–95. [PubMed: 8272873]
18. Finbloom DS, Winestock KD. IL-10 induces the tyrosine phosphorylation of tyk2 and Jak1 and the differential assembly of STAT1 alpha and STAT3 complexes in human T cells and monocytes. J Immunol 1995;155:1079–1090. [PubMed: 7543512]
19. Bacon CM, McVicar DW, Ortaldo JR, Rees RC, O'Shea JJ, Johnston JA. Interleukin 12 (IL-12) induces tyrosine phosphorylation of JAK2 and TYK2: differential use of Janus family tyrosine kinases by IL-2 and IL-12. J Exp Med 1995;181:399–404. [PubMed: 7528775]
20. Welham MJ, Learmonth L, Bone H, Schrader JW. Interleukin-13 signal transduction in lymphohemopoietic cells. Similarities and differences in signal transduction with interleukin-4 and insulin. J Biol Chem 1995;270:12286–12296. [PubMed: 7744881]
21. Parham C, Chirica M, Timans J, Vaisberg E, Travis M, Cheung J, Pflanz S, Zhang R, Singh KP, Vega F, To W, Wagner J, O'Farrell AM, McClanahan T, Zurawski S, Hannum C, Gorman D, Rennick DM, Kastelein RA, de Waal Malefyt R, Moore KW. A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J Immunol 2002;168:5699–5708. [PubMed: 12023369]

NIH-PA Author Manuscript NIH-PA Author Manuscript NIH-PA Author Manuscript

22. Leonard WJ, O'Shea JJ. Jaks and STATs: biological implications. Annu Rev Immunol 1998;16:293–322. [PubMed: 9597132]

23. Shimoda K, Kato K, Aoki K, Matsuda T, Miyamoto A, Shibamori M, Yamashita M, Numata A, Takase K, Kobayashi S, Shibata S, Asano Y, Gondo H, Sekiguchi K, Nakayama K, Nakayama T, Okamura T, Okamura S, Niho Y, Nakayama K. Tyk2 plays a restricted role in IFN alpha signaling, although it is required for IL-12-mediated T cell function. Immunity 2000;13:561–571. [PubMed: 11070174]

24. Shaw MH, Boyartchuk V, Wong S, Karaghiosoff M, Ragimbeau J, Pellegrini S, Muller M, Dietrich WF, Yap GS. A natural mutation in the Tyk2 pseudokinase domain underlies altered susceptibility of B10.Q/J mice to infection and autoimmunity. Proc Natl Acad Sci U S A 2003;100:11594–11599. [PubMed: 14500783]

25. Ortmann R, Smeltz R, Yap G, Sher A, Shevach EM. A heritable defect in IL-12 signaling in B10.Q/J mice. I. In vitro analysis. J Immunol 2001;166:5712–5719. [PubMed: 11313413]

26. Yap GS, Ortmann R, Shevach E, Sher A. A heritable defect in IL-12 signaling in B10.Q/J mice. II. Effect on acute resistance to Toxoplasma gondii and rescue by IL-18 treatment. J Immunol 2001;166:5720–5725. [PubMed: 11313414]

27. Gazzinelli RT, Wysocka M, Hieny S, Scharton-Kersten T, Cheever A, Kuhn R, Muller W, Trinchieri G, Sher A. In the absence of endogenous IL-10, mice acutely infected with Toxoplasma gondii succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. J Immunol 1996;157:798–805. [PubMed: 8752931]

28. Vyse TJ, Todd JA. Genetic analysis of autoimmune disease. Cell 1996;85:311–318. [PubMed: 8616887]

29. Becker KG. The common genetic hypothesis of autoimmune/inflammatory disease. Curr Opin Allergy Clin Immunol 2001;1:399–405. [PubMed: 11964719]

30. Sigurdsson S, Nordmark G, Goring HH, Lindroos K, Wiman AC, Sturfelt G, Jonsen A, Rantapaa-Dahlqvist S, Moller B, Kere J, Koskenmies S, Widen E, Eloranta ML, Julkunen H, Kristjansdottir H, Steinsson K, Alm G, Ronnblom L, Syvanen AC. Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus. Am J Hum Genet 2005;76:528–537. [PubMed: 15657875]

31. Graham DS, Akil M, Vyse TJ. Association of polymorphisms across the tyrosine kinase gene, TYK2 in UK SLE families. Rheumatology (Oxford) 2007;46:927–930. [PubMed: 17384181]

32. Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, Kwiatkowski DP, McCarthy MI, Ouwehand WH, Samani NJ, Todd JA, Donnelly P, Barrett JC, Davison D, Easton D, Evans DM, Leung HT, Marchini JL, Morris AP, Spencer CC, Tobin MD, Attwood AP, Boorman JP, Cant B, Everson U, Hussey JM, Jolley JD, Knight AS, Koch K, Meech E, Nutland S, Prowse CV, Stevens HE, Taylor NC, Walters GR, Walker NM, Watkins NA, Winzer T, Jones RW, McArdle WL, Ring SM, Strachan DP, Pembrey M, Breen G, St Clair D, Caesar S, Gordon-Smith K, Jones L, Fraser C, Green EK, Grozeva D, Hamshere ML, Holmans PA, Jones IR, Kirov G, Moskivina V, Nikolov I, O'Donovan MC, Owen MJ, Collier DA, Elkin A, Farmer A, Williamson R, McGuffin P, Young AH, Ferrier IN, Ball SG, Balmforth AJ, Barrett JH, Bishop TD, Iles MM, Maqbool A, Yuldasheva N, Hall AS, Braund PS, Dixon RJ, Mangino M, Stevens S, Thompson JR, Bredin F, Tremelling M, Parkes M, Drummond H, Lees CW, Nimmo ER, Satsangi J, Fisher SA, Forbes A, Lewis CM, Onnie CM, Prescott NJ, Sanderson J, Matthew CG, Barbour J, Mohiuddin MK, Todhunter CE, Mansfield JC, Ahmad T, Cummings FR, Jewell DP, Webster J, Brown MJ, Lathrop MG, Connell J, Dominiczak A, Marcano CA, Burke B, Dobson R, Gungadoo J, Lee KL, Munroe PB, Newhouse SJ, Onipinla A, Wallace C, Xue M, Caulfield M, Farrall M, Barton A, Bruce IN, Donovan H, Eyre S, Gilbert PD, Hilder SL, Hinks AM, John SL, Potter C, Silman AJ, Symmons DP, Thomson W, Worthington J, Dunger DB, Widmer B, Frayling TM, Freathy RM, Lango H, Perry JR, Shields BM, Weedon MN, Hattersley AT, Hitman GA, Walker M, Elliott KS, Groves CJ, Lindgren CM, Rayner NW, Timpson NJ, Zeggini E, Newport M, Sirugo G, Lyons E, Vannberg F, Hill AV, Bradbury LA, Farrar C, Pointon JJ, Wordsworth P, Brown MA, Franklyn JA, Heward JM, Simmonds MJ, Gough SC, Seal S, Stratton MR, Rahman N, Ban M, Goris A, Sawcer SJ, Compston A, Conway D, Jallow M, Rockett KA, Bumpstead SJ, Chaney A, Downes K, Ghori MJ, Gwilliam R, Hunt SE, Inouye M, Keniry A, King E, McGinnis R, Potter S, Ravindrarajah R, Whittaker P, Widden C, Withers D, Cardin NJ, Ferreira T, Pereira-Gale J,

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Hallgrimsdo'ttir IB, Howie BN, Su Z, Teo YY, Vukcevic D, Bentley D, Mitchell SL, Newby PR, Brand OJ, Carr-Smith J, Pearce SH, Reveille JD, Zhou X, Sims AM, Dowling A, Taylor J, Doan T, Davis JC, Savage L, Ward MM, Learch TL, Weisman MH, Brown M. Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. Nat Genet 2007;39:1329–1337. [PubMed: 17952073]

33. Abdul-Majid KB, Jirholt J, Stadelmann C, Stefferl A, Kjellen P, Wallstrom E, Holmdahl R, Lassmann H, Olsson T, Harris RA. Screening of several H-2 congenic mouse strains identified H-2(q) mice as highly susceptible to MOG-induced EAE with minimal adjuvant requirement. J Neuroimmunol 2000;111:23–33. [PubMed: 11063818]
34. Noubade R, Milligan G, Zachary JF, Blankenhorn EP, del Rio R, Rincon M, Teuscher C. Histamine receptor H1 is required for TCR-mediated p38 MAPK activation and optimal IFN-gamma production in mice. J Clin Invest 2007;117:3507–3518. [PubMed: 17965772]
35. Teuscher C, Noubade R, Spach K, McElvany B, Bunn JY, Fillmore PD, Zachary JF, Blankenhorn EP. Evidence that the Y chromosome influences autoimmune disease in male and female mice. Proc Natl Acad Sci U S A 2006;103:8024–8029. [PubMed: 16702550]
36. Butterfield RJ, Blankenhorn EP, Roper RJ, Zachary JF, Doerge RW, Sudweeks J, Rose J, Teuscher C. 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 1999;162:3096–3102. [PubMed: 10072563]
37. Butterfield RJ, Blankenhorn EP, Roper RJ, Zachary JF, Doerge RW, Teuscher C. Identification of genetic loci controlling the characteristics and severity of brain and spinal cord lesions in experimental allergic encephalomyelitis. Am J Pathol 2000;157:637–645. [PubMed: 10934166]
38. Butterfield RJ, Sudweeks JD, Blankenhorn EP, Korngold R, Marini JC, Todd JA, Roper RJ, Teuscher C. New genetic loci that control susceptibility and symptoms of experimental allergic encephalomyelitis in inbred mice. J Immunol 1998;161:1860–1867. [PubMed: 9712054]
39. Blankenhorn EP, Butterfield RJ, Rigby R, Cort L, Giambrone D, McDermott P, McEntee K, Solowski N, Meeker ND, Zachary JF, Doerge RW, Teuscher C. Genetic analysis of the influence of pertussis toxin on experimental allergic encephalomyelitis susceptibility: an environmental agent can override genetic checkpoints. J Immunol 2000;164:3420–3425. [PubMed: 10706738]
40. Hofstetter HH, Shive CL, Forsthuber TG. Pertussis toxin modulates the immune response to neuroantigens injected in incomplete Freund's adjuvant: induction of Th1 cells and experimental autoimmune encephalomyelitis in the presence of high frequencies of Th2 cells. J Immunol 2002;169:117–125. [PubMed: 12077236]
41. Ryan M, McCarthy L, Rappuoli R, Mahon BP, Mills KH. Pertussis toxin potentiates Th1 and Th2 responses to co-injected antigen: adjuvant action is associated with enhanced regulatory cytokine production and expression of the co-stimulatory molecules B7-1, B7-2 and CD28. Int Immunol 1998;10:651–662. [PubMed: 9645613]
42. Sewell WA, Munoz JJ, Vadas MA. Enhancement of the intensity, persistence, and passive transfer of delayed-type hypersensitivity lesions by pertussigen in mice. J Exp Med 1983;157:2087–2096. [PubMed: 6304228]
43. Millward JM, Caruso M, Campbell IL, Gauldie J, Owens T. IFN-gamma-induced chemokines synergize with pertussis toxin to promote T cell entry to the central nervous system. J Immunol 2007;178:8175–8182. [PubMed: 17548656]
44. Watford WT, Hissong BD, Bream JH, Kanno Y, Muul L, O'Shea JJ. Signaling by IL-12 and IL-23 and the immunoregulatory roles of STAT4. Immunol Rev 2004;202:139–156. [PubMed: 15546391]
45. Seto Y, Nakajima H, Suto A, Shimoda K, Saito Y, Nakayama KI, Iwamoto I. Enhanced Th2 cell-mediated allergic inflammation in Tyk2-deficient mice. J Immunol 2003;170:1077–1083. [PubMed: 12517976]
46. Cua DJ, Sherlock J, Chen Y, Murphy CA, Joyce B, Seymour B, Lucian L, To W, Kwan S, Churakova T, Zurawski S, Wiekowski M, Lira SA, Gorman D, Kastelein RA, Sedgwick JD. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. Nature 2003;421:744–748. [PubMed: 12610626]

NIH-PA Author Manuscript

47. Ferber IA, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, Dalton D, Fathman CG. Mice with a disrupted IFN-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). J Immunol 1996;156:5–7. [PubMed: 8598493]
48. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005;201:233–240. [PubMed: 15657292]
49. Willenborg DO, Fordham S, Bernard CC, Cowden WB, Ramshaw IA. IFN-gamma plays a critical down-regulatory role in the induction and effector phase of myelin oligodendrocyte glycoprotein-induced autoimmune encephalomyelitis. J Immunol 1996;157:3223–3227. [PubMed: 8871615]
50. Linker RA, Luhder F, Kallen KJ, Lee DH, Engelhardt B, Rose-John S, Gold R. IL-6 transsignalling modulates the early effector phase of EAE and targets the blood-brain barrier. J Neuroimmunol 2008;205:64–72. [PubMed: 18950871]
51. Proudfoot AE, de Souza AL, Muzio V. The use of chemokine antagonists in EAE models. J Neuroimmunol 2008;198:27–30. [PubMed: 18550179]
52. Tokumasa N, Suto A, Kagami S, Furuta S, Hirose K, Watanabe N, Saito Y, Shimoda K, Iwamoto I, Nakajima H. Expression of Tyk2 in dendritic cells is required for IL-12, IL-23, and IFN-gamma production and the induction of Th1 cell differentiation. Blood 2007;110:553–560. [PubMed: 17395783]
53. Carrieri PB, Provitera V, De Rosa T, Tartaglia G, Gorga F, Perrella O. Profile of cerebrospinal fluid and serum cytokines in patients with relapsing-remitting multiple sclerosis: a correlation with clinical activity. Immunopharmacol Immunotoxicol 1998;20:373–382. [PubMed: 9736442]
54. McQualter JL, Darwiche R, Ewing C, Onuki M, Kay TW, Hamilton JA, Reid HH, Bernard CC. Granulocyte macrophage colony-stimulating factor: a new putative therapeutic target in multiple sclerosis. J Exp Med 2001;194:873–882. [PubMed: 11581310]
55. Hedegaard CJ, Krakauer M, Bendtzen K, Lund H, Sellebjerg F, Nielsen CH. T helper cell type 1 (Th1), Th2 and Th17 responses to myelin basic protein and disease activity in multiple sclerosis. Immunology 2008;125:161–169. [PubMed: 18397264]
56. Wiesemann E, Deb M, Hemmer B, Radeke HH, Windhagen A. Early identification of interferon-beta responders by ex vivo testing in patients with multiple sclerosis. Clin Immunol 2008;128:306–313. [PubMed: 18539537]
57. Sanna A, Fois ML, Arru G, Huang YM, Link H, Pugliatti M, Rosati G, Sotgiu S. Glatiramer acetate reduces lymphocyte proliferation and enhances IL-5 and IL-13 production through modulation of monocyte-derived dendritic cells in multiple sclerosis. Clin Exp Immunol 2006;143:357–362. [PubMed: 16412061]
58. Kaslow HR, Burns DL. Pertussis toxin and target eukaryotic cells: binding, entry, and activation. FASEB J 1992;6:2684–2690. [PubMed: 1612292]
59. Huang TT, Zong Y, Dalwadi H, Chung C, Miceli MC, Spicher K, Birnbaumer L, Braun J, Aranda R. TCR-mediated hyper-responsiveness of autoimmune Galphai2(−/−) mice is an intrinsic naive CD4(+) T cell disorder selective for the Galphai2 subunit. Int Immunol 2003;15:1359–1367. [PubMed: 14565934]
60. Chen X, Howard OM, Oppenheim JJ. Pertussis toxin by inducing IL-6 promotes the generation of IL-17-producing CD4 cells. J Immunol 2007;178:6123–6129. [PubMed: 17475838]
61. He J, Gurunathan S, Iwasaki A, Ash-Shaheed B, Kelsall BL. Primary role for Gi protein signaling in the regulation of interleukin 12 production and the induction of T helper cell type 1 responses. J Exp Med 2000;191:1605–1610. [PubMed: 10790434]
62. Tonon S, Goriely S, Aksoy E, Pradier O, Del Giudice G, Trannoy E, Willems F, Goldman M, De Wit D. Bordetella pertussis toxin induces the release of inflammatory cytokines and dendritic cell activation in whole blood: impaired responses in human newborns. Eur J Immunol 2002;32:3118–3125. [PubMed: 12385032]
63. Hou W, Wu Y, Sun S, Shi M, Sun Y, Yang C, Pei G, Gu Y, Zhong C, Sun B. Pertussis toxin enhances Th1 responses by stimulation of dendritic cells. J Immunol 2003;170:1728–1736. [PubMed: 12574336]

NIH-PA Author Manuscript

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64. Denkinger CM, Denkinger MD, Forsthuber TG. Pertussis toxin-induced cytokine differentiation and clonal expansion of T cells is mediated predominantly via costimulation. Cell Immunol 2007;246:46–54. [PubMed: 17601518]
65. Wakatsuki A, Borrow P, Rigley K, Beverley PC. Cell-surface bound pertussis toxin induces polyclonal T cell responses with high levels of interferon-gamma in the absence of interleukin-12. Eur J Immunol 2003;33:1859–1868. [PubMed: 12811846]
66. Shimoda K, Tsutsui H, Aoki K, Kato K, Matsuda T, Numata A, Takase K, Yamamoto T, Nukina H, Hoshino T, Asano Y, Gondo H, Okamura T, Okamura S, Nakayama K, Nakanishi K, Niho Y, Harada M. Partial impairment of interleukin-12 (IL-12) and IL-18 signaling in Tyk2-deficient mice. Blood 2002;99:2094–2099. [PubMed: 11877284]
67. Gutcher I, Urich E, Wolter K, Prinz M, Becher B. Interleukin 18-independent engagement of interleukin 18 receptor-alpha is required for autoimmune inflammation. Nat Immunol 2006;7:946–953. [PubMed: 16906165]
68. Stockinger B, Veldhoen M. Differentiation and function of Th17 T cells. Curr Opin Immunol 2007;19:281–286. [PubMed: 17433650]
69. Liu J, Plotnikov A, Banerjee A, Suresh Kumar KG, Ragimbeau J, Marijanovic Z, Baker DP, Pellegrini S, Fuchs SY. Ligand-independent pathway that controls stability of interferon alpha receptor. Biochem Biophys Res Commun 2008;367:388–393. [PubMed: 18166147]
70. Ebers GC, Bulman DE, Sadovnick AD, Paty DW, Warren S, Hader W, Murray TJ, Seland TP, Duquette P, Grey T, et al. A population-based study of multiple sclerosis in twins. N Engl J Med 1986;315:1638–1642. [PubMed: 3785335]
71. Freedman DM, Dosemeci M, Alavanja MC. Mortality from multiple sclerosis and exposure to residential and occupational solar radiation: a case-control study based on death certificates. Occup Environ Med 2000;57:418–421. [PubMed: 10810132]
72. Goldacre MJ, Seagroatt V, Yeates D, Acheson ED. Skin cancer in people with multiple sclerosis: a record linkage study. J Epidemiol Community Health 2004;58:142–144. [PubMed: 14729897]
73. van der Mei IA, Ponsonby AL, Blizzard L, Dwyer T. Regional variation in multiple sclerosis prevalence in Australia and its association with ambient ultraviolet radiation. Neuroepidemiology 2001;20:168–174. [PubMed: 11490162]
74. van der Mei IA, Ponsonby AL, Dwyer T, Blizzard L, Simmons R, Taylor BV, Butzkueven H, Kilpatrick T. Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study. BMJ 2003;327:316. [PubMed: 12907484]
75. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006;296:2832–2838. [PubMed: 17179460]
76. Embry AF, Snowdon LR, Vieth R. Vitamin D and seasonal fluctuations of gadolinium-enhancing magnetic resonance imaging lesions in multiple sclerosis. Ann Neurol 2000;48:271–272. [PubMed: 10939587]
77. VanAmerongen BM, Dijkstra CD, Lips P, Polman CH. Multiple sclerosis and vitamin D: an update. Eur J Clin Nutr 2004;58:1095–1109. [PubMed: 15054436]
78. Soilu-Hanninen M, Laaksonen M, Laitinen I, Eralinna JP, Lilius EM, Mononen I. A longitudinal study of serum 25-hydroxyvitamin D and intact parathyroid hormone levels indicate the importance of vitamin D and calcium homeostasis regulation in multiple sclerosis. J Neurol Neurosurg Psychiatry 2008;79:152–157. [PubMed: 17578859]
79. Cantorna MT, Hayes CE, DeLuca HF. 1,25-Dihydroxyvitamin D3 reversibly blocks the progression of relapsing encephalomyelitis, a model of multiple sclerosis. Proc Natl Acad Sci U S A 1996;93:7861–7864. [PubMed: 8755567]
80. Muthian G, Raikwar HP, Rajasingh J, Bright JJ. 1,25 Dihydroxyvitamin-D3 modulates JAK-STAT pathway in IL-12/IFNgamma axis leading to Th1 response in experimental allergic encephalomyelitis. J Neurosci Res 2006;83:1299–1309. [PubMed: 16547967]
81. Orton SM, Herrera BM, Yee IM, Valdar W, Ramagopalan SV, Sadovnick AD, Ebers GC. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol 2006;5:932–936. [PubMed: 17052660]

82. Muthian G, Bright JJ. Quercetin, a flavonoid phytoestrogen, ameliorates experimental allergic encephalomyelitis by blocking IL-12 signaling through JAK-STAT pathway in T lymphocyte. J Clin Immunol 2004;24:542–552. [PubMed: 15359113]

Abbreviations used in this paper

CIA	collagen-induced arthritis
$MOG_{79–96}$	myelin oligodendrocyte glycoprotein $peptide_{79–96}$
PTX	pertussis toxin
Tyk2	tyrosine kinase 2

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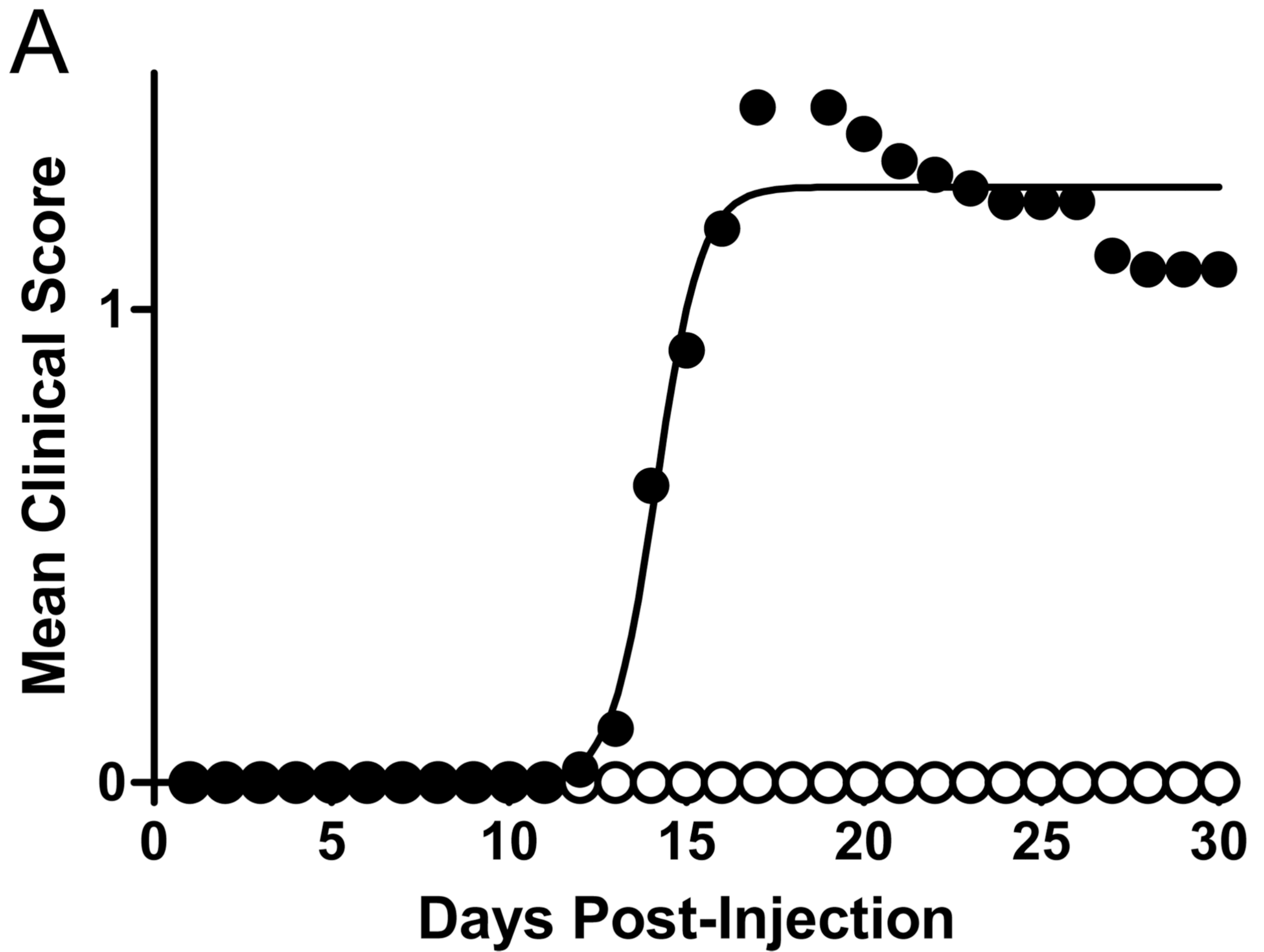

Figure 1. *Tyk2* alleles determine EAE susceptibility

(**A**) EAE was elicited by immunization with $MOG_{79–96}$-CFA on days 0 and 7. B10.Q/Ai mice (●, n = 36) were susceptible to EAE whereas B10.D1 mice bearing the $Tyk2^A$ allele (○, n = 46) were resistant to EAE. (**B**) (B10.Q/Ai × B10.D1) (Δ, n = 31) and (B10.D1 × B10.Q/Ai) F_1 hybrids (◊, n = 18) were also fully susceptible to EAE, indicating that $Tyk2^A$ is recessive. Regression lines and mean clinical scores are shown.

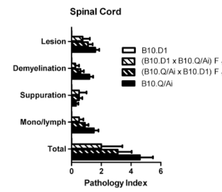

Figure 2. Quantification of EAE pathology in B10.Q/Ai, B10.D1 and reciprocal F_1 hybrid mice

EAE was elicited by immunization with $MOG_{79–96}$-CFA on days 0 and 7. EAE pathology in the spinal cords of B10.Q/Ai mice was more severe on day 30 compared with B10.D1 mice for lesion severity, monocyte/lymphocyte infiltration and total EAE pathology ($p < 0.001$) with B10.D1 < (B10.D1 × B10.Q/Ai) F_1 = (B10.Q/Ai × B10.D1) F_1 = B10.Q/Ai (n = 4–11/group). Significance of differences was assessed using the Kruskal-Wallis test followed by Dunn's multiple comparisons.

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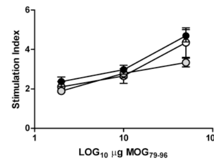

Figure 3. ***Ex vivo*** **MOG_{79-96}-specific cytokine profiles and proliferation of MOG_{79-96}-CFA immunized B10.D1, B10.Q/Ai and F_1 hybrid mice**

Cytokine production was assessed by stimulating splenocytes and lymphocytes from draining lymph nodes on day 10 post MOG_{79-96}-CFA immunization with 50 µg MOG_{79-96} for 72 hr and measuring levels in supernatants by Bioplex assay (n = 8–10/group). The significance of differences was determined using the Kruskal-Wallis test followed by Dunn's post hoc multiple comparisons, * p<0.05, **p<0.01, ***p<0.001. IL-4 (**A**), TNFα (**B**) and IL-17 (**C**) did not differ significantly with EAE susceptibility whereas IFNγ (**D**) (F=4.1; p<0.05), RANTES (**E**) (F=9.9, p<0.01) and IL-6 (**F**) (F=7.3; p<0.01) increased significantly with increasing EAE susceptibility. Data for B10.D1 (open bars), B10.Q/Ai (blank bars) and F_1 hybrid (gray bars) from one representative experiment of two is shown. **(G)** Proliferation was assessed by stimulating splenocytes and lymphocytes from draining lymph nodes on day 10 post MOG_{79-96}-CFA immunization with and without MOG_{79-96} for 72 hrs total and [^{3}H]-thymidine incorporation measured in the final 18 h. Stimulation indices were calculated as average counts / background counts. B10.D1 (○), B10.Q/Ai (●) and reciprocal F_1 hybrid (●) samples (n = 8–10/group) were analyzed by two-way ANOVA (comparison: concentration of MOG_{79-96}, p<0.0001; strain differences, p=0.11; interaction, p=0.49). Data from one representative experiment of two is shown.

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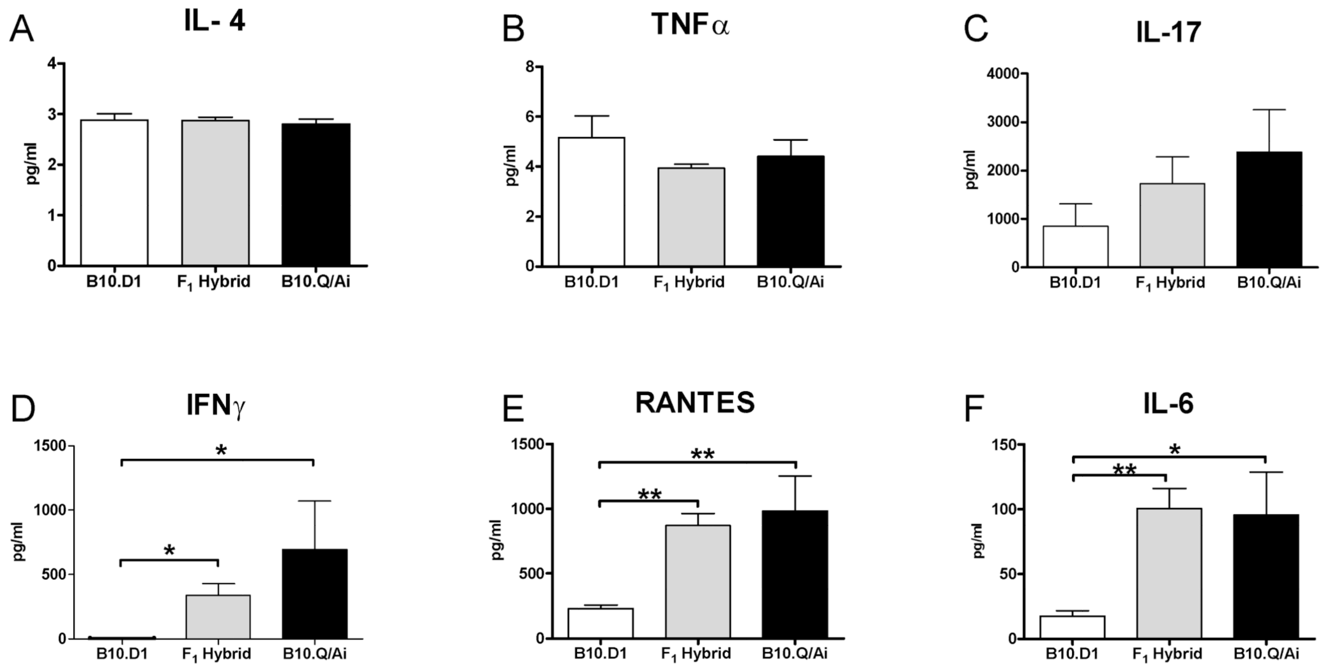

Figure 4. PTX complements EAE susceptibility of B10.D1 mice

EAE was induced by immunization with $MOG_{79–96}$-CFA + PTX on day 0. (**A**) B10.D1 (○, n = 37) were susceptible to EAE induced by $MOG_{79–96}$-CFA + PTX, in contrast to their resistance when immunized with $MOG_{79–96}$-CFA alone (●, n = 46) (also shown in Fig. 1A). (**B**) Both B10.Q/Ai (●, n = 44) and B10.D1 (○, n = 37) mice were susceptible to $MOG_{79–96}$-CFA + PTX induced EAE. The significance of differences in the course of clinical disease was assessed by regression analysis. Regression lines and daily mean clinical scores are shown.

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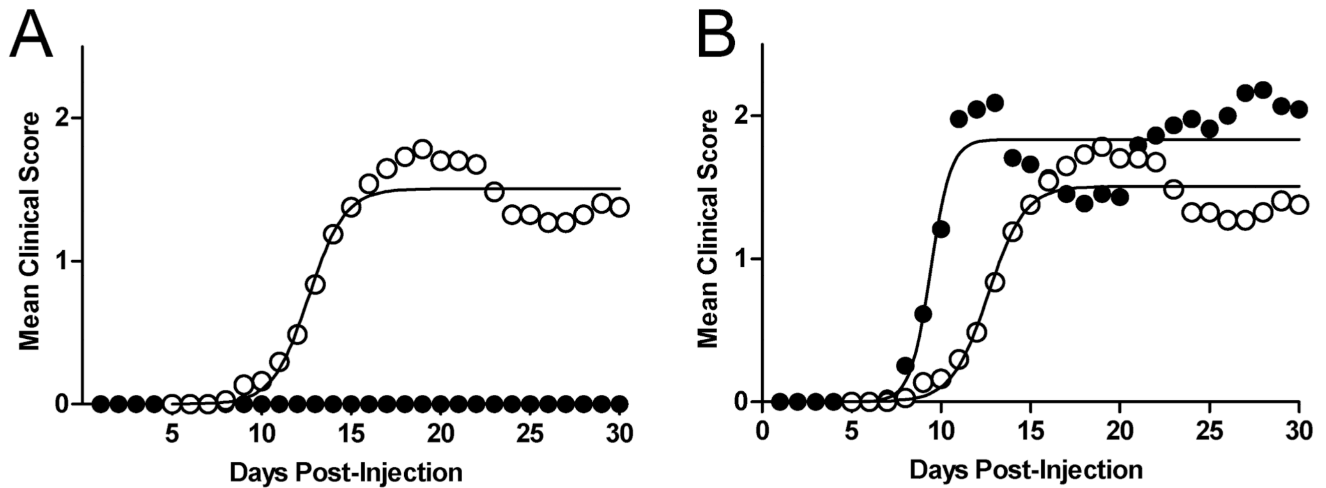

Figure 5. $MOG_{79–96}$-CFA + PTX immunization increased *ex vivo* $MOG_{79–96}$-specific cytokine and chemokine production compared to $MOG_{79–96}$-CFA in B10.D1 mice but proliferative responses did not differ between strains

Cytokine production and proliferation were assessed by stimulating splenocytes and lymphocytes from draining lymph nodes on day 10 after immunization with $MOG_{79–96}$-CFA or $MOG_{79–96}$-CFA + PTX. (**A**) Single cell suspensions were stimulated with 50μg $MOG_{79–96}$ for 72 hr and measuring levels in supernatants by cytometric bead assay. Samples (n=10/group) were analyzed by one way ANOVA. Cytokines that changed significantly have their average fold change represented in graph **A** above, $p<0.05$. (**B**) Proliferation was assessed by stimulating splenocytes and lymphocytes with and without $MOG_{79–96}$ for 72 hrs total and [^{3}H]-thymidine incorporation measured in the final 18 h. Stimulation indices were calculated as average counts / background counts. Samples (n = 10/group) from B10.D1 mice immunized with $MOG_{79–96}$-CFA with PTX (○) or without PTX (●) were analyzed by two -way ANOVA (comparison: concentration of $MOG_{79–96}$, p=0.002; strain, p=0.3; interaction, p=0.44). Data from one representative experiment of two is shown.

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Table I

Summary of clinical disease traits in B10.D1, B10.Q/Ai and reciprocal F_1 hybrids following MOG_{79-96}-CFA immunization[A].

Strain		Overall Incidence[B]		Day of Onset	Cumulative disease score	Peak Severity	Severity Index[C]	Days Affected
B10.D1		0/46		N/A	0.0	0.0	0.0	0.0
B10.Q/Ai		31/36		14.8	20.5	1.7	1.3	13.0
(Ai × D1)		26/31		14.9	21.5	1.5	1.5	13.5
(D1 × Ai)		16/18		15.4	18.7	1.3	1.3	13.7
	χ^2	89*	F	415.3*	32.0*	43.5*	74.2*	67.6*

[A] Trait values among B10.Q/Ai and the reciprocal F_1 hybrids were not significantly different whereas all were significantly different from B10.D1 mice, $p<0.001$. Means are shown. The significance of differences for the trait values among the strains was assessed by Chi-square or Kruskal-Wallis followed by Dunn's post hoc multiple comparisons,

* $p<0.0001$.

[B] Animals were considered affected that showed clinical signs of ≥ 1 for two or more consecutive days.

[C] The severity index is the cumulative disease score/days affected.

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Table II

Summary of EAE clinical traits in B10.D1 and B10.Q/Ai mice with and without PTX included in the immunization protocol[A].

Strain	Immunization protocol	Overall Incidence	Day of Onset	CDS[B]	Peak Severity	Severity Index	Days Affected
B10.D1	MOG_{79-96}-CFA	0/46	N/A	0.0	0.0	0.0	0.0
B10.D1	MOG_{79-96}-CFA+PTX	33/37*	13.8*	27.1*	2.3*	1.9*	12.9*
B10.Q/Ai	MOG_{79-96}-CFA	31/36	14.8	20.5	1.7	1.3	13.0
B10.Q/Ai	MOG_{79-96}-CFA+PTX	44/44*	10.1*	38.8*	3.1*	2.0*	19.3*

[A] Clinical traits were calculated as detailed in Table I legend. Within each strain, trait values for both B10.D1 and B10.Q/Ai were significantly different between MOG_{79-96}-CFA + PTX and MOG_{79-96}-CFA protocols as assessed by Chi-square or Student's *t* test,

* $p<0.001$. Means are shown.

[B] CDS, cumulative disease score.