Factors Which Predispose to the Onset of Autoimmune Disease

A Senior Honors Thesis

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by

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Abstract:

Multiple sclerosis (MS) is a human demyelinating disease of the central nervous system (CNS). Experimental autoimmune encephalomyelitis (EAE) is a commonly used animal model to study MS. EAE is induced by injecting mice with myelin proteins or peptides combined with immunologic adjuvants. The animals direct an immune response against the injected myelin, which results in attacks against their own CNS tissue. T-helper lymphocytes, bearing the CD4 molecule, have been shown to cause EAE. Clinically, the signs observed in mice with EAE are similar to relapsing-remitting MS (RRMS), in which the disease expresses itself in cycles. Mouse strains typically used are the B10.PL, SJL and C57BL/6, which are susceptible to EAE, but show different patterns of clinical disease.

In this study, we compared CD4 lymphocyte levels between males and females in each of these three strains to elucidate any immunologic differences either between the sexes or strains. We used flow cytometry to analyze cell populations on the basis of surface marker combinations. SJL mice have lymphocyte populations composed of 54.5% (females) and 51.8% (males) CD4 cells. These values were found to be 20% higher than the CD4 levels of the other two strains, in both sexes. Within a strain, sex based immunologic differences were minimal (<4%). These findings offer one possible explanation for the increased susceptibility of SJL mice to EAE compared with other strains. The findings also suggest that the sexual dimorphism seen after induction of disease is not due to an immunologic predisposition of either sex. The application of these findings will serve to aid future studies in EAE by providing baseline values. More importantly, these results will also be helpful in delineating different human immunologic states which predispose to the onset of autoimmune disease. (Supported by Dean's Undergraduate Research Fund Award - College of Biological Sciences, Undergraduate Research Scholarship - Colleges of the Arts and Sciences)

Introduction:

Multiple sclerosis (MS) is the most common of the demyelinating diseases of the central nervous system (CNS) and affects over 300,000 persons in the United States (Steinman, 1996). In studying MS, the murine animal model of experimental autoimmune encephalomyelitis (EAE) is commonly used. EAE is mediated through the action of CD4+ T-helper cells in response to activation with neuroantigens derived from CNS myelin. Neuroantigens include myelin oligodendrocyte glycoprotein (MOG), proteolipid protein (PLP) and myelin basic protein (MBP). The disease can only be induced in select strains of laboratory mice by injection of neuroantigens and adjuvants. Susceptible strains have been shown to have a bias towards T-helper 1 cytokines (IFN-γ, IL-2) amongst CD4+ T-cells (Duong et al., 1994; Butler et al., 2002). Strains commonly used in studying EAE are the B10.PL ($H\text{-}2^u$), SJL ($H\text{-}2^s$) and C57BL/6 ($H\text{-}2^b$).

As with many autoimmune diseases, sexual dimorphism is observed in MS with females showing a greater prevalence of the disease than males (Whitacre, 2001). The disease presents in various clinical forms including relapsing-remitting MS (RRMS), which is more prevalent in females and primary progressive MS (PPMS), which is more prevalent in males (Lublin and Reingold, 1996). EAE, like MS, shows sexual dimorphism but also differences in disease course depending on the strain selected (Papenfuss et al., 2004). The SJL strain exhibits a relapsing-remitting disease pattern similar to RRMS and is more severe in females. The B10.PL shows a disease pattern similar to PPMS with males showing greater disease severity than females while the C57BL/6 strain shows progressive disease with no sex differences (Papenfuss et al., 2004) (Fig.1).

The purpose of this study is to elaborate on these findings by comparing naïve mice for strain and sex differences in order to discern which cell populations predispose a strain/sex to a particular pattern of autoimmune disease. We compared the cell populations in lymph nodes and spleens of naïve SJL, B10.PL and C57BL/6 male and female mice using flow cytometry. Our results indicate that prior to immunization for EAE, there are no sex differences in T-cell populations, nor are differences observed in naïve splenocyte populations. However, basal levels of CD4+ cells are higher in the lymph nodes of the SJL strain relative to the B10.PL and C57BL/6 strains, while immuno-regulatory cell populations are unchanged across strains.

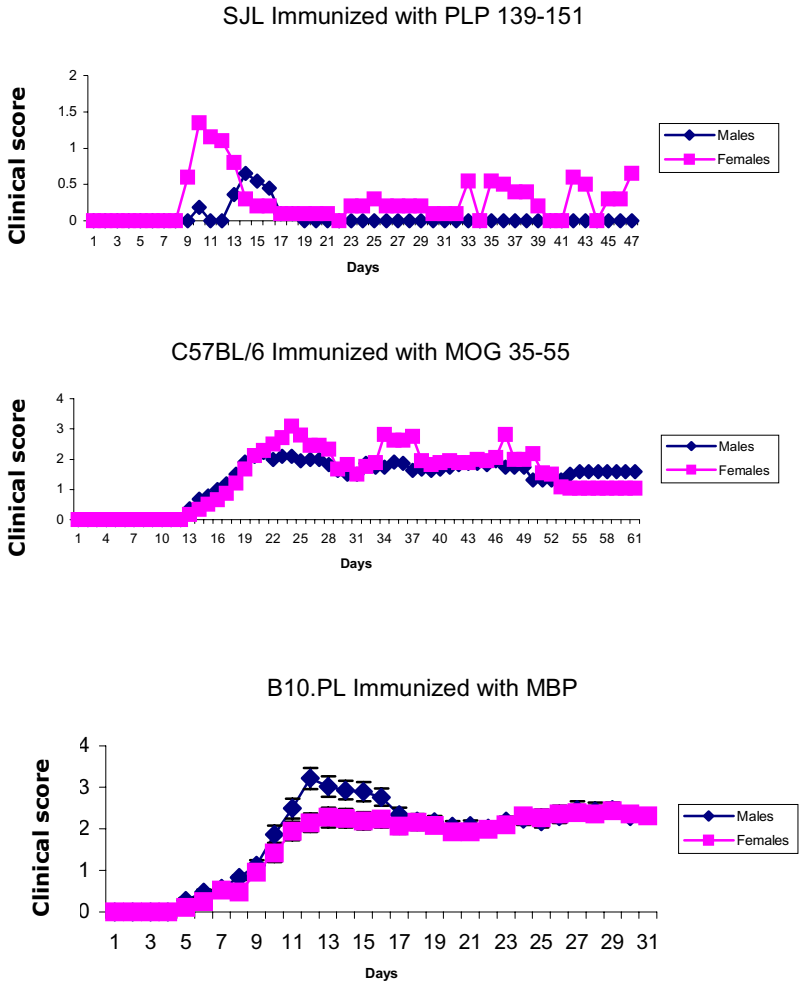

Figure1
Representative graphs of disease course of SJL, C57BL/6 and B10.PL strains upon induction of EAE.
(Papenfuss TL, Rogers CJ, Gienapp I, Valo J, McClain M, Damico N, Song FS, Whitacre CC. Sex differences in experimental autoimmune encephalomyelitis in multiple murine strains. *J Neuroimmunol*, 150:59-69, 2004.)

Methods/Materials:

Mice:

Male and female B10.PL (H-2^u), SJL (H-2^s) and C57BL/6 (H-2^b) mice, between 5-9 weeks of age were purchased from Jackson Laboratories (Bar Harbor, ME). Mice were housed 5 per cage and were allowed an acclimation period before experimental use.

Isolation of lymph node and spleen:

Naïve male and female mice of each strain were sacrificed. Spleens and lymph nodes were harvested and single cell suspensions were prepared for analysis using flow cytometry.

Flow Cytometry:

Single-cell suspensions were obtained from spleen and lymph nodes and stained using fluorescein isothiocyanate- (FITC), phycoerythrin- (PE) and allophycocyanin- (APC) conjugated antibodies. Staining was carried out as follows: CD4, CD11b, CD19 markers were identified using FITC-conjugated mouse antibodies (mAb); CD62L, CD28, CD69, CD8, B220, CD80 markers were identified using PE-conjugated mAb; CD25, CD3, CD11c were labelled with APC-conjugated mAb (Table 1). Isotype controls were matched for florochrome to control for non-specific binding. Cell suspensions (10^6 cells per tube) were incubated with labelled mAb in RPMI medium containing 5% serum for 40 minutes at 4°C. After incubation, cell suspensions were washed and fixed with 1% para-formaldehyde. Stained cells were analyzed on a flow cytometer (FACS Calibur, Becton-Dickinson, San Jose, CA) 24 hours later. All antibodies were purchased from Pharmingen.

Marker	Description
CD3	Pan T-cell marker
CD4	T-helper cells
CD8	cytotoxic T-cells
CD62L	naïve marker, homing receptor for lymph nodes
CD25	α-chain of IL-2 receptor, found on activated and regulatory T-cells
CD69	activation marker
CD28	costimulatory molecule on T-cells
CD19	B-cell marker
CD11b	macrophage marker
CD11c	dendritic cell marker
B220	B-cell and plasmacytoid dendritic cell marker
CD80	costimulatory molecule on antigen presenting cells

Table 1
Description of cell surface markers.

Statistical Analysis:

Error bars on graphs represent the standard error of the mean. A one-way ANOVA followed by the Newman-Keuls Multiple Comparison Test was used in determining statistical significance between strains.

Results:

1. *Lymph node CD4 levels are elevated in the SJL strain*

As can be seen in Table 2, lymph node CD4 levels are higher in both male and female SJL mice, compared to levels in B10.PL and C57BL/6 strains. The SJL strain also has a greater percentage of CD3+ cells. Thus, the SJL strain has an increased level of all T-cells, including the CD4+ T-helper cells, which mediate EAE. Interestingly, the SJL strain shows a lower proportion of cells bearing the CD19 marker which is specific for B-cells. A graphical representation of the data can be seen in Fig. 2.

The differences between the strains were only seen in lymph nodes and not in the spleen (data not shown). Also, no differences were seen in populations of T-regulatory cells (bearing CD25), dendritic cells or macrophages (data not shown).

Strain	CD4	CD8	CD3	CD19
SJL (F)	53.97**	32.03	84.96*	2.17**
SJL (M)	52.16**	31.86	84.46*	2.93**
C57 (F)	34.07	26.52	61.36	8.45
C57 (M)	33.09	25.54	62.12	9.62
B10 (F)	31.93	20.89	49.85	10.62
B10 (M)	32.59	22.43	56.38	13.97*

Table 2
The SJL strain shows statistically significant i
percentage of CD4+ and CD3+ cell populatio
males and females. The SJL strain has a statis
significant decrease relative to the other two s
CD19+ cells.
(n=4 for CD4, n=2 for all other markers)
* denotes p < .05
** denotes p < .001
* denotes p < .01 (increase in B10.PL male ov
female and C57BL/6 male))

2. *Differences are not seen between sexes*

While clear differences can be seen between the sexes when noting the clinical disease pattern (Fig. 1), no significant differences were noted in T-cell phenotypes between the sexes (Fig. 2). As EAE is mediated through the action of T-cells, this suggests that the sexual dimorphism seen during disease is due to factors that occur after immunization for EAE. However, it should be noted that greater percentages of CD19+ cells were seen in B10.PL males compared to females.

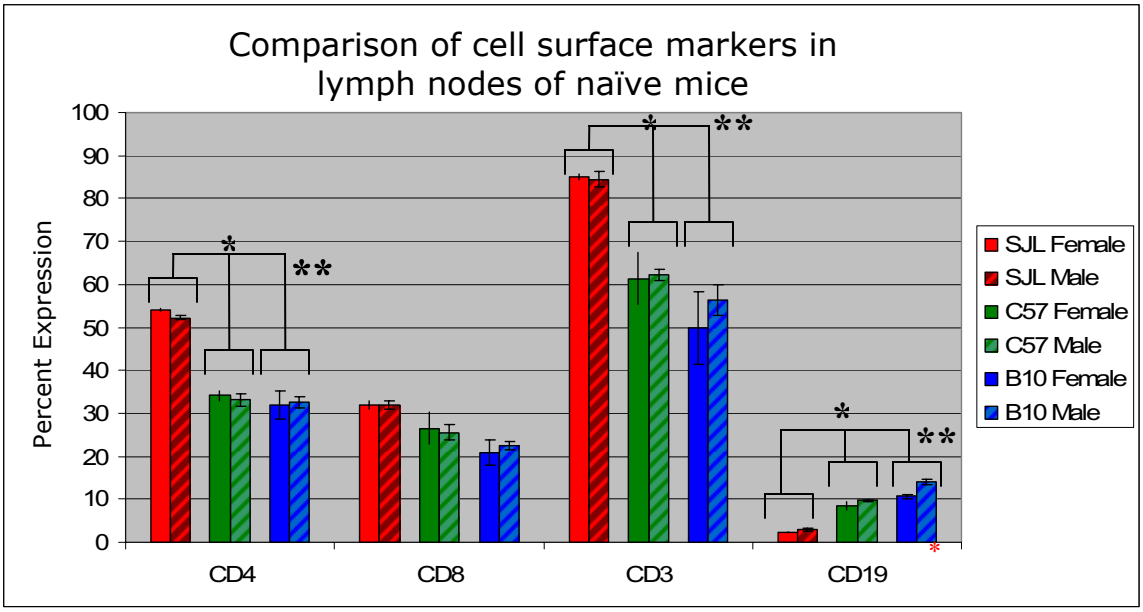

Figure 2

Levels of CD4+ (T-helper cells), CD8+ (cytotoxic T-cells), CD3+ (T-cells) and CD19+ (B-cells) populations in lymph nodes of SJL, C57BL/6 and B10.PL mice.
* denotes significant difference between SJL and C57BL/6 (Females and Males)
** denotes significant difference between SJL and B10.PL (Females and Males)
* denotes significant difference between B10.PL male and B10.PL female or C57BL/6 male

Discussion:

Naïve male and female mice of the SJL strain have significantly greater numbers of CD3+ and CD4+ lymph node cells and fewer lymph node B-cells relative to mice of the C57BL/6 and B10.PL (B cells only) strains. No sex differences are seen in lymph node or spleen cell populations of naïve mice from the three strains.

The differences we observed in the SJL strain can be explained in part by some anomalies reported for the strain. The SJL strain has a reduced repertoire of T-cell receptors (TcR), as well as a deficiency in CD4+, NK1.1+ T-cells which produce the immunosuppressive cytokine IL-4 (Yoshimoto et al., 1995). However, within this reduced pool of receptor specificity, there is an increase in T-cells which recognize the neuroantigen peptide PLP (139-151), used to induce EAE (Anderson et al., 2000). The present findings can be explained in the context of these factors. Due to the reduced repertoire of T-cell types, our hypothesis is that there is an overcompensation of CD4+ cells to counter balance the loss in receptor diversity. A similar homeostatic mechanism may also account for the comparative decrease in B-cells which corresponds to an increase in T-cells.

It has been shown that the SJL strain develops EAE and still exhibits sexual dimorphism, without the use of the adjuvant pertussis toxin (PT) (Papenfuss et al., 2004). We speculate that because of the increase in CD4+ cells, the SJL strain is susceptible to disease without the need for PT to amplify the immune response. The cytokine IL-10 has been suggested as a mechanism that protects the SJL from spontaneous EAE (Anderson et al., 2004).

B10.PL and C57BL/6 strains present few differences in the naïve state. This can be linked to their clinical pattern by noting that these strains show few periods of relapse and remission during their disease course. Therefore, the increased CD4+ populations seen in the SJL strain may play a role in the relapsing-remitting disease pattern observed for this mouse.

In this study, we have used three highly inbred mouse strains. We view these mouse strains as examples of individual human responses, with the SJL representing an individual highly susceptible to autoimmunity. The implications of this study aid in designing a model which could be used to analyze susceptible individuals and to predict their likely disease course for autoimmune disease. These findings suggest that individuals with increased numbers of T-cells could proceed along a RRMS disease course, similar to the SJL strain. Susceptible individuals with more normal T-cell numbers may demonstrate a disease pattern with less fluctuation, such as PPMS, as seen in the B10.PL and C57BL/6 strains.

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