

Systematic Review and Meta-Analysis of the role of Th17 in the Pathophysiology of the Animal Model for Multiple Sclerosis, Experimental Autoimmune Encephalomyelitis

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Abstract

Background: Multiple Sclerosis (MS) is a complex disease and little is understood on the underlying mechanisms of the disease. Treatment that has shown to be successful in mice for the animal model of MS, Experimental Autoimmune Encephalomyelitis (EAE), seems to show little success in humans. In understanding the pathophysiology of EAE one hopes to increase the likelihood of developing an effective treatment in humans for MS. At present there is a great deal of interest in the newly discovered T helper cell 17, Th17, thought to play a significant role in the pathophysiology of MS. Here we use a systematic review and meta-analysis to describe the role of Th17 and describe the efficacy of the studies pertained to Th17 in the pathophysiology of EAE.

Method: A systematic review and meta-analysis of studies describing the role of Th17, in the pathophysiology of the animal model of MS, EAE. Individual outcomes were collected and assessed against the change in clinical outcome for specified genotypes.

Results: 19 publications described a role for Th17 in the pathophysiology of EAE. 315 outcomes were collected and assessed against EAE severity.

Conclusion: Current literature states that Th17 is a major component in EAE. These results thus confirm there is a relation between Th17 cells and the severity of EAE. However, this conclusion may be qualified because of the presence of potential source of bias and the relatively limited amount of data.

Keywords: systematic review, meta-analysis, experimental autoimmune encephalomyelitis, Th17, IL-17

Abbreviations: EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; IL-17, interleukin-17; Th17, T helper 17

Conflict of Interest The author states no conflict of interest.

Introduction

Multiple sclerosis (MS) is believed to be the most common disabling neurological condition affecting young adults (Sospedra, *et al.*, 2005) and is considered to be an autoimmune disease. It is often described as the result of damage to the myelin surrounding axons in the central nervous system, disrupting the ability of nerves to conduct action potentials and thus interfering with messages sent within the brain. It is understood that currently over two and a half million people around the world suffer from MS (Fox *et al.*, 2006)

Although research in MS has progressed at a staggering pace there is still an incomplete understanding of the underlying mechanisms of the disease and current research suggests an ongoing search for preventive treatment against MS. MS is often classed into one of four categories: Progressive Relapsing (PRMS); Relapsing/Remitting (RRMS); Primary Progressive (PPMS); and Secondary Progressive MS (SPMS). One form of therapeutic intervention is the use of interferon-beta to prevent the inflammatory symptoms of PRMS. PRMS is characterised by a steady progression of clinical neurological damage superimposed by relapses and remission (Sospedra, *et al.*, 2005). There is significant recovery immediately following a relapse but between relapses there is a gradual worsening of symptoms (Figure.1) (Sospedra, *et al.*, 2005). Interferon-beta acts by reducing the number and severity of relapses and may delay or reduce the chance of disability in people with PRMS. However interferon-beta has shown only to produce small benefits (McKee, 1998) and can cause a wide range of adverse affects (Arnason, 1999). Thus the search for a therapeutic treatment in humans still continues.

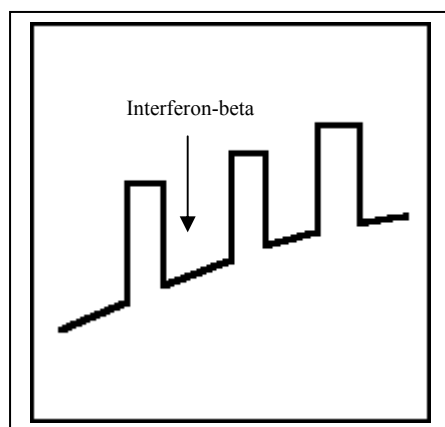


Figure 1 a graphical model of PRMS, illustrating the time at which interferon-beta is administered.

Unfortunately MS is exceptionally difficult to research as it is very unpredictable and varies greatly. Depending on which areas of the CNS are affected and how badly damaged they are can determine the type and severity of symptoms. It is generally argued to be a combination of genetic, immunological and environmental factors (Ashton, 2004). However, until the identification of the most common factors that set off autoimmunity has been uncovered the understanding of the ensuing pathogenesis is incomplete.

Animal models are thought to play an effective role in understanding the mechanisms of a disease, including autoimmune diseases. Experimental autoimmune encephalomyelitis (EAE) has received the most attention as a model for MS. The model exhibits many clinical and histological features of MS and is induced by intravenous injection of an autoimmune antigen i.e. MOG, PLP or MBP followed by an adjuvant such as CFA and the exotoxin Pertussis Toxin known to establish infection (Baker *et al.*, 2007). EAE has proven to be a very effective model for MS in that it is easy to detect and can relapse unlike most other experimental autoimmune models, so is therefore often used (Baker *et al.*, 2007). However, there are drawbacks to using EAE as a model for MS.

A mouse model, as in any reductionist approach, is potentially invalid, because it provides only a partial representation of the real biological complexity underlying the human disease. It is important to consider that any given response in a mouse, the most commonly used animal in experimental medicine, may not represent sufficient fidelity to the pathophysiology of the disease (Mestas *et al.*, 2004). It has been argued that the animal model has limited contribution to the understanding of MS (Sriram *et al.*, 2005) and that the number of pitfalls of using EAE as a model for MS outnumbers the benefits. This suggests some limitation in trying to uncover the pathogenesis of MS. Nonetheless, animal models are at the core of autoimmune research, and a large body of literature reflects the many advances brought by these models in terms of deciphering disease mechanisms (Morel, 2004; Gold *et al.*, 2006).

Over 9000 publications have been conducted to describe the use of the model of EAE. It is thus difficult to obtain an overview of the literature and thus suggests tackling it in smaller segments. A systematic review is a useful technique to compare and evaluate the data on any particular characteristic of EAE. Also non-systematic reviews have been shown to overstate biological effects. A full spectrum of all the data collected on any one area of EAE will allow us to review what components have been identified and to what extent. A stratified meta-analysis, in which dose, species or immunising agent is analysed and can describe the impact of study quality and study design characteristics (Sena, 2007) is useful in assessing the quality of the data.

EAE is often characterised by an infiltration of inflammatory lymphocytes. As EAE is often described as a CD4⁺ T cell-mediated disease model, it is believed that CD4⁺ cells are the main effector cells (Kuchroo *et al.*, 2002). It was believed for a long time that the antigen-driven differentiation of naïve T cells into effector, CD4⁺, T cells were assigned to either the T helper type 1 (Th1) or (Th2) lineage with each playing a distinct role in protective immunity (Bottomly, 1998). These conclusions were based on the observation of their cytokine profiles, with Th2 cells producing IL-4 and Th1 cells producing IL-2 and IFN- γ (Harrington *et al.*, 2005; Park *et al.*, 2005) (Figure 2). It was argued that this Th1 type CD4⁺ T cell was the predominant mediator in the initiation and predominance of autoimmunity, in EAE (Liblau *et al.*, 1995; O'Garra *et al.* 1997). However, subsequent evidence of mice developing severe EAE when lacking Th1-associated molecules, such as IFN- γ (Ferber *et al.*, 1996), was calling into question the relevance of Th1 in this autoimmune disease. Thus, key data demonstrating the newly described Th17 lineage of CD4⁺ T cells has emerged to be the major effector cell for the development of EAE (Lanrigh *et al.*, 2005; Axtell *et al.*, 2006; Park *et al.*, 2005). Its role and the role of IL-17, another molecule receiving considerable attention, are currently the topics of scrutiny in the pathophysiology of

MS. Here we conduct a meta-analysis to identify the quality of studies based on Th17 in the animal model, EAE, and a systematic review to describe the evidence for a role of Th17 and its subsequent cytokine IL-17 in the pathophysiology of EAE as well as the contributing evidence made in regards to MS

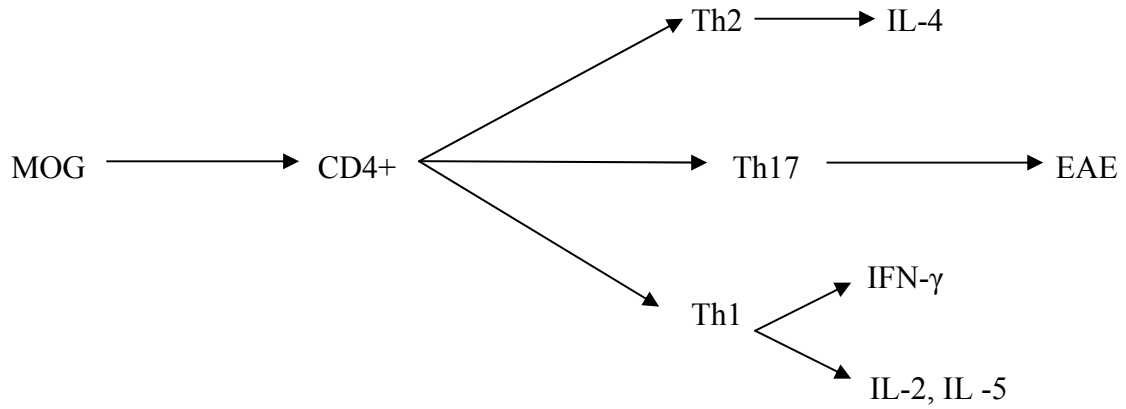


figure 2 cytokine profile of the distinct T helper cells believed to play a role in the pathophysiology of EAE

Methods

Identification of Relevant Studies

We performed an electronic search on PubMed for all studies of Multiple Sclerosis (MS) based on the animal model Experimental Autoimmune Encephalomyelitis (EAE). The following search terms were used: Experimental Autoimmune EAE AND Animals OR Experimental Allergic EAE AND Animals OR Experimental Autoimmune Encephalomyelitis AND Animals OR Experimental Allergic Encephalomyelitis AND Animals OR Multiple Sclerosis AND Animals OR Autoimmune Demyelinating Disease AND Animals OR Preclinical EAE Trials AND Animals. These search terms were confirmed with a knowledgeable investigator (M.M)

Categorizing Studies

The results from PubMed were converted to MEDLINE format and transferred to Reference Manager. Two investigators (M.H., H.V.) independently read through each abstract and classified publications as: pathophysiology of the animal model of MS; drug treatment of the animal model of MS; both the pathophysiology and drug treatment of the animal model of MS or Neither. Only primary data based on controlled animal studies of MS were extracted. Disagreement or confusion was resolved in discussion with a third investigator (M.M)

Focus of Pathophysiology Data

Cytokines was the basis of interest focused on within the pathophysiology publications. An electronic search on Reference Manager was carried out specific to the action and requirements of cytokines in the pathophysiology of the animal model for MS. Search was performed solely on extracted publications based on the "pathophysiology" AND "pathophysiology and drug treatment" on the animal model of MS. A search of the following terms to appear in the Abstract or the Title of the publication was performed: interleukin* OR IL* OR tumor* OR TNF* OR interferon* OR INF* OR transforming* OR TGF* OR cytokine* OR chemokine*.

Pathophysiology Data on Th17 and IL-17

As a multitude of cytokines are involved with EAE, the amount of data was extensive. As many of the cytokines seemed to have a connection with the Th17 subset and it's main producing cytokine IL-17, these two mediators became the basis for a new search within the pathophysiology of the animal model of MS. A further search including the following terms in the abstract or the title was made: IL-17* OR Th17* OR interleukin-17* OR interleukin17* OR T helper 17* OR Th17* OR T17* OR Thelper17*. To increase the probability of capturing all sources published another search was carried out on PubMed. This search did not specify EAE. The search terms used were as follows: IL-17* OR Th17* OR interleukin-17* OR interleukin 17* OR T helper 17* OR Th17* OR T17*.

Outcome under Review

The quality and characteristics of data were measured and analysed. Due to the inconsistency of outcome measurements, only those measurements related to EAE traits or T helper cells were extracted and only those where EAE was induced in the animal. All maximum clinical outcomes were measured for a specified genotype against a control. The change in clinical outcome was then recorded against the individual outcomes in regards to the specified genotype.

Methods of Review

Quality of Assessment

There were no published criteria for assessing the quality of study on animal, experimental, models of the pathophysiology of disease. Assessment quality was based on a published ten item checklist (Macleod, 2004) excluding: statement of control temperature; avoidance of anaesthetics with marked intrinsic neuroprotective properties; and the use of animals with hypertension or diabetes, as these do not pertain to EAE. The following categories however, were used: (1) publication in peer reviewed journal; (2) randomization to treatment or control; (3) blinded assessment of outcome; (4) sample size calculation; (5) statement of compliance with regulatory requirements; and (6) statement regarding possible conflicts of interest. Thus, giving a maximum quality score of six.

Data Extraction

Data extracted from each publication was based on individual comparisons, in which outcome was measured in a group of specified genotype animals, which had been induced with EAE, and compared against a control. These individual comparisons were then assessed against the change in EAE severity that corresponded to the genotype assessed. The specific location from which cell cultures were extracted was recorded, along with day outcome was measured after EAE immunization. It was also recorded as to whether a significant difference had been calculated per individual outcome. Clinical score was recorded as maximum clinical score obtained per group of animals against WT control and was used as the basis for EAE severity. Where numerical data was available it was recorded and when given graphically, it was measured with the use of a Jruler. The Jruler was downloaded from the following website: <http://mrswizard.com/jruler.html>. The Jruler's dimensions were in pixels and the journal was set at x400 magnification in pdf format on a PC computer. We also collected other relevant data including antigen peptide and adjuvant administered to induce EAE. As well as strain and sex of animal employed in the study and the individual items of the quality checklist.

Analysis

Our null hypothesis was that Th17 levels will show no difference in increased EAE severity or inversely that Th17 levels will show no difference in decreased EAE severity. Our hypothesis stated that increased Th17 levels will give increased EAE severity and decreased Th17 levels will show decreased EAE severity. According to our null hypothesis, the chance that an experiment reports an increase in EAE severity with an increase in Th17 should be the same as the chance that an experiment reports a reduction in EAE with increased Th17 levels. If the null hypothesis is false then there should be more experiments reporting an association. We can therefore test the

significance of an increase in Th17 levels being related to an increase in EAE severity or in reverse a decrease in Th17 levels being related to a decrease in EAE severity by using the binomial distribution as shown below:

$$P(X = k) = \binom{n}{k} p^k (1-p)^{n-k}$$

where $n =$

$$\binom{n}{k} = \frac{n!}{k!(n-k)!}$$

n = number of trials with result of interest

p = probability of outcome under the null hypothesis (0.5)

k = total number of trials

This same theorem can be used to assess the all outcomes measured against EAE severity. Significance was $p < 0.05$.

*** the search includes the word either independently or with other letters, symbols etc.. included.**

□

Results

Search Results

Our electronic search identified 9654 publications related to the animal model, EAE. 2795 of these publications were related to the pathophysiology of EAE. The search based on the role of cytokines in the pathophysiology of EAE gave a result of 1187 publications. Given that there was a vast amount of literature solely based on cytokines the focus of research was restricted to Th17 and IL-17. Existing data supports the idea that Th17 cells are key in the initiation of EAE (Weaver *et al.*, 2006) as well as what is believed to be its main producing mediator, IL-17. There were 1763 publications based on the role of these components within the pathophysiology of EAE. The second search found 432 papers. After manually reading through all abstracts of the 432 journals, an additional four papers were found to show a role for Th17 in the pathophysiology of EAE. In total there were 19 papers published describing the roles of Th17 in the pathophysiology of EAE. This analysis is therefore based on 19 publications, all of which the full articles are available (appendix 1). Within these 19 publications, 315 individual outcomes were measured against EAE severity. All studies were based on a transgenic, gene knock out or treated animal group compared against a control. One author did not consistently use a control group throughout the study; therefore, this data was excluded. A good deal of outcomes did not include the S.D. or S.E.M. and/or the number of animals in a group so data could not be normalized. Nor was significance of measurement recorded for all outcomes. Therefore, measurement data was recorded and described simply as a decrease, increase or no difference with significance of data supplied where available.

Study quality and Publication Bias (Appendix 1)

No study described a sample size calculation. Two publications stated there was a potential conflict of interest, three had no statements of potential conflict of interest and a further 15 stated no potential conflict of interest. Two of the 19 studies were directly funded by the industries the publishers worked for, while, 17 were funded by either governmental or NGO. Only one study was blinded for assessment of outcome and all publications were peer reviewed. All authors complied with animal welfare regulations via one.

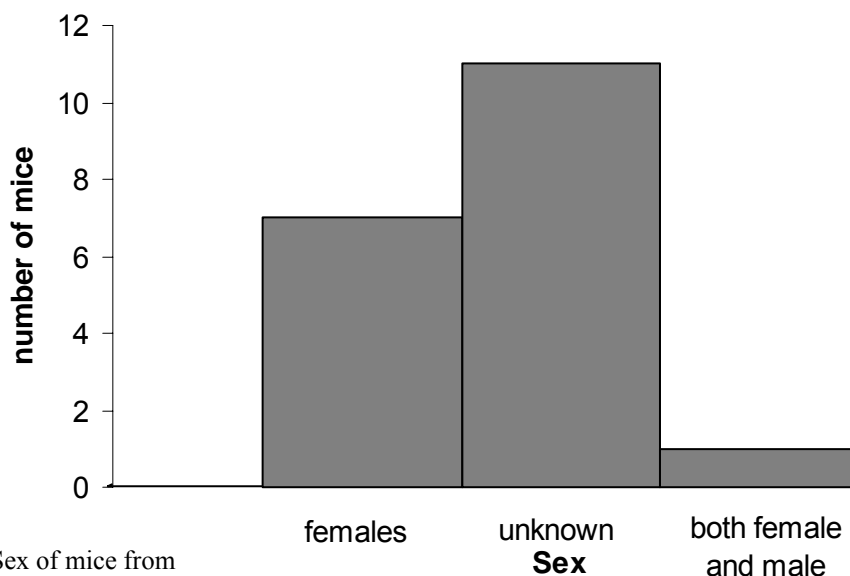


Figure 3 Sex of mice from individual publications induced with EAE. 19 publications in total.

Method of EAE Induction (Appendix 2)

All 19 studies used mice in their studies, 15 used C57BL/6 strain of mouse and four used the SJL mouse strain (Figure 3). Seven studies used female mice and one study used both male and female mice while eleven did not state the sex of the mouse (Figure 4). All publications except three induced EAE with the myelin antigen Myelin Oligodendrocyte Glycoprotein (MOG); the remaining three used the antigen, Proteolipid Protein (PLP).

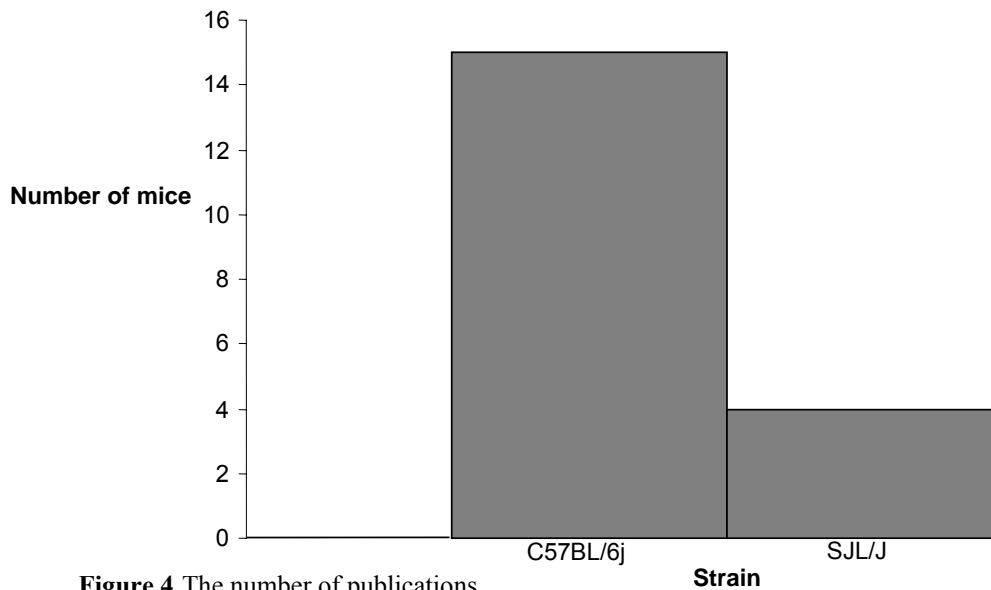


Figure 4 The number of publications using C57BL/6j or SJL/J to induce EAE

Review of Outcomes (Appendix 3)

In total 315 outcomes were measured against disease severity. 95 studies looked at further evidence of EAE characteristics (see method). 220 outcomes looked at evidence for a role of Th17 and IL-17 or for further evidence appraising the role of T helper cell lineages, Th1 or Th2.

Traits of EAE (Appendix 3)

In total, 11 studies out of 14 demonstrated a decrease in incidence with a decrease in EAE severity. As well as 11 studies out of 13 showing a delayed onset of EAE with an increase in disease severity. Thus, a decrease in EAE severity is significantly ($p=0.022$) correlated to a reduction in incidence of EAE while an increase in EAE severity is significantly ($p=0.0095$) associated with a delay on the onset of EAE. There were 6 studies carried out on axonal loss, 7 studies on inflammation and 13 carried out on demyelination, all primarily in the spinal cord of the CNS. All were significantly ($p=0.016$, $p=0.0078$, $p=0.0095$ respectively) concordant to EAE severity in that axonal loss decreased with decreased EAE severity, and both inflammation and demyelination showed an increase with increased EAE severity and a decrease with decreased EAE severity. This is in accordance with previously described traits of EAE. (Picard-Riera *et al.*, 2002; Kerschensteiner *et al.*, 2004). 21 studies alone were carried out on infiltration of T cells in the CNS and in all 21 studies there was a significant ($p<0.001$) correspondence with an increase

of T cell infiltration with increased EAE severity or a decrease in infiltration with a decreased severity. 19 studies demonstrated a difference in T cell frequency in both the spinal cord and lymph node cells. There was a significant association of an increase or decrease in T cells with an increase or decrease in EAE severity respectively, in particular the CD4⁺, as well as the CD45⁺CD11b⁺ and CD45⁻CD11b⁺ T cells (macrophages and microglia). EAE has often been described as a CD4⁺ T cell mediated disease model because of the increased infiltration of CD4⁺ T cells, (Becher *et al.*, 2002; Cua *et al.*, 2003).

Th17 and Th1 T cells (Appendix 3)

32 studies were performed on the changes of Th17 levels with an increase or decrease in EAE severity. There was a significant ($p < 0.001$) correlation between the changes of Th17 and EAE severity in that Th17 levels increased during increased EAE severity and decreased in relation to decreased EAE severity (Figure 5 & 6). The Th1 levels had a varied outcome. There was no significant correlation between the changes in Th1 levels and the severity of EAE, showing further evidence that Th17 rather than Th1 are the prime T helper cells in the induction of EAE. ThIFN- γ ⁺IL-17⁺ cells displayed a significant ($p = 0.016$) association between decreased severity of EAE and decreased level of the ThIFN- γ ⁺IL-17⁺ cells however it is uncertain whether these cells are distinct from Th17 but it is generally believed that they are (Infante-Duarte *et al.*, 2000; Axtell *et al.*, 2006).

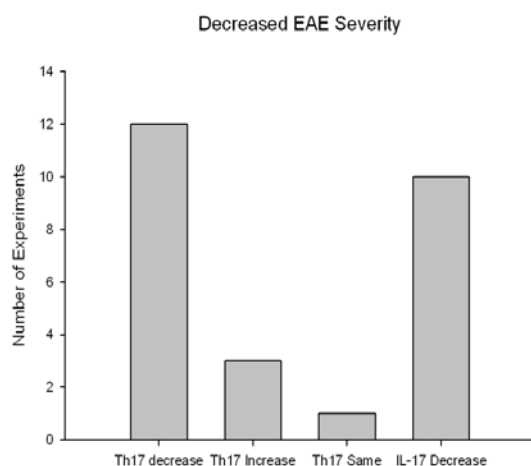


Figure 5 The number of studies illustrating an increase or decrease in Th17 and IL-17 levels in association with decreased EAE severity

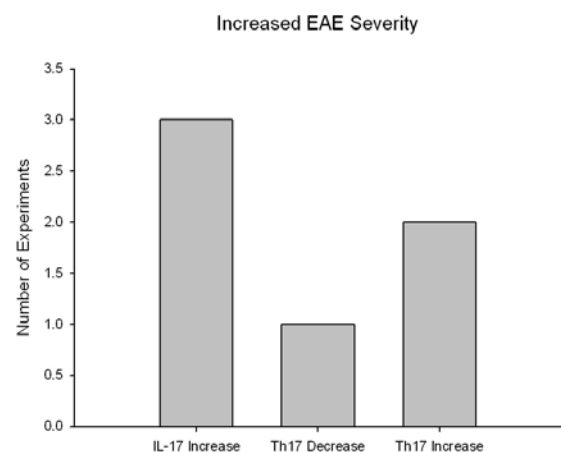


Figure 6 The number of studies illustrating an increase or decrease in Th17 and IL-17 levels in association with increased EAE severity

Cytokines (Appendix 3)

47 studies were carried out based on the level of cytokines thought to be associated with Th17 (Figure 5 & 6) (Iwakura *et al.*, 2006) in the pathophysiology of EAE. The levels of IL-17 ($p < 0.001$), TNF ($p = 0.027$), IL-23p40 ($p < 0.001$) and IL-6 ($p = 0.043$) were all significantly associated to EAE severity, showing an increase in cytokine levels along with an increase in EAE severity. Although, IL-17F, IL-1, TGF- β and GM-CSF all expressed a 100% correlation to EAE severity, in respect that they either increased with increased EAE severity or they decreased with decreased EAE severity there was not sufficient data to obtain significance. IL-10 also did not express any significance again contributing to lack of data. The levels of mRNA for IL-17, IL-17F, IL-23, TNF, IL-6 all showed a parallel to an increase or decrease in EAE severity but

again there was inadequate amount of data available to show significance. In the assessment of Th1 associated cytokines IFN- γ did not show any signs of correlation with EAE severity, IFN- γ levels both increased and decreased with an increased EAE severity. An inadequate amount of data was available to sustain the role of IL-2 and IL-5, both believed to be Th1 mediated cytokines. Nor was there sufficient data for the Th2 cytokine, IL-4.

Chemokines (Appendix 3)

21 studies were performed on the levels of chemokines and chemokine receptors. Chemokines have been thought to be upregulated by IL-17 secreted from Th17 cells and induce the inflammation during EAE (Park *et al.*, 2005). The 21 studies illustrated showed a decrease in chemokine and chemokine receptors when EAE severity was decreased, thus showing a significant ($p < 0.001$) evidence for a relation between reduced chemokines and a reduction in EAE severity.

Immunoglobulin and NO (Appendix 3)

There was shown to be no apparent correlation between the severity of EAE and the elevation of antibodies, suggesting they are not directly involved in the development of EAE. Nitric oxide (NO) was also shown to have no correlation with EAE severity but few studies were carried out so further investigation would be suggested.

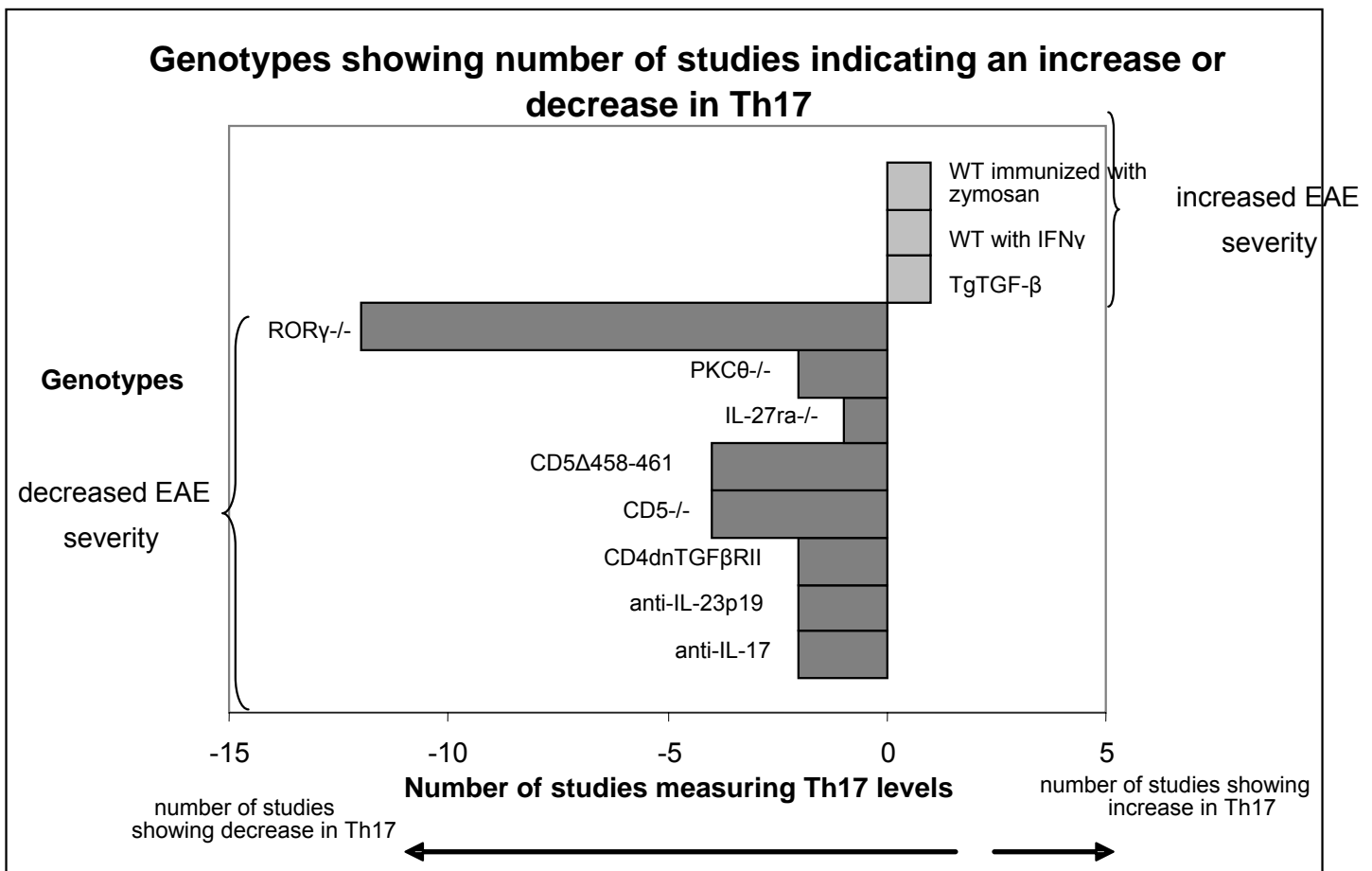


Figure 7 The number of individual genotypes showing an increase or decrease in Th17 cells during an increase or decrease in EAE severity.

The genotypes in Figure 7 illustrate a host of abundant contributors to the development of Th17 cells during an increase or decrease in EAE severity. However, ROR γ deficient mice were shown to illustrate the largest number of studies showing a decrease in Th17 cells with decreased EAE severity.

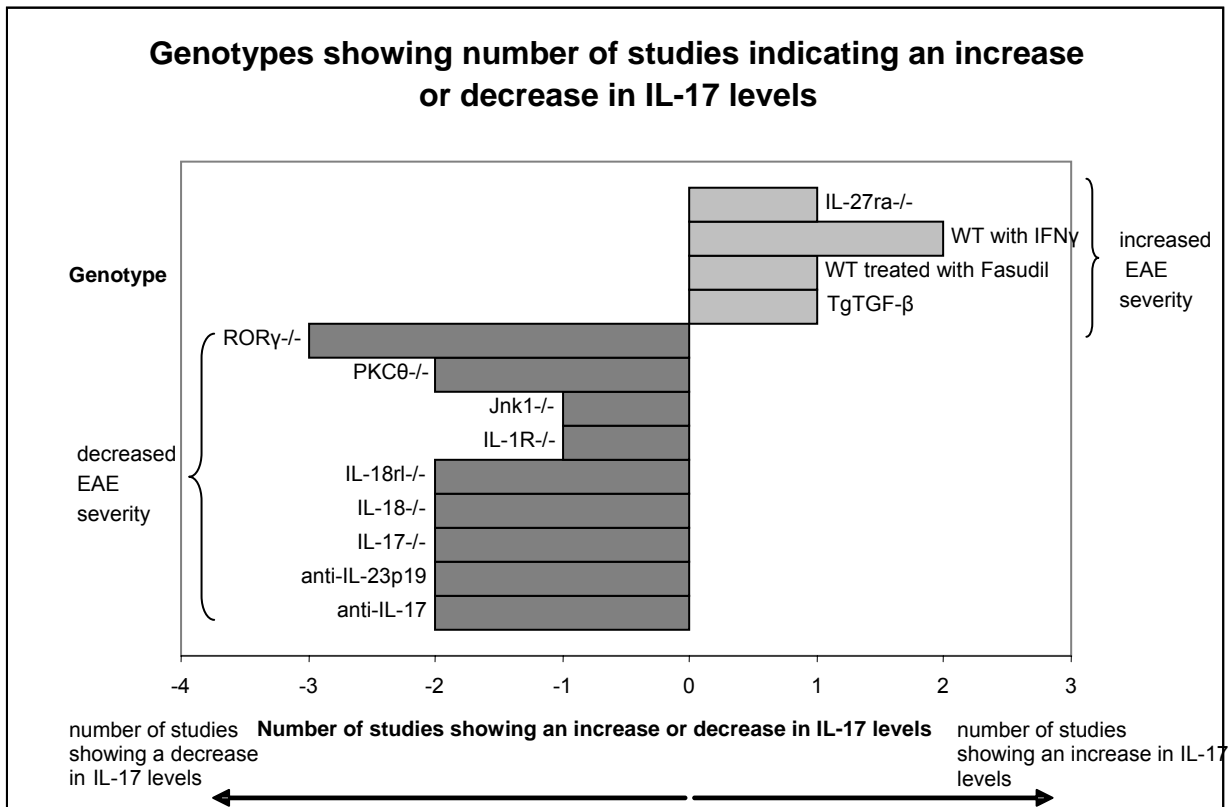


Figure 8 The number of individual genotypes showing an increase or decrease in IL-17 levels in accordance with EAE severity

ROR γ deficient mice again was showed the largest number of studies for decreased IL-17 levels in relation to reduced EAE severity (Figure 8). However, surprisingly IFN γ treated WT mice expressed the largest number of studies showing an increase in IL-17 levels with an increase in EAE severity. However, it should be kept in mind that these are number of studies, and more studies in general could have been carried out with these genotypes than any other but to have consistent studies show the same result in outcome does bear some implication.

Study Quality on the role of Th17 and IL-17 in EAE

On the basis of recent evidence it has been proposed that Th17 cells play an important role in the induction of EAE. 19 publications in total have investigated the role of Th17 as well as largely examining the role of IL-17. However, this evidence must be interpreted with some caution. The average quality score was two out of six on our quality checklist items and it has previously been shown that low study quality is associated with higher estimates of efficacy (Lythgoe *et al.*, 1990). In particular concern was the use of unblinded assessment of outcome in the majority of publications and no report of random allocation to group in any of the studies. In research there is a particular risk of expectation influencing findings, especially in cases where there is some subjectivity in assessment leading to biased results (Day *et al.*, 2000). Blinding is often effective to eliminate such bias. Previous studies that have not used appropriate levels of blinding showed increased efficacy in comparison to randomised and blinded studies (Iwakura *et al.*, 2006; Macleod *et al.*, 2004; Schulz *et al.*, 1995). Random allocation is a tenet that has also been included in trial strategies to avoid selection bias (Schulz *et al.*, 1995). These are features to be considered in conducting future trials in the investigation of pathophysiology of any disease or disease model. As acknowledged in this meta-analysis the size of an experiment is important: too small and the result will be imprecise, too large and the costs, both in terms of financial and in animal use will be unnecessarily large (Sena *et al.*, 2007). Sample size calculations are another key feature of trial strategies as it provides some reassurance that sample size has not been increased during analyses, an approach that substantially increases the risk of falsely concluding that an observed difference is real (Sena *et al.*, 2007). Not one of the 19 publications calculated sample size calculations.

Importance of Study Characteristics

EAE has been characterised in certain strains of mice to have a gender difference that parallels to that of multiple sclerosis with males shown to be less susceptible than females (Palaszynski *et al.*, 2004; Sookyun *et al.*, 1999). The result of increased resistance in males is not well understood but is thought to be associated to the protective effects of testosterone in male mice. (Bebo Jr. *et al.*, 1999; Palaszynski *et al.*, 2004). Female mice were stated as being used more times than not but in the majority of publications the sex of the mouse was not stated (Figure 3) suggesting further caution in evaluating data. The clinical course of EAE can vary according to the immunising agent (i.e. PLP, MOG) and strain of mouse (i.e. SJL, C57BL/6) applied. For example SJL mice can develop relapsing EAE when induced by whole myelin whereas C57BL/6 is resistant. However, C57BL/6 can develop chronic, paralytic EAE when induced with MOG (Baker *et al.*, 2007). Thus, EAE is not a single model but a number of models that have different degrees of similarity to MS, again stressing that the animal model, EAE, is not an absolute parallel to MS and that myelin antigens can effect outcome. The C57BL/6 mouse was significantly used in the 19 publications studied. It has been found that this is the most commonly used strain in transgenesis and it has often shown itself to be superior in effectively and reliably developing knockout (KO) models (Morel, 2004). While attaining effective genotypes for research other factors should be taken into consideration when choosing a strain of mouse.

Th17 shows a primary role over Th1 in the pathophysiology of EAE

EAE demonstrates many traits of MS, including inflammation, demyelination, axonal loss and infiltration, in particular the increase in CD4⁺ T cell frequency as well as macrophages and microglia (Becher *et al.*, 2002; Cua *et al.*, 2003). However, this does not provide sufficient evidence for EAE to be an efficient model of MS.

It was suspected for a long time that Th1 was the predominant T helper cell in mediating EAE. This was based on evidence of high levels of IFN- γ and IL-12 detected in inflammatory sites (Gately *et al.*, 1998). In addition, treatment with mAbs against IL-12p40, or IL-12p40 deficient mice, suppressed EAE development (Gately *et al.*, 1998; Becher *et al.*, 2002; Chen *et al.*, 2006). However, data showed that mice deficient in IL-12p35, IL-12 receptor β 2 (IL-12R β 2) and IFN- γ did not suppress EAE but on the contrary caused an increase in severity of disease and in accordance with our data IFN- γ showed no significant correlation to EAE severity. Not enough data was available in our results to show the function of IL-12. Recent data has, although, shown that IL-12 shares the p40 and p19 subunit with IL-23 (Oppmann *et al.*, 2000), and our data has shown a significant ($p < 0.001$) parallel with EAE severity and IL-23p40, thus suggesting a role for IL-23 rather than IL-12 in the pathophysiology of EAE. The literature also states that IL-23 is not required for Th17 commitment and early IL-17 production, but instead appears to be important for amplifying and/or stabilizing the Th17 phenotype (Weaver *et al.*, 2006). We, however, did not have sufficient data to test for this. On the other hand, there were high levels of significance between Th17 and severity of EAE. Both Th17 and IL-17 showed a significance of $p < 0.001$ and was in agreement with the current literature on its role in EAE severity. Also concordant with our results is the role for IL-6 and TNF in relating to the severity of EAE, both cytokines have been shown to have an inflammatory role and are thought to be upregulated by Th17 cells (Cua *et al.*, 2003; Liang *et al.*, 2006; Wheeler *et al.*, 2006; Wraith, 2006). Both TNF and IL-6 were shown to be significantly ($p = 0.027$ and $p = 0.043$ respectively) related to EAE, decreasing with a decreased EAE severity and increasing with an increased EAE severity. We also found an upregulation of chemokines, which are believed to have an inflammatory role in EAE. While it is thought that Th17 plays a role in inducing the inflammatory chemokines (Wheeler *et al.*, 2006; Korner *et al.*, 1997) this can not be elucidated in our data but a significant ($p < 0.001$) link of chemokines to EAE severity was demonstrated, showing a decrease in EAE severity with a decrease in chemokine levels. It is also critical to note, however, the identity of the ThIFN- γ ⁺IL-17⁺ cells which are thought to be distinct from the Th17 subset. They too secrete IL-17 in addition to Th17 cells and are shown to increase with increased EAE severity. Thus high levels of IL-17 can not be assumed to be derived from Th17 cells.

There was a large amount of studies performed by ROR γ deficient mice expressing a decrease in Th17 and IL-17 with a decrease in EAE severity. Recently published observations reveal that ROR γ is possibly the transcription factor thought to be responsible for directing differentiation of Th17 cells (Ivanov *et al.*, 2006; Wraith, 2006) much as T-bet and GATA-3 are the differentiation factors for Th1 and Th2 cells (O'Garra, 2000). However, we do not have sufficient data to prove this and evaluating the pathway at this detail is not possible within this systematic review. A summary of the results from figures 7 & 8 reveal just how complicated the pathway in EAE can become.

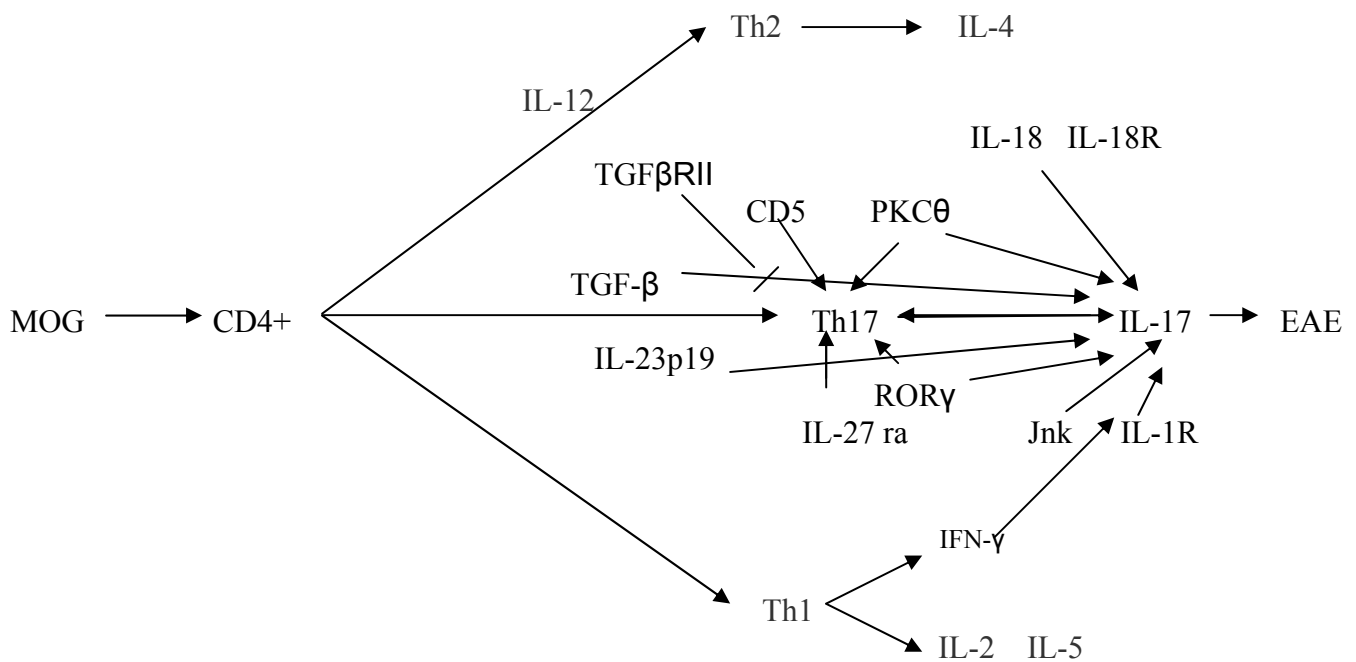


Figure 9 Illustration of results from for Th17 and IL-17 levels shown by genotype in relation to EAE severity

The pathway involved in EAE is very complex and is best understood by breaking it down into smaller sections but with such a mass of detail it can still be difficult to elucidate the overall pathophysiology of EAE. When performing a meta-analysis and systematic review on the pathophysiology of a disease or disease model it is important to keep in mind the depth one can delve into. With a large number of pathways and huge number of factors involved in the pathophysiology of EAE the complexity of the model can become overwhelming. However, what is central to our understanding, from the data we collected is that there is a significant relationship between Th17 and the severity of EAE.

Robustness of Data

Although we prespecified our choice of stratification variables and tested the significance of the results some results may have been accredited to the play of chance and thus evidence should be interpreted with caution. This meta-analysis does include other weaknesses. It is only attributed to available data and thus publication bias may result in our analysis overestimating the role of Th17 and its relation to EAE. Also our search strategy did not follow any standard criteria and although we validated the search results with a subsequent search employing more specific terms our search outcome has yet to be confirmed. Nor was our significance level set at a highly stringent level. Also our data collection was only observational and although most studies collected did test significance not all did. Further, while we did collect data on cytokines presumed to be associated with Th17 in EAE, this data was only that of what was present in the papers assessing Th17. It would be interesting to conduct a meta-analysis and systematic review on all the data available on the role of all cytokines in the pathophysiology of EAE.

References

- Armason BGW (1999). Treatment of multiple sclerosis with interferon β . *Biomed & Pharmacotherapy* **53**:8.
- Ashton, E (2004). The Multiple Factors of Multiple Sclerosis: A Darwinian Perspective. *J of Nutr & Envir Medicine* **14**: 307-317.
- Axtell, Robert C, Xu, Liang, Barnum, Scott R, Raman, Chander (2006) CD5-CK2 Binding/Activation-Deficient Mice Are Resistant to Experimental Autoimmune Encephalomyelitis: Protection Is Associated with Diminished Populations of IL-17-Expressing T Cells in the Central Nervous System. *J Immunol* **177**: 8542-8549.
- Baker D, Jackson SJ (2007). Models of Multiple Sclerosis. *ACER* **6**: 6
- Bebo Jr. BF, Schuster JC, Vandenbark AA, Offner H (1999). Androgens alter the cytokine profile and reduce encephalitogenicity of myelin-reactive T cells. *Journal of Immunology* **162**: 35–40.
- Becher B, Durell BG, Noelle RJ (2002) Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. *J Clin Invest* **110**:493–497.
- Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK (2006). Reciprocal developmental pathways for the generation of pathogenic effector T_H17 and regulatory T cells. *Nature* **441**: 235-238.
- Bottomly K (1988). A functional dichotomy in CD4⁺ T lymphocytes. *Immunol Today* **9**:268.
- Chen Y, Langrish CL, McKenzie B, Joyce-Shaikh B, Stumhofer JS, McClanahan T, Blumenschein W, Churakovsa T, Low J, Presta L, Hunter CA, Kastelein RA, Cua DJ (2006). Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. *J Clin Invest* **116**: 1317–1326.
- 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, Gormank D, Kasteleink RA, Sedgwick JD (2003). Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* **421**: 744–748
- Day SJ, Douglas GA (2000) Statistics Notes: Blinding in clinical trials and other studies Statistics *BMJ* **321**:504
- Ferber IA, Brocke S, Taylor-Edwards C, Ridgway W, Dinisco C, Steinman L, Dalton D, Fathman CG (1996). Mice with a disrupted INF-gamma gene are susceptible to the induction of experimental autoimmune encephalomyelitis (EAE). *J Immunol* **156**:5–7.
- Fox RJ, Bethoux F, Goldman MD, Cohen JA (2006). multiple sclerosis: advances in understanding, diagnosing, and treating the underlying disease. *cleveland clinic journal of medicine* **73**:91-102

- Gately MK, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, Presky DH (1998) The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* **16**:495–521.
- Gold R, Linington C, 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**:1953–71.
- Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM Weaver CT (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.
- Infante-Duarte C, Horton HF, Byrne MC, Kamradt T (2000). Microbial lipopeptides induce the production of IL-17 in Th cells. *J Immunol* **165**: 6107–6115.
- Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, Cua DJ, Littman DR (2006). The Orphan Nuclear Receptor ROR γ t Directs the Differentiation Program of Proinflammatory IL-17⁺ T Helper Cells. *Cell* **126**: 1121-1133.
- Iwakura Y, Ishigame H (2006). The IL-23/IL-17 axis in inflammation. *J Clin Invest* **116**: 1218-1222.
- Kerschensteiner M, Stadelmann C, Buddeberg BS, Merkler D, Bareyre FM, Anthony DC, Linington C, Brück W, Schwab ME (2004). Targeting Experimental Autoimmune Encephalomyelitis Lesions to a Predetermined Axonal Tract System Allows for Refined Behavioral Testing in an Animal Model of Multiple Sclerosis. *Am J Pathol* **164**: 1455–1469.
- Kollias G, Kontoyiannis D (2002). Role of TNF/TNFR in autoimmunity: specific TNF receptor blockade may be advantageous to anti-TNF treatments. *Cytokine Growth Factor Rev* **13**:315–321.
- Korner H, Lemckert FA, Chaudhri G, Etteldorf S, Sedgwick JD (1997). Tumour necrosis factor blockade in actively induced experimental autoimmune encephalomyelitis prevents clinical disease despite activated T cell infiltration to the central nervous system. *Eur J Immunology* **27**: 1973-81
- Kuchroo VK, Anderson AC, Waldner H, Munder M, Bettelli E, Nicholson LB (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.
- Laman J, Thompson J, Kappos L (1998). Balancing the TH1/TH2 concept in multiple sclerosis. *Immunol Today* **19**: 489–490
- Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ (2005). IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.* **201**: 233-240.

- Liang SC, Tan XY, Luxenberg, Karim K, Dunussi-Joannopoulos M, Collins LA Fouser (2006). Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med* **203**: 2271-2279
- Liblau R, Singer S, McDevitt H (1995). Th1 and Th2 CD4+ T cells in the pathogenesis of organ-specific autoimmune diseases. *Immunol Today* **16**:34-38.
- Lythgoe DJ, Little RA, O'Shaughnessy CT, Steward MC (1990). Effect of U-74006-F on edema and infarct volumes following permanent occlusion of the middle cerebral artery in the rat. *Br J Pharmacol* **100**:454P.
- McKee L (1998). Interferon beta produces only small benefits in multiple sclerosis *BMJ* **316**:1407.
- Macleod MR, O'Collins T, Howells DW, Donnan GA (2004). Pooling of animal experimental data reveals influence of study design and publication bias. *Stroke* **35**:1203–1208.
- Mestas J, Hughes CC (2004). Of mice and not men: differences between mouse and human immunology. *J. Immunol* **172**: 2731–2738.
- Morel L (2004). Mouse models of human autoimmune diseases: essential tools that require the proper controls. *PLoS Biol* **2**:E241.
- O'Garra A, Arai N (2000). The molecular basis of T helper 1 and T helper 2 cell differentiation. *Trends Cell Biol* **10**: 542-550
- O'Garra A, Steinman L, Gijbels K (1997). CD4+ T-cell subsets in autoimmunity. *Curr Opin Immunol* **9**: 872–883.
- Oppmann, Lesley R, Blom B, Timans JC, Xu Y, Hunte B, Vega F, Yu N, Wang J, Singh K, Zonin F, Vaisberg E, Churakova T, Liu MR, Gorman D, Wagner J, Zurawski S, Liu YJ, Abrams JS, Moore KW, Rennick D, de Waal-Malefyt R, Hannum C, Bazan JF, Kastelein RA (2000). Novel p19 protein engages IL-12p40 to form a cytokine, IL-23, with biological activities similar as well as distinct from IL-12. *Immunity* **13**: 715–725
- Palaszynski KM, Loo KK, Ashouri JF, Liu H, Voskuhl RR (2004). Androgens are protective in experimental autoimmune encephalomyelitis: implications for multiple sclerosis *Journal of Neuroimmunology* **146**: 144-152
- Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C (2005). A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17, *Nat Immunol* **6**: 1133–1141.
- Picard-Riera N, Decker L, Delarasse C, Goude K, Nait-Oumesmar B, Liblau R, Pham-Dinh D, Evercooren AB (2002). Experimental autoimmune encephalomyelitis mobilizes neural progenitors from the subventricular zone to undergo oligodendrogenesis in adult mice. *Proc Natl Acad Sci USA* **99**:13211–13216.

- Schulz KF, Chalmers I, Hayes R, Altman DG. (1995). Empirical evidence of bias: dimensions of methodological quality associated with estimates of treatment effects in controlled trials. *JAMA* **273**:408-12.
- Sena E, Bart van der Worp H, Howells D, Macleod M (2007) Improving the process of drug development in stroke. (to be published).
- Sena E, Wheble P, Sandercock P, Macleod M (2007). Systematic Review and Meta-Analysis of the Efficacy of Tirilazad in Experimental Stroke. *Stroke* **38**: 388-394.
- Sookhyun K, Voskuhl RR (1999). Decreased IL-12 production underlies the decreased ability of male lymph node cells to induce experimental autoimmune encephalomyelitis. *Journal of Immunology* **162**: 5561– 5568.
- Sospedra M, Martin R (2005). Immunology of multiple sclerosis. *Annual Review of Immunology* **23**: 683-747
- Sriram S, Steiner I (2005). Experimental allergic encephalomyelitis: a misleading model of multiple sclerosis. *Ann Neurol* **58**:939–945.
- Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM (2006) Th17: An Effector CD4 T Cell Lineage with Regulatory T Cell Ties. *Immunity*. **24**:677-88.
- Wheeler RD, Zehntner SP, Kelly LM, Bourbonnière L, Owens T (2006) Elevated interferon gamma expression in the central nervous system of tumour necrosis factor receptor 1-deficient mice with experimental autoimmune encephalomyelitis *Immunology* **118**: 527–538.
- Wraith DC (2006). Anti-cytokine vaccines and the immunotherapy of autoimmune diseases. *Euro J Immunol* **36**:2844-2848

Appendix 1: MS Study Quality Table

<i>Name and Year</i>	<i>Peer Review Publication</i>	<i>Random Allocation to Group</i>	<i>Blinded Assessment of Outcome</i>	<i>Sample Size Calculation</i>	<i>Compliance with Animal Welfare Regulations</i>	<i>Statement of Potential Conflicts of Interest</i>	<i>Quality Score</i>
Axtell <i>et al.</i> , 2006	+				+		2
Batten <i>et al.</i> , 2006	+		+		+	+	4
Bettelli <i>et al.</i> , 2006	+				+		2
Chen <i>et al.</i> , 2006	+				+		2
Gran <i>et al.</i> , 2004	+						1
Gutcher <i>et al.</i> , 2006	+				+		2
Hofstetter <i>et al.</i> , 2005	+				+		2
Ivanov <i>et al.</i> , 2006	+				+		2
Langrish <i>et al.</i> , 2005	+				+		2
Park <i>et al.</i> , 2005	+				+		2
Rohn <i>et al.</i> , 2006	+				+	+	3
Tan <i>et al.</i> , 2006	+				+		2
Sun <i>et al.</i> , 2006	+				+		2
Sutton <i>et al.</i> , 2006	+				+		2
Tran <i>et al.</i> , 2006	+				+		2

<i>Name and Year</i>	<i>Peer Review Publication</i>	<i>Random Allocation to Group</i>	<i>Blinded Assessment of Outcome</i>	<i>Sample Size Calculation</i>	<i>Compliance with Animal Welfare Regulations</i>	<i>Statement of Potential Conflicts of Interest</i>	<i>Quality Score</i>
Uyttenhove <i>et al.</i> , 2004	+				+		2
Veldhoen <i>et al.</i> , 2006	+				+		2
Wheeler <i>et al.</i> , 2006	+				+		2
Komiyama <i>et al.</i> , 2006	+				+		2

Reference List

1. AXTELL,R.C., XU,L., BARNUM,S.R. & RAMAN,C. 2006. CD5-CK2 binding/activation-deficient mice are resistant to experimental autoimmune encephalomyelitis: protection is associated with diminished populations of IL-17-expressing T cells in the central nervous system. *J. Immunol.*, **177**, 8542-8549.
2. BATTEN,M., LI,J., YI,S., KLJAVIN,N.M., DANILENKO,D.M., LUCAS,S., LEE,J., DE SAUVAGE,F.J. & GHILARDI,N. 2006. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat. Immunol.*, **7**, 929-936.
3. BETTELLI,E., CARRIER,Y., GAO,W., KORN,T., STROM,T.B., OUKKA,M., WEINER,H.L. & KUCHROO,V.K. 2006. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature.*, **441**, 235-238.
4. CHEN,Y., LANGRISH,C.L., MCKENZIE,B., JOYCE-SHAIKH,B., STUMHOFER,J.S., MCCLANAHAN,T., BLUMENSCHNEIN,W., CHURAKOVSA,T., LOW,J., PRESTA,L., HUNTER,C.A., KASTELEIN,R.A. & CUA,D.J. 2006. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. *J. Clin. Invest.*, **116**, 1317-1326.
5. GRAN,B., CHU,N., ZHANG,G.X., YU,S., LI,Y., CHEN,X.H., KAMOUN,M. & ROSTAMI,A. 2004. Early administration of IL-12 suppresses EAE through induction of interferon-gamma. *J. Neuroimmunol.*, **156**, 123-131.
6. GUTCHER,I., URICH,E., WOLTER,K., PRINZ,M. & BECHER,B. 2006. Interleukin 18-independent engagement of interleukin 18 receptor-alpha is required for autoimmune inflammation. *Nat. Immunol.*, **7**, 946-953.
7. HOFSTETTER,H.H., IBRAHIM,S.M., KOCZAN,D., KRUSE,N., WEISHAUPT,A., TOYKA,K.V. & GOLD,R. 2005. Therapeutic efficacy of IL-17 neutralization in murine experimental autoimmune encephalomyelitis. *Cell Immunol.*, **237**, 123-130.
8. IVANOV,I.I., MCKENZIE,B.S., ZHOU,L., TADOKORO,C.E., LEPELLEY,A., LAFAILLE,J.J., CUA,D.J. & LITTMAN,D.R. 2006. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. *Cell.*, **126**, 1121-1133.
9. KOMIYAMA,Y., NAKAE,S., MATSUKI,T., NAMBU,A., ISHIGAME,H., KAKUTA,S., SUDO,K. & IWAKURA,Y. 2006. IL-17 plays an important role in the development of experimental autoimmune encephalomyelitis. *J. Immunol.*, **177**, 566-573.
10. LANGRISH,C.L., CHEN,Y., BLUMENSCHNEIN,W.M., MATTSON,J., BASHAM,B., SEDGWICK,J.D., MCCLANAHAN,T., KASTELEIN,R.A. & CUA,D.J. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J. Exp. Med.*, **201**, 233-240.
11. PARK,H., LI,Z., YANG,X.O., CHANG,S.H., NURIEVA,R., WANG,Y.H., WANG,Y., HOOD,L., ZHU,Z., TIAN,Q. & DONG,C. 2005. A distinct lineage of CD4 T cells

- regulates tissue inflammation by producing interleukin 17. *Nat. Immunol.*, **6**, 1133-1141.
12. ROHN,T.A., JENNINGS,G.T., HERNANDEZ,M., GREY,P., BECK,M., ZOU,Y., KOPF,M. & BACHMANN,M.F. 2006. Vaccination against IL-17 suppresses autoimmune arthritis and encephalomyelitis. *Eur. J. Immunol.*, **36**, 2857-2867.
 13. SUN,X., MINOHARA,M., KIKUCHI,H., ISHIZU,T., TANAKA,M., PIAO,H., OSOEGAWA,M., OHYAGI,Y., SHIMOKAWA,H. & KIRA,J. 2006. The selective Rho-kinase inhibitor Fasudil is protective and therapeutic in experimental autoimmune encephalomyelitis. *J. Neuroimmunol.*, **180**, 126-134.
 14. SUTTON,C., BRERETON,C., KEOGH,B., MILLS,K.H. & LAVELLE,E.C. 2006. A crucial role for interleukin IL-1 in the induction of IL-17-producing T cells that mediate autoimmune encephalomyelitis. *J. Exp. Med.*, **203**, 1685-1691.
 15. TAN,S.L., ZHAO,J., BI,C., CHEN,X.C., HEPBURN,D.L., WANG,J., SEDGWICK,J.D., CHINTALACHARUVU,S.R. & NA,S. 2006. Resistance to experimental autoimmune encephalomyelitis and impaired IL-17 production in protein kinase C theta-deficient mice. *J. Immunol.*, **176**, 2872-2879.
 16. TRAN,E.H., AZUMA,Y.T., CHEN,M., WESTON,C., DAVIS,R.J. & FLAVELL,R.A. 2006. Inactivation of JNK1 enhances innate IL-10 production and dampens autoimmune inflammation in the brain. *Proc. Natl. Acad. Sci. U. S. A*, **103**, 13451-13456.
 17. UYTENHOVE,C., ARENDSE,B., STROOBANT,V., BROMBACHER,F. & VAN,S.J. 2004. Development of an anti-IL-12 p40 auto-vaccine: protection in experimental autoimmune encephalomyelitis at the expense of increased sensitivity to infection. *Eur. J. Immunol.*, **34**, 3572-3581.
 18. VELDHOEN,M., HOCKING,R.J., FLAVELL,R.A. & STOCKINGER,B. 2006. Signals mediated by transforming growth factor-beta initiate autoimmune encephalomyelitis, but chronic inflammation is needed to sustain disease. *Nat. Immunol.*, **7**, 1151-1156.
 19. WHEELER,R.D., ZEHNTNER,S.P., KELLY,L.M., BOURBONNIERE,L. & OWENS,T. 2006. Elevated interferon gamma expression in the central nervous system of tumour necrosis factor receptor 1-deficient mice with experimental autoimmune encephalomyelitis. *Immunology*, **118**, 527-538.

Appendix 2: MS Study Characteristics Query

<i>Name and Year</i>	<i>Animal</i>	<i>Strain</i>	<i>Sex</i>	<i>EAE Induction</i>	<i>Dose</i>	<i>Myobacterium Tuberculosis</i>	<i>Freunds incomplete adjuvant</i>	<i>CFA</i>	<i>Pertussis Toxin</i>
Axtell et al., 2006	Mouse	C57BL/6j	Unknown	MOG	150µg			+	+
Batten et al., 2006	Mouse	C57BL/6j	Unknown	MOG	200µg	+		+	+
Becher et al., 2003	Mouse	C57BL/6j	Female	MOG	200µg	+		+	+
Chen et al., 2006	Mouse	C57BL/6j	Female	PLP	80µg	+		+	+
Gran et al., 2004	Mouse	C57BL/6j	Unknown	MOG	300µg	+		+	+
Gutcher et al., 2006	Mouse	SJL/J	Unknown	MOG	200µg			+	+
Hofstetter et al., 2005	Mouse	SJL/J	Unknown	MOG	200µg	+	+		+
Ivanov et al., 2006	Mouse	SJL/J	Unknown	MOG	150µg	+		+	+
Langrish et al., 2004	Mouse	C57BL/6j	Female	MOG	100µg			+	
Park et al., 2005	Mouse	C57BL/6j	Female	MOG	unknown				
Rohn et al., 2006	Mouse	SJL/J	Female	MOG	200µg			+	+
Seng-Lai et al., 2006	Mouse	C57BL/6j	Female	MOG	300µg	+		+	
Sun et al., 2006	Mouse	C57BL/6j	Female	PLP	200µg	+		+	+
Sutton et al., 2006	Mouse	C57BL/6j	Unknown	MOG	150µg	+		+	+
Tran et al., 2006	Mouse	C57BL/6j	Both	MOG	50µg	+		+	+
Uyttenhove et al., 2006	Mouse	C57BL/6j	Unknown	PLP	150µg	+		+	
Vledhoen et al., 2006	Mouse	C57BL/6j	Unknown	MOG	250µg	+	+		+
Wheeler et al., 2006	Mouse	C57BL/6j	Unknown	MOG	unknown				
Yutaka et al., 2006	Mouse	C57BL/6j	Unknown	MOG	300µg	+	+		+

Appendix 3: MS Study Results against Disease Severity

Genotype No. of Studies	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Ref- erence
incidence <i>anti-IL-12p40</i> 1	decrease		decreased severity	2	N/A	N/A	N/A	NS	Chen <i>et al.</i> , 2006
<i>anti-IL-23p19</i> 1	decrease		decreased severity	2	N/A	N/A	N/A	NS	Chen <i>et al.</i> , 2006
<i>CD5^{-/-}</i> 1	decrease		decreased severity	2	N/A	N/A	N/A	NS	Axtell <i>et al.</i> , 2006
<i>CD5Δ458-461</i> 1	decrease		decreased severity	2	N/A	N/A	N/A	NS	Axtell <i>et al.</i> , 2006
<i>IL12a^{-/-}IL-18^{-/-}</i> 1	decrease		increased severity	2	N/A	N/A	N/A	NS	Gutcher <i>et al.</i> , 2006
<i>IL-17^{-/-}</i> 2	decrease		decreased severity	2	N/A	N/A	40	Significant	Yutaka <i>et al.</i> , 2006
<i>IL18^{-/-}</i> 1	increase		decreased severity	2	N/A	N/A	N/A	NS	Gutcher <i>et al.</i> , 2006
<i>IL18rl^{-/-}</i> 1	decrease		decreased severity	2	N/A	N/A	N/A	NS	Gutcher <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
<i>IL-1R-/-</i>									
1	decrease		decreased severity	2	N/A	N/A	N/A	NS	Sutton <i>et al.</i> , 2006
<i>IL-27ra-/-</i>									
1	decrease		increased severity	4	N/A	N/A	N/A	NS	Batten <i>et al.</i> , 2006
<i>WT (Fasudil)</i>									
1	decrease		decreased severity	2			-	NS	Sun <i>et al.</i> , 2006
<i>WT (IL-17-/-)</i>		received IL-17-/- CD4+ T cells							
1	decrease		decreased severity	2	N/A	N/A	N/A	Significant	Yutaka <i>et al.</i> , 2006
<i>WT (Qβ-IL-17)</i>									
1	decrease		decreased severity	3			-	NS	Rohn <i>et al.</i> , 2006
disease onset									
<i>anti-IL-12p40</i>									
1	increase		decreased severity	2	N/A	N/A	N/A	NS	Chen <i>et al.</i> , 2006
<i>anti-IL-17</i>									
1	increase		decreased severity	2	N/A	N/A	N/A	NS	Park <i>et al.</i> , 2005
<i>anti-IL-23p19</i>									
1	increase		decreased severity	2	N/A	N/A	N/A	NS	Chen <i>et al.</i> , 2006
<i>CD5-/-</i>									
1	increase		decreased severity	2	N/A	N/A	N/A	NS	Axtell <i>et al.</i> , 2006

Genotype <i>No. of Studies</i>	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
<i>IL12a-/-IL-18-/-</i> 1	increase		increased severity	2	N/A	N/A	N/A	NS	Gutcher <i>et al.</i> , 2006
<i>IL18-/-</i> 1	increase		decreased severity	2	N/A	N/A	N/A	NS	Gutcher <i>et al.</i> , 2006
<i>IL18rl-/-</i> 1	increase		decreased severity	2	N/A	N/A	N/A	NS	Gutcher <i>et al.</i> , 2006
<i>IL-27ra-/-</i> 1	same		increased severity	4	N/A	N/A	N/A	NS	Batten <i>et al.</i> , 2006
<i>RORγ-/-</i> 3	increase		decreased severity	2	N/A	N/A	N/A	NS	Ivanov <i>et al.</i> , 2006
<i>WT (Fasudil)</i> 1	decrease		decreased severity	2	N/A	N/A	N/A	NS	Sun <i>et al.</i> , 2006
<i>WT (Qβ-IL-17)</i> 1	increase		decreased severity	3	N/A	N/A	N/A	NS	Rohn <i>et al.</i> , 2006
axonal loss									
<i>WT (Fasudil)</i> 1	dramatic reduction of axon transaction	given before immunization: chronic phase	decreased severity	2	immunohistochemistry	spinal cord NF	42	NS	Sun <i>et al.</i> , 2006
3	decrease	given before immunization: acute phase given after immunization: chronic phase	decreased severity	2	immunostaining	spinal cord	14-67	Significant	Sun <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
No. of Studies 2	reduction of axon transaction	given before immunization: acute phase given after immunization: chronic phase	decreased severity	2	immunohistochemistry	spinal cord APP spinal cord NF	14-67	Significant	Sun <i>et al.</i> , 2006
infiltration									
<i>anti-IL-12p40</i>									
1	no infiltration		decreased severity	2	immunohistochemistry	spinal cord	30-40	NS	Chen <i>et al.</i> , 2006
<i>anti-IL-17</i>									
1	intense infiltration of inflammatory cells into lumbar region of spinal cord		decreased severity	2	immunohistochemistry	spinal cord	-	NS	Chen <i>et al.</i> , 2006
1	no obvious cellular infiltration in spinal cord		decreased severity	2	immunohistochemistry	spinal cord	6	NS	Park <i>et al.</i> , 2005
<i>anti-IL-23p19</i>									
1	no inflammation in white matter		decreased severity	2	immunohistochemistry	spinal cord	-	NS	Chen <i>et al.</i> , 2006
<i>anti-IL-23p19</i>									
1	no infiltration		decreased severity	2	immunohistochemistry	spinal cord	30-40	NS	Chen <i>et al.</i> , 2006
<i>CD5Δ458-461</i>									
1	infiltration only in grey matter, not present in white matter as in WT		decreased severity	2	immunohistochemistry	spinal cord	17	NS	Axtell <i>et al.</i> , 2006
<i>IL-17-/-</i>									
2	infiltration was sig reduced		decreased severity	2	N/A	N/A	42	Significant	Yutaka <i>et al.</i> , 2006
<i>IL18-/-</i>									
1	Before disease onset the number of IFN-gamma-secreting cells invading the CNS was similar, whereas Th17 cells were nearly completely		decreased severity	2	ELISA	lymph node cells	9	NS	Gutcher <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
No. of Studies									
1	typical infiltration of EAE		decreased severity	2	immunohistochemistry	spinal cord	28	NS	Gutcher <i>et al.</i> , 2006
<i>IL18rl-/-</i>									
1	CNS was devoid of inflammatory infiltrates		decreased severity	2	immunohistochemistry	spinal cord	28	NS	Gutcher <i>et al.</i> , 2006
1	Before disease onset the number of IFN-gamma-secreting cells invading the CNS was similar, whereas Th17 cells were nearly completely		decreased severity	2	ELISA	lymph node cells	14	NS	Gutcher <i>et al.</i> , 2006
<i>IL-27ra-/-</i>									
1	greater proportion of infiltrating CD4+ T cells produced IL-17		increased severity	4	flow cytometry; intracellular cytokine staining	spinal cord & brain	-	Significant	Batten <i>et al.</i> , 2006
1	increase		increased severity	4	immunohistochemistry	spinal cord	14	NS	Batten <i>et al.</i> , 2006
<i>Jnk1-/-</i>									
1	reduced activated microglia/macrophages (CD11b/Mac-1+) infiltration		decreased severity	2	immunohistochemistry	spinal cord	-	NS	Tran <i>et al.</i> , 2006
1	reduced inflammatory infiltration		decreased severity	2	immunohistochemistry	spinal cord & brain	-	NS	Tran <i>et al.</i> , 2006
<i>PKCθ-/-</i>									
1	no visible signs of cellular infiltrates		decreased severity	2	immunohistochemistry	spinal cord	21	NS	Seng-Lai <i>et al.</i> , 2006
<i>TNFR1-/-</i>									
1	no sig different between infiltrates		decreased severity	2	immunohistochemistry	spinal cord	-	NS	Wheeler <i>et al.</i> , 2006
<i>WT (Fasudil)</i>									
2	reduction of leukocyte infiltration	given before immunization: acute phase) given after immunization: chronic phase)	decreased severity	2	immunohistochemistry	spinal cord CD45	14-67	Significant	Sun <i>et al.</i> , 2006

Genotype <i>No. of Studies</i>	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
1	dramatic reduction of leukocyte infiltration	given before immunization: chronic phase)	decreased severity	2	immunohistochemistry	spinal cord CD45	42	NS	Sun <i>et al.</i> , 2006
WT (IFN-γ)									
1	intense infiltration of inflammatory cells into lumbar region of spinal cord		increased severity	2	immunohistochemistry	spinal cord	-	NS	Chen <i>et al.</i> , 2006
WT (IL-12)									
1	decreased mononuclear cell infiltration		decreased severity	1	immunohistochemistry	spinal cord	21	NS	Gran <i>et al.</i> , 2004
inflammation									
IL18^{-/-}									
1	typical inflammation of EAE		decreased severity	2	immunohistochemistry	spinal cord	28	NS	Gutcher <i>et al.</i> , 2006
IL18^{rl}^{-/-}									
1	decreased inflammation		decreased severity	2	immunohistochemistry	spinal cord	28	NS	Gutcher <i>et al.</i> , 2006
IL-27^{ra}^{-/-}									
1	increase		increased severity	4	immunohistochemistry	spinal cord	14	Significant	Batten <i>et al.</i> , 2006
1	increase		increased severity	4	quantitative assessment	spinal cord	14	Significant	Batten <i>et al.</i> , 2006
WT (Fasudil)									
3	decrease	given before immunization: acute phase) given after immunization: chronic phase)	decreased severity	2	immunostaining	spinal cord	14-67	Significant	Sun <i>et al.</i> , 2006
demyelination									
IL18^{-/-}									
1	typical demyelination of EAE		decreased severity	2	immunohistochemistry	spinal cord	28	NS	Gutcher <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
<i>IL18rl-/-</i>									
1	decreased demyelination		decreased severity	2	immunohistochemistry	spinal cord	28	NS	Gutcher <i>et al.</i> , 2006
<i>IL-27ra-/-</i>									
1	increase		increased severity	4	immunohistochemistry	spinal cord	14	Significant	Batten <i>et al.</i> , 2006
1	increase		increased severity	4	quantitative assessment	spinal cord	14	Significant	Batten <i>et al.</i> , 2006
<i>PKCθ-/-</i>									
1	no visible signs of demyelinating lesions		decreased severity	2	immunohistochemistry	spinal cord	21	NS	Seng-Lai <i>et al.</i> , 2006
<i>WT (Fasudil)</i>									
3	decrease	given before immunization: acute phase) given after immunization: chronic phase)	decreased severity	2	immunostaining	spinal cord	14-67	Significant	Sun <i>et al.</i> , 2006
2	reduction of demyelination	given before immunization: acute phase) given after immunization: chronic phase)	decreased severity	2	immunohistochemistry	spinal cord MBP	14-67	Significant	Sun <i>et al.</i> , 2006
1	dramatic reduction of demyelination	given before immunization: chronic phase)	decreased severity	2	immunohistochemistry	spinal cord MBP	42	NS	Sun <i>et al.</i> , 2006
<i>WT (IL-12)</i>									
1	decrease		decreased severity	1	immunohistochemistry	spinal cord	21	Significant	Gran <i>et al.</i> , 2004
1	decreased mononuclear demyelination		decreased severity	1	immunohistochemistry	spinal cord	21	NS	Gran <i>et al.</i> , 2004

Genotype <i>No. of Studies</i>	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
T cell frequency									
<i>anti-IL-17</i>									
1	CD45-CD11b+ decrease		decreased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
1	CD45+CD11b+ decrease		decreased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
1	CD4+ increase		decreased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
<i>anti-IL-23p19</i>									
1	CD45-CD11b+ increase		decreased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
1	CD45+CD11b+ decrease		decreased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
1	CD4+ decrease		decreased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
<i>IL-17-/-</i>									
2	lymph node cells		decreased severity	2	FACS	lymph node cells	-	Significant	Yutaka <i>et al.</i> , 2006
2	lymph node cells decrease	no PTx	decreased severity	2	flow cytometry	lymph node cells	10	Significant	Yutaka <i>et al.</i> , 2006
2	CD62L-CD44+ increase	no PTx	decreased severity	2	FACS	lymph node cells	10	NS	Yutaka <i>et al.</i> , 2006
2	CD45RB-CD44+ decrease	no PTx	decreased severity	2	FACS	lymph node cells	10	NS	Yutaka <i>et al.</i> , 2006
<i>IL18-/-</i>									
1	CD45hi decrease		decreased severity	2	flow cytometry	CNS	7	NS	Gutcher <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
IL18rl-/-									
1	CD45hi decrease		decreased severity	2	flow cytometry	CNS	7	NS	Gutcher <i>et al.</i> , 2006
WT (IFN-γ)									
1	CD45-CD11b+ increase		increased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
1	CD45+CD11b+ increase		increased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
1	CD4+ increase		increased severity	2	FACS analysis	spinal cord	12	NS	Chen <i>et al.</i> , 2006
Th1									
anti-IL-17									
1	Th1 increase		decreased severity	2	intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
1	Th1 same		decreased severity	2	intracellular cytokine staining	CNS	-	NS	Chen <i>et al.</i> , 2006
anti-IL-23p19									
1	Th1 same		decreased severity	2	intracellular cytokine staining	CNS	-	NS	Chen <i>et al.</i> , 2006
1	Th1 decrease		decreased severity	2	intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
CD4dnTGFβRII									
2	Th1 decrease		decreased severity	2	AutoMACS	spinal cord	18	NS	Vledhoen <i>et al.</i> , 2006
CD5-/-									
2	Th1 increase		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes	4-9	NS	Axtell <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
2	Th1 decrease		decreased severity	2	flow cytometry; intracellular cytokine staining	spinal cord	17-9	NS	Axtell <i>et al.</i> , 2006
CD5Δ458-461									
1	Th1 increase		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes	4	NS	Axtell <i>et al.</i> , 2006
3	Th1 decrease		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes spinal cord	17-9	NS	Axtell <i>et al.</i> , 2006
IL-17-/-									
2	Th1 increase		decreased severity	2	intracellular cytokine staining	lymph node cells	-	Significant	Yutaka <i>et al.</i> , 2006
2	Th1 same		decreased severity	2	intracellular cytokine staining	lymph node cells	-	Significant	Yutaka <i>et al.</i> , 2006
IL-27ra-/-									
1	Th1 increase		increased severity	4	flow cytometry	spleen cells	-	NS	Batten <i>et al.</i> , 2006
RORγ-/-									
3	Th1	Rorgamma-/- bone marrow cells reconstituted into RAG2-/- mice	decreased severity	2	FACS plot; intracellular staining	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	Th1 decrease	Rorgamma-/- spleen cells reconstituted into RAG2-/- mice	decreased severity	2	FACS plot; intracellular staining	spinal cord	21	NS	Ivanov <i>et al.</i> , 2006
WT (IFN-γ)									
1	Th1 increase		increased severity	2	intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
WT (zymosan)									
1	Th1 decrease		decreased severity	2	AutoMACS	spinal cord	42	NS	Vledhoen <i>et al.</i> , 2006

Genotype <i>No. of Studies</i>	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Ref-erence
Th17									
<i>anti-IL-17</i> 2	Th17 decrease		decreased severity	2	intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
<i>anti-IL-23p19</i> 2	Th17 decrease		decreased severity	2	intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
<i>CD4dnTGFβRII</i> 2	Th17 decrease		decreased severity	2	AutoMACS	spinal cord	18	NS	Vledhoen <i>et al.</i> , 2006
<i>CD5-/-</i> 1	Th17 decrease		decreased severity	2	flow cytometry; intracellular cytokine staining	spinal cord	9	NS	Axtell <i>et al.</i> , 2006
3	Th17 increase		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes spinal cord	17-9	NS	Axtell <i>et al.</i> , 2006
<i>CD5Δ458-461</i> 2	Th17 decrease		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes spinal cord	9	NS	Axtell <i>et al.</i> , 2006
1	Th17 increase		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes	4	NS	Axtell <i>et al.</i> , 2006
1	Th17 same		decreased severity	2	flow cytometry; intracellular cytokine staining	spinal cord	17	NS	Axtell <i>et al.</i> , 2006
<i>IL-27ra-/-</i> 1	Th17 decrease		increased severity	4	flow cytometry	spleen cells	-	NS	Batten <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
PKCθ^{-/-}									
1	Th17 decrease		decreased severity	2	ELISPOT	spleen cells	-	NS	Seng-Lai <i>et al.</i> , 2006
1	Th17 decrease		decreased severity	2	ELISPOT	spleen cells	-	Significant	Seng-Lai <i>et al.</i> , 2006
RORγ^{-/-}									
6	Th17 decrease	Rorgamma ^{-/-} spleen cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	FACS plot; intracellular staining	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	Th17 decrease	Rorgamma ^{-/-} spleen cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	FACS plot; intracellular staining	spinal cord	21	NS	Ivanov <i>et al.</i> , 2006
3	Th17	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	FACS plot; intracellular staining	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
TgTGF-β									
1	Th17 increase		increased severity	2	intracellular cytokine staining	CNS	-	NS	Becher <i>et al.</i> , 2003
WT (IFN-γ)									
1	Th17 increase		increased severity	2	intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
WT (zymosan)									
1	Th17 decrease		decreased severity	2	AutoMACS	spinal cord	42	NS	Vledhoen <i>et al.</i> , 2006
ThIFNγ+IL-17+ anti-IL-17									
1	ThIFN γ +IL-17+ decrease		decreased severity	2	intracellular cytokine staining	CNS	-	NS	Chen <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
anti-IL-23p19									
1	ThIFN γ +IL-17+ decrease		decreased severity	2	intracellular cytokine staining	CNS	-	NS	Chen <i>et al.</i> , 2006
CD5^{-/-}									
3	ThIFN γ +IL-17+ decrease		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes spinal cord	17-9	NS	Axtell <i>et al.</i> , 2006
1	ThIFN γ +IL-17+ increase		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes	4	NS	Axtell <i>et al.</i> , 2006
CD5Δ458-461									
1	ThIFN γ +IL-17+ decrease		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes	9	Significant	Axtell <i>et al.</i> , 2006
2	ThIFN γ +IL-17+ decrease		decreased severity	2	flow cytometry; intracellular cytokine staining	spinal cord	17-9	NS	Axtell <i>et al.</i> , 2006
1	ThIFN γ +IL-17+ increase		decreased severity	2	flow cytometry; intracellular cytokine staining	draining lymph nodes	4	NS	Axtell <i>et al.</i> , 2006
RORγ^{-/-}									
3	ThIFN γ +IL-17+ decrease	Ror γ ^{-/-} spleen cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	FACS plot; intracellular staining	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	ThIFN γ +IL-17+ decrease	Ror γ ^{-/-} spleen cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	FACS plot; intracellular staining	spinal cord	21	NS	Ivanov <i>et al.</i> , 2006
CCL2									
anti-IL-17									
1	CCL2 decrease		decreased severity	2	Taqman PCR	brain	-	NS	Park <i>et al.</i> , 2005
1	CCL7 decrease		decreased severity	2	Taqman PCR	brain	-	NS	Park <i>et al.</i> , 2005

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Ref-erence
RORγ^{-/-}									
3	CCL11 decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	CCL9 decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	CCL24 decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	CCL20 decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	CCL6 decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
CXCL1									
anti-IL-17									
1	CXCL1 decrease		decreased severity	2	Taqman PCR	brain	-	NS	Park <i>et al.</i> , 2005
IL-2									
anti-IL-17									
1	IL-2 decrease		decreased severity	2	ELISA	lymph node cells & spleen cells	18	NS	Park <i>et al.</i> , 2005
1	TNF increase		decreased severity	2	intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
1	TNF decrease		decreased severity	2	FACS analysis		12	NS	Chen <i>et al.</i> , 2006
1	TNF increase		decreased severity	2	ELISA	lymph node cells & spleen cells	18	NS	Park <i>et al.</i> , 2005
1	IL-17 decrease		decreased severity	2	FACS analysis		12	NS	Chen <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
No. of Studies									
1	IL-17 decrease		decreased severity	2	ELISA	lymph node cells & spleen cells	18	NS	Park <i>et al.</i> , 2005
1	IFN- γ increase		decreased severity	2	ELISA	lymph node cells & spleen cells	18	NS	Park <i>et al.</i> , 2005
1	IFN- γ decrease		decreased severity	2	FACS analysis		12	NS	Chen <i>et al.</i> , 2006
anti-IL-23p19									
2	TNF decrease		decreased severity	2	FACS analysis intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
1	IFN- γ decrease		decreased severity	2	FACS analysis		12	NS	Chen <i>et al.</i> , 2006
2	IL-17 decrease		decreased severity	2	ELISA FACS analysis	N/A	12-N/A	NS	Chen <i>et al.</i> , 2006
IL-17-/-									
2	IL-17 decrease		decreased severity	2	ELISA	lymph node cells	-	NS	Yutaka <i>et al.</i> , 2006
2	IL-4 same		decreased severity	2	ELISA	lymph node cells	-	NS	Yutaka <i>et al.</i> , 2006
2	IFN- γ increase		decreased severity	2	ELISA	lymph node cells	-	NS	Yutaka <i>et al.</i> , 2006
IL18-/-									
4	IL-23p40 decrease		decreased severity	2	ELISA	spleen cells	-	NS	Gutcher <i>et al.</i> , 2006
2	IL-17 decrease		decreased severity	2	ELISA	lymph node cells	7	NS	Gutcher <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
<i>IL18rl-/-</i>									
2	IL-23p40 decrease		decreased severity	2	ELISA	spleen cells	-	NS	Gutcher <i>et al.</i> , 2006
2	IL-23p40 decrease		decreased severity	2	ELISA	spleen cells	-	Significant	Gutcher <i>et al.</i> , 2006
2	IL-17 decrease		decreased severity	2	ELISA	lymph node cells	7	Significant	Gutcher <i>et al.</i> , 2006
<i>IL-1R-/-</i>									
1	IL-6 increase		decreased severity	2	ELISA	Spleen cells	24	NS	Sutton <i>et al.</i> , 2006
1	TNF- α decrease		decreased severity	2	ELISA	Spleen cells	24	NS	Sutton <i>et al.</i> , 2006
1	IL-17 decrease		decreased severity	2	ELISA	Spleen cells	24	Significant	Sutton <i>et al.</i> , 2006
1	IL-10 increase		decreased severity	2	ELISA	Spleen cells	24	NS	Sutton <i>et al.</i> , 2006
<i>IL-27ra-/-</i>									
1	TNF increase		increased severity	4	ELISA	spinal cord	14	Significant	Batten <i>et al.</i> , 2006
1	IL-6 increase		increased severity	4	ELISA	spinal cord	14	Significant	Batten <i>et al.</i> , 2006
1	IL-17 increase		increased severity	4	ELISA	spinal cord	14	Significant	Batten <i>et al.</i> , 2006
1	GM-CSF increase		increased severity	4	ELISA	spinal cord	14	Significant	Batten <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
No. of Studies									
1	IL-17F increase		increased severity	4	ELISA	spinal cord	14	Significant	Batten <i>et al.</i> , 2006
<i>Jnk1</i>^{-/-}									
1	IL-17 decrease		decreased severity	2	ELISA	lymphoid cells	2-4	NS	Tran <i>et al.</i> , 2006
<i>PKCθ</i>^{-/-}									
2	IL-17 decrease		decreased severity	2	ELISA	spleen cells	14-21	Significant	Seng-Lai <i>et al.</i> , 2006
<i>RORγ</i>^{-/-}									
3	IL-6 decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	IL-17F decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	IL-17 decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
3	CCR1 decrease	Rorgamma ^{-/-} bone marrow cells reconstituted into RAG2 ^{-/-} mice	decreased severity	2	RT-PCR	spinal cord	21	Significant	Ivanov <i>et al.</i> , 2006
<i>TgTGF-β</i>									
1	total TGF-β increase		increased severity	2	ELISA	spleen cells	-	NS	Becher <i>et al.</i> , 2003
1	IFN-γ decrease		increased severity	2	ELISA	spleen cells	-	NS	Becher <i>et al.</i> , 2003
1	IL-10 decrease		increased severity	2	ELISA	spleen cells	-	NS	Becher <i>et al.</i> , 2003
1	IL-17 increase		increased severity	2	ELISA	spleen cells	-	NS	Becher <i>et al.</i> , 2003

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Ref-erence
No. of Studies									
1	IL-4 decrease		increased severity	2	ELISA	spleen cells	-	NS	Becher <i>et al.</i> , 2003
1	IL-6 decrease		increased severity	2	ELISA	spleen cells	-	NS	Becher <i>et al.</i> , 2003
1	TNF- α decrease		increased severity	2	ELISA	spleen cells	-	NS	Becher <i>et al.</i> , 2003
<i>TNFR1-/-</i>									
1	increase		decreased severity	2	MFI	spinal cord & brain	-	NS	Wheeler <i>et al.</i> , 2006
1	increase		decreased severity	2	MFI	spinal cord & brain	-	Significant	Wheeler <i>et al.</i> , 2006
<i>WT (Fasudil)</i>									
1	GM-CSF decrease		decreased severity	2	ELISA	spleen cells	10	NS	Sun <i>et al.</i> , 2006
1	TNF- α decrease		decreased severity	2	ELISA	spleen cells	10	NS	Sun <i>et al.</i> , 2006
1	IL-10 decrease		decreased severity	2	ELISA	spleen cells	10	NS	Sun <i>et al.</i> , 2006
1	IL-17 decrease		decreased severity	2	ELISA	spleen cells	10	NS	Sun <i>et al.</i> , 2006
1	IL-1 decrease		decreased severity	2	ELISA	spleen cells	10	NS	Sun <i>et al.</i> , 2006
1	IL-5 decrease		decreased severity	2	ELISA	spleen cells	10	NS	Sun <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
No. of Studies									
1	IL-2 decrease		decreased severity	2	ELISA	spleen cells	10	NS	Sun <i>et al.</i> , 2006
1	IL-4 increase		decreased severity	2	ELISA	spleen cells	10	NS	Sun <i>et al.</i> , 2006
1	IFN- γ decrease		decreased severity	2	ELISA	spleen cells	10	Significant	Sun <i>et al.</i> , 2006
WT (IFN-γ)									
2	TNF increase		increased severity	2	FACS analysis intracellular cytokine staining	CNS	12	NS	Chen <i>et al.</i> , 2006
2	IFN- γ increase		increased severity	2	intracellular cytokine staining FACS analysis	brain & spinal cord	12	NS	Chen <i>et al.</i> , 2006
2	IL-17 increase		increased severity	2	ELISA FACS analysis	N/A	12-N/A	NS	Chen <i>et al.</i> , 2006
WT (zymosan)									
4	IL-6 decrease		decreased severity	2	RT-PCR	spleen cells draining lymph node	18-42	NS	Vledhoen <i>et al.</i> , 2006
4	IL-23 decrease		decreased severity	2	RT-PCR	spleen cells draining lymph node	18-42	NS	Vledhoen <i>et al.</i> , 2006
IL-17 mRNA									
IL18^{-/-}									
2	IL-17 mRNA increase		decreased severity	2	RT-PCR	lymph node cells	7	NS	Gutcher <i>et al.</i> , 2006
IL18^{rl-/-}									
2	IL-17 mRNA decrease		decreased severity	2	RT-PCR	lymph node cells	7	Significant	Gutcher <i>et al.</i> , 2006

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
<i>PKCθ-/-</i>									
1	IL-17 mRNA same	IL-2	decreased severity	2	ELISA	spleen cells	-	Significant	Seng-Lai <i>et al.</i> , 2006
2	IL-17 mRNA decrease	IL-2	decreased severity	2	ELISA Taqman RT-PCR	spleen cells spinal cord	21	Significant	Seng-Lai <i>et al.</i> , 2006
<i>TNFR1-/-</i>									
1	IFN-γ mRNA increase		decreased severity	2	RT-PCR	spinal cord	-	Significant	Wheeler <i>et al.</i> , 2006
1	increase		decreased severity	2	RT-PCR	spinal cord	-	Significant	Wheeler <i>et al.</i> , 2006
1	IL-17 mRNA increase		decreased severity	2	RT-PCR	spinal cord	-	NS	Wheeler <i>et al.</i> , 2006
<i>WT (IL-12)</i>									
1	IL-17 mRNA decrease		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004
1	IL-12Rbeta2 mRNA increase		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004
1	IL-17F mRNA decrease		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004
1	IL-23R mRNA decrease		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004
1	IFN-γ mRNA increase		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004
1	IL-6 mRNA decrease		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
1	Granzyme G mRNA increase		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004
1	IL-17 mRNA decrease		decreased severity	1	RT-PCR	spleen cells	7	NS	Gran <i>et al.</i> , 2004
1	TNF mRNA decrease		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004
immunoglobulin frequency									
<i>IL-17^{-/-}</i>									
2	IgM decrease		decreased severity	2	ELISA	sera	42	NS	Yutaka <i>et al.</i> , 2006
2	IgG3 increase		decreased severity	2	ELISA	sera	42	NS	Yutaka <i>et al.</i> , 2006
2	IgG2b increase		decreased severity	2	ELISA	sera	42	NS	Yutaka <i>et al.</i> , 2006
2	IgG2a increase		decreased severity	2	ELISA	sera	42	NS	Yutaka <i>et al.</i> , 2006
4	IgG increase		decreased severity	2	ELISA	sera	-1-20	NS	Yutaka <i>et al.</i> , 2006
4	IgG increase		decreased severity	2	ELISA	sera	<40-42	Significant	Yutaka <i>et al.</i> , 2006
2	IgG1 increase		decreased severity	2	ELISA	sera	42	Significant	Yutaka <i>et al.</i> , 2006
<i>WT (IL-12)</i>									
1	Integrin-alpha3 mRNA decrease		decreased severity	2	Taqman RT-PCR	draining lymph nodes	-	Significant	Langrish <i>et al.</i> , 2004

Genotype	Change in Outcome	Co-treatments	Severity of Disease	Quality	Method of Assessment	Where Assessed	Day of Assess	Statement of Significant difference	Reference
NO levels									
WT (IL-12)									
1	decrease		decreased severity	1	Griss reagent	spleen cells	21	Significant	Gran <i>et al.</i> , 2004
2	increase		decreased severity	1	Griss reagent	spleen cells	21	NS	Gran <i>et al.</i> , 2004