



## **Analysis of spontaneous genetic mutations as a possible cause of Multiple Sclerosis**

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### **Summary**

Various features of MS, particularly the lack of complete concordance for MS in monozygotic twins, indicate that the disease is not solely caused by inherited genetic factors. Other risk factors must be involved, which could be either environmental influences (toxins, nutrition, pathogens, or trauma) or spontaneous genetic mutations. This report assesses the various types of evidence for and against the involvement of spontaneous mutations in the development of MS.

It is not yet clear whether or how spontaneous mutations increase the risk of MS. Circumstantial evidence exists connecting exposure to mutagenic environmental factors with higher risk of MS, but it is inconclusive. Similarly, various types of spontaneous genetic effects, such as chromosomal abnormalities and T cell receptor gene repertoire patterns, have been linked with MS but it is not yet clear whether these features are present prior to disease onset or whether they are brought about by disease events.

### **Hypothesis**

Spontaneous genetic mutations (as distinguished from stably inherited genetic factors) either cause MS or influence susceptibility to MS.

### **Experimental tests of the hypothesis**

Mechanisms by which spontaneous genetic mutations might cause MS or influence the risk of developing MS include both normal, nonpathogenic processes (T cell receptor and immunoglobulin gene rearrangements) and pathogenic processes (viral, chemical or radiation-induced mutagenesis).

#### **1. T cell receptor gene rearrangement**

The T cell receptor (TCR) is encoded by a series of genetic sequences that are stochastically rearranged during T cell maturation to create a functional protein. This normal somatic rearrangement process creates in each individual a diverse and unique T cell repertoire with receptors able to bind to a wide variety of antigens.

TCRs are composed of heterodimers consisting of paired alpha and beta chains, or paired gamma and delta chains. The majority of T cells express an alpha beta heterodimer; the rest express a gamma delta heterodimer. Each chain originates from a germline TCR gene containing multiple versions of variable (V), joining (J), and constant (C) segments (as well as diversity (D) segments in the case of beta and gamma chains). Through a process of splicing and rejoining, some segments are removed and remaining ones recombined to yield a functional gene.

Diversity in an individual's T cell repertoire results from the variety of random combinations of genetic segments that are joined together through this process as well as from the pairing of two different chains (alpha with beta and gamma with delta) to create heterodimers. Additional mechanisms involving variable gene segment joining (junctional flexibility) and the addition of nucleotides in the complementarity-determining region 3 (CDR3) allow for even more diversity in the repertoire. While this process is nonpathogenic and indeed beneficial, it is nevertheless possible that the makeup of the T cell repertoire based on the outcome of the rearrangement process may influence the risk of developing MS. Several aspects of T cell receptor rearrangement have been studied for possible contributions of this genetic process to MS:

- Repertoire differences between identical twins or family members: One way to analyze whether T cell receptor gene rearrangement plays a role in the development of MS is to compare T cell repertoires in concordant and discordant monozygotic twin pairs. Although two studies showed a high degree of similarity in gene usage for healthy monozygotic twins<sup>1, 2</sup>, several studies of MS or MBP-specific cells have demonstrated that twins with an otherwise "identical" genetic background are capable of generating different TCR repertoires, as demonstrated by different gene rearrangements, recognition of different epitopes, or selection of different TCR chains in response to a specific epitope<sup>3-6</sup>. Three of these studies showed that twins discordant for MS were more likely than healthy or concordant pairs to possess divergent repertoires<sup>3, 4, 6</sup>, one demonstrating dissimilarities in naïve T cells, purportedly less affected by disease<sup>6</sup>. Additionally, a study of multiplex MS families showed little overlap in fine specificities to MBP epitopes between individual family members, even HLA-identical siblings<sup>7</sup>.
- Oligoclonality/overexpression of gene sequences: Many studies have investigated T cell oligoclonality (a proliferation of T cells stemming from a small number of originating cells) as a feature of MS<sup>8-50</sup>. The majority of these analyses found some type of amplification or predominant rearrangement in people with MS, and comparisons of MS subjects with healthy controls tended to find a higher degree of oligoclonality in subjects. One study of healthy identical twins of MS subjects found that they, like people with MS, have shifts in their CDR3 repertoires, indicating that these shifts may predispose to MS<sup>51</sup>. However, oligoclonality is not a defining characteristic of MS, as other studies have found either a heterogeneous pattern of T cell receptor genes in MS subjects or similar degrees of clonal expansion in subjects and controls. Variations in the experimental strategies used include what types of subjects were included, whether T cells were taken from peripheral blood or CNS tissue, which TCR element was examined (alpha, beta, gamma, or delta chains, or CDR3 motifs), what methods were used to generate clone lines, whether cells studied were restricted by HLA type or epitope recognition, and what measurement techniques were used to assess the degree of clonal expansion. Some studies reported

biases toward or against various gene sequences or combinations in people with MS<sup>11, 14, 17, 34, 37, 38, 41, 42</sup>, but there does not seem to be an overall rearrangement pattern characteristic of the disease.

- Gene sequence specificity to certain epitopes: Another question that has been investigated is whether specific TCR sequences react preferentially with specific epitopes, with the goal of finding particular sequence/epitope combinations that could possibly be linked with MS. Investigations looking for biased expression of alpha or beta chain sequences in response to MBP or MBP epitopes produced evidence for the predominance of several V beta and V alpha chains, such as V beta 5.2<sup>20, 39, 52-55</sup>. Other epitopes studied include HSP70, for which one study found V delta 2-J delta 3 to be predominant<sup>24</sup>, and PLP, for which V beta 2 and J beta 2.5 were found to be overutilized<sup>31</sup>. However, later studies failed to confirm some of these overexpressions, for instance, studies finding V beta 5.2 not to be overexpressed in MBP-specific T cell lines<sup>19, 56</sup> or detecting no evidence for clonal expansion in MOG-reactive cell lines<sup>57</sup>. Moreover, a meta-analysis found no statistically significant differences in V beta, V alpha, or J beta gene usage among human MBP-reactive T cell clones, clones derived from MS brains, and controls<sup>30</sup>.
- Dynamic nature of T cell repertoire: Variations in T cell repertoires in MS over time have been explored in multiple ways. Studies have demonstrated dynamism as well as persistence, and their findings indicate that expansion can begin early in the course of the disease (for instance, in the first two years after onset); that gene biases can change over time or with exacerbations and remissions; that expansion can decline over time back to normal levels; and that particular rearrangements and overall patterns of usage can persist for years<sup>19, 21, 23, 32, 36, 41, 44, 46, 48, 54, 58-63</sup>.

Other studies seeking to connect T cell repertoire to MS have found an increased frequency of gamma-delta T cells in MS subjects compared with controls<sup>18, 26</sup> and evidence for and against a relationship between expansions and subclinical measures such as IgG bands and MRI<sup>42, 45, 46</sup>.

## **2. Immunoglobulin gene rearrangement and further mutation**

In a process similar to T cell receptor gene rearrangement, the genes encoding immunoglobulins (Igs, also known as antibodies) also somatically rearrange during B cell maturation to encode functioning Ig proteins. In addition, when B cells proliferate in response to an immune challenge, the Ig genes undergo further somatic mutation to continue improving their specificity for the antigen(s) being targeted.

Several studies have found evidence for oligoclonal expansion and/or continued mutation of Ig genes in brain tissue and CSF of people with MS<sup>64-73</sup>. These features have also been found in the CSF of people with a clinically isolated syndrome (initial MS symptom), and can even precede development of oligoclonal bands and multiple MRI lesions, indicating that B cell expansion is an early event in the course of MS<sup>74</sup>. Patterns of rearrangement were found in two studies to be different between MS subjects and non-MS controls<sup>66, 67</sup>, whereas another study showed similar characteristics in people with MS and subacute sclerosing panencephalitis (SSPE)<sup>69</sup>. Certain sequences were found in some studies to be more frequently used, in particular VH4<sup>64-66</sup>. However,

overall Ig repertoires appear to differ from person to person<sup>69, 75</sup>. Expansion and rearrangement of Ig genes in MS may be somewhat localized to the CNS as demonstrated by studies comparing CNS and peripheral blood repertoires<sup>67, 71</sup>, and there is evidence that the B cell differentiation process may even be recapitulated within the CNS during inflammatory reactions<sup>72</sup>.

In contrast with T cell receptor gene repertoires, which have been found to differ in twins discordant for MS, Ig repertoires and/or usage may actually be similar in discordant twins. Support for this idea comes from a study of VH gene repertoires in rheumatoid arthritis showing that twins discordant for RA still used highly similar VH repertoires<sup>76</sup>. In addition, healthy siblings of people with MS were found in one study to be more likely than controls to have oligoclonal bands in their CSF, indicating underlying similarities in humoral immune processes<sup>77</sup>.

### **3. Retroviral integration/viral mutagenic effects**

The possibility that the integration of retroviruses or other viruses into the human genome is involved in the development of MS has been investigated from a number of angles. Several studies have searched for evidence of the presence of known RNA-based exogenous human retroviruses (HTLV-I, HIV). The results of these studies have been mixed, with some providing evidence such as the presence of HTLV-I antibodies<sup>78</sup>, cells expressing antigens that react with HTLV antibodies<sup>79</sup>, or HTLV gene sequences<sup>80</sup> in MS subjects, and several others finding no such evidence<sup>81-87</sup>.

Two studies detected integration of the DNA-encoded HHV-6 viral genome in the DNA of peripheral blood mononuclear cells of certain MS subjects as well as subjects with lymphoproliferative disorders<sup>88, 89</sup>. A follow-up study mapped an HHV integration site to the telomeric region of chromosome 17p, a potentially destabilizing site<sup>90</sup>.

Other studies have focused on the presence and activity of endogenous retroviruses (ERVs) in the blood and CNS tissue of people with MS. A few studies comparing the expression of known ERV sequences in MS subjects with controls were not able to find substantial differences<sup>91-93</sup>. However, other studies involving people with MS detected and investigated novel endogenous retrovirus sequences, often found in retroviral particles, which appear to be associated with MS. These include the MS-related retrovirus, or MSR/V, a related human ERV family, HERV-W, and an MS-specific retrovirus with high homology to RGH<sup>94-109</sup>. Unlike most other endogenous retroviruses, evidence suggests that these retroviruses may actually be replication-competent and transmissible<sup>95, 109, 110</sup>. Supporting a role for ERV replication in the development of MS is one study in which cytogenetic analysis using the FISH technique on blood cells detected increased copy numbers of the MSR/V *pol* sequence in chromosomes of MS subjects compared with controls<sup>111</sup>. Increased MSR/V gene expression in MS (and other inflammatory diseases) and cytotoxic or superantigenic effects of certain ERV proteins also suggest a possible role for these retroviruses in MS<sup>112-115</sup>.

For more information on the evidence that infectious agents may influence the development of MS, please refer to our document "Analysis of specific pathogens as possible triggers of Multiple Sclerosis" in the infectious agents track of the Cure Map.

#### 4. Chemical/radiation-induced mutagenesis

Several observations have been reported of increased chromosomal abnormalities in people with MS (see “Analysis of chromosomal abnormalities as a possible cause of Multiple Sclerosis”), although the significance of this phenomenon is not known. These observations raised the question of whether inherent sensitivity to radiation was a characteristic of people with MS. Results have been mixed, with one study finding an increased level of cellular radiosensitivity in MS subjects<sup>116</sup> and two others finding no difference between subjects and controls in X-radiation sensitivity<sup>117</sup> or DNA repair or survival<sup>118</sup>. Exposure to radiation through radiological work and/or X-rays was shown to increase the risk of MS in two studies<sup>119-121</sup>; however, a study of Danish utility workers did not associate risk of MS with exposure to electromagnetic fields<sup>122</sup>. Similarly, exposure to organic solvents has been positively associated with MS in some studies<sup>120</sup> but not in others<sup>123, 124</sup>. A meta-analysis of 13 studies found a Mantel-Haenszel ratio of 2.1 for exposure to organic solvents and frequency of MS<sup>125</sup>. Cigarette smoke, which contains genotoxic and carcinogenic agents, has been shown in recent studies to increase the risk of MS<sup>126-129</sup>. Interestingly, it has been suggested that exposure to UV radiation, which is associated with increased risk of skin cancer, may actually reduce the risk of MS<sup>130, 131</sup>.

For more information on the evidence that toxic agents may influence the development of MS, please refer to our document “Analysis of specific toxic agents as possible triggers of Multiple Sclerosis” in the toxic agents track of the Cure Map.

#### 5. Other

Other types of somatic mutations or epigenetic phenomena noted for a possible connection with MS include imprinting, which was proposed by one set of authors not to affect risk based on an observed lack of maternal influence on risk<sup>132</sup>; trinucleotide repeat expansion, for which studies of the SCA genes have found no evidence of involvement in MS<sup>133-136</sup>; and microchimerism, which has been associated with scleroderma and Hashimoto’s thyroiditis<sup>137</sup> and is being investigated as a risk factor for MS with mixed results to date<sup>138, 139</sup>.

### Conclusions

This report analyzes the possibility that susceptibility to MS is influenced by spontaneous mutations occurring for the first time in an individual who then goes on to either develop MS or bear a child who inherits the mutation and eventually develops MS. These spontaneous genetic changes may either be pathogenic in nature or come about as a result of normal physiological events. The involvement of spontaneous mutations, particularly somatic mutations, in MS is suggested by two epidemiological phenomena: (a) the fact that the majority of cases of MS are nonfamilial, and (b) the lack of complete concordance between genetically “identical” twins (20-30% as opposed to 100%). It is important to note, though, that these phenomena can also be explained by a multifactorial model involving environmental factors that affect some but not all family members.

Various types of evidence have been produced for and against the involvement of certain classes of spontaneous mutations implicated in human disease. This includes evidence concerning common underlying causes of spontaneous mutations and evidence (direct or circumstantial) concerning the actual presence of mutations in MS.

**Evidence concerning the cause of spontaneous mutations:** Factors that can cause or influence the development of spontaneous mutations or genomic instability include mutagenic chemicals and radiation, inherent defects in DNA editing and repair, and replication-competent retroviruses. Conflicting or insufficient evidence and lack of demonstrated connections to actual biological effects in MS make it impossible at this time to assign a causal contribution to any one factor.

Mutagenic chemicals and radiation: A few epidemiological studies have looked for possible associations between exposure to radiation or organic solvents and risk of MS. However, no strong consensus has yet emerged showing that exposure to radiation or solvents affects risk of MS. Four studies have shown that cigarette smoking increases the risk of MS, raising the possibility that DNA mutations caused by chemical agents in cigarette smoke contribute to the development of MS in some people.

Chromosomal instability: Reports of chromosomal damage in people with MS have led to suggestions that people with MS have higher inherent cellular susceptibility to radiation, which could lead to more frequent spontaneous mutations. However, only a few studies have been conducted to test this hypothesis, and these have produced conflicting results.

Viral genetic insertion: Another cause of spontaneous mutations in humans is the insertion of DNA into endogenous genes by exogenous or endogenous retroviruses that are capable of this activity. A few studies have reported the genetic presence of the exogenous retrovirus HTLV or the insertion of HHV-6 DNA into the genome of MS subjects, potentially in a region that would lead to chromosomal instability. Endogenous retroviruses have also been linked with MS, and although most endogenous retroviruses are thought to be incapable of becoming pathogenic through replication and insertion into the genome, there is evidence that MS-linked retroviruses may be capable of replication and transmission. This evidence is all circumstantial in nature, however: no concrete demonstrations have yet been made of actual damage to or disruption of the genome of MS subjects attributable to retroviruses.

**Evidence concerning the actual development and presence of spontaneous mutations in MS:** In addition to evaluating factors promoting the induction of spontaneous mutations in people with MS, it is also necessary to consider evidence for the development of such mutations in a manner that leads to MS.

Gamete/germ cell mutations: A spontaneous mutation can affect either the person in which the mutation takes place or it can affect his/her offspring, if it occurs during gametogenesis or is located in a germ cell. Regarding the latter situation, little evidence, either genetic or epidemiological, has yet been produced suggesting that MS results primarily from mutations in gametes or germ cells that do not affect the parent but go on to induce MS in offspring. One study based on a Swedish MS registry did correlate increased paternal age with higher risk of MS<sup>140</sup>. However, special classes of genetic errors introduced during gametogenesis, such as imprinting and triplet repeat expansions, have not yet been associated with MS. Genetic imprinting errors do not

appear to be a factor in MS, as MS is transmitted by parents of both sexes, whereas disease caused by imprinting errors are generally specific to maternal or paternal transmission. No evidence has yet been discovered for involvement of a triplet repeat gene expansion in MS; for instance, MS has not been associated with anticipation (earlier onset and higher severity in affected offspring of affected parents), a key feature of many triplet repeat expansion diseases. (One study conducted in Sardinia did report that the age of onset of MS had decreased in younger generations, but a genetic basis for this phenomenon was deemed unlikely<sup>141</sup>.)

Mosaicism: A spontaneous mutation affecting the person in which it takes place could occur during the early development process, leading to mosaicism. No evidence has yet been produced that mosaicism is a causal factor for MS. It is theoretically possible that a mutation occurring early in development could affect only part of the body (e.g., the CNS) in such a way as to predispose to later development of MS. However, no studies have yet identified tissue-to-tissue germline genomic differences in people with MS.

Pathogenic somatic mutations: Somatic mutations occurring throughout an individual's life are often harmless or even helpful (see discussion of T cell receptor and immunoglobulin genes below). However, there are mechanisms whereby somatic mutations could lead to disease. Two examples of pathogenic models for somatic mutations include the induction of cellular proliferation leading to tumors, and the accumulation of defects in mitochondrial DNA through oxidative damage. These types of models are now being considered for significant roles in common multifactorial diseases such as coronary heart disease<sup>142</sup>. Types of evidence that would indicate a role for post-embryonic somatic mutation as a cause of MS include the presence of uncontrolled cell division or instability or damage to chromosomal or mitochondrial DNA. No unusual clonal expansion indicative of uncontrolled cell division has been commonly observed in MS tissues, aside from patterns of T and B cell line expansion (see below). Evidence does suggest that somatic chromosomal aberrations in peripheral blood lymphocytes and other cells are found at a higher frequency in people with MS than in controls. However, it is not clear whether or how this contributes to the initial development of MS. (See "Analysis of chromosomal abnormalities as a possible cause for Multiple Sclerosis" for further information.) MS has also been associated with damage to mitochondrial DNA – however, as with chromosomal abnormalities, this may be an effect rather than a cause. (See "Analysis of mitochondrial DNA mutations as a possible cause for Multiple Sclerosis" for further information.)

Normal spontaneous mutations contributing to immune system diversity: In addition to spontaneous mutations traditionally thought of as pathogenic in nature, the normal somatic genetic changes in immune cells that contribute to the diversity of immune responses available to humans may also play a role in MS. For instance, the process of T cell receptor gene rearrangement during maturation, combined with P- and N-region nucleotide addition and joining flexibility, has been estimated to produce as many as  $10^{13}$  possible amino acid combinations in the TCR junctional regions. It is possible that certain rearrangements or repertoires might make it possible for MS to develop in the presence of a critical antigen, whereas others may protect against the development of MS.

Various special aspects of T cell receptor usage have been analyzed in people with MS. The results of studies in this area, although not conclusive, do indicate that the composition of these molecules may affect the pathogenesis of MS. Of particular note

are the increased presence of oligoclonal rearrangements in people with MS, specificity of repertoires to certain epitopes, increased usage of gamma-delta chains in people with MS, etc. However, the fact that the subjects of studies investigating T cell gene usage in MS are generally already several years into the course of their disease creates problems for determining whether these features of gene usage are a cause or an effect of MS – particularly because it has been demonstrated that T cell repertoires can be dynamic and are almost certainly affected by the course of events taking place in MS.

Some help with this problem comes in the form of evidence showing greater levels of discrepancy in T cell repertoires in discordant than in concordant twins. In addition, one study was conducted specifically to eliminate the confounding factors of the effects of the disease process on T cells. Researchers compared the naïve T cells (thought to be less affected by the course of the disease) of discordant monozygotic twins, showing a significant difference between populations. Based on this evidence, they concluded that a shift in T cell repertoire precedes the onset of MS. However, corroborating evidence from further studies of this type and evidence from earlier stages of the disease is required to be more definite about a causal role for T cell receptor genes in MS.

A similar situation exists for somatic rearrangement and subsequent mutation of immunoglobulin genes. Enhanced rearrangement, clonal expansion and extensive somatic mutation of antibody sequences, compared to controls, have also been found in several studies of tissue from MS subjects. It is possible that these diversification mechanisms, producing and allowing for the amplification of some but not all possible combinations at any one time in a person's immune system, may help account for individual differences in the development of MS. However, at this time there is no firm evidence linking pre-existing antibody repertoires to risk of MS.

## References

- 1 J. A. Loveridge, W. M. Rosenberg, T. B. Kirkwood and J. I. Bell, *The genetic contribution to human T-cell receptor repertoire*. Immunology, 1991. **74**(2): p. 246-50. PubMed ID: 1836200.

An analysis of normal human TCR repertoires revealed that in most people, all members of the V beta family are used; that amounts of the V beta families differ from person to person; and that the normal V beta repertoire is more concordant in identical twins than in unrelated individuals.

- 2 G. E. Hawes, L. Struyk and P. J. van den Elsen, *Differential usage of T cell receptor V gene segments in CD4+ and CD8+ subsets of T lymphocytes in monozygotic twins*. J Immunol, 1993. **150**(5): p. 2033-45. PubMed ID: 8436833.

High concordance was found for V beta, and to a lesser extent, V alpha gene usage in monozygotic twins; more variation in V alpha/beta gene usage was found between unrelated individuals. Skewing of certain V alpha or V beta families to either CD4+ or CD8+ cells was observed; some skewing patterns were found that applied to the entire sample set and some were specific for individual twin pairs.

- 3 R. Martin, R. Voskuhl, M. Flerlage, D. E. McFarlin and H. F. McFarland, *Myelin basic protein-specific T-cell responses in identical twins discordant or concordant for multiple sclerosis*. *Ann Neurol*, 1993. **34**(4): p. 524-35. PubMed ID: 7692808.

A study of the MBP-specific T cell response in 3 twin pairs concordant for MS and three discordant twin pairs found similar frequencies of MBP-specific cells in affected and unaffected individuals, and no differences with respect to HLA restriction. However, differences in specificities in some discordant twins indicate possible differences in MBP repertoires.

- 4 U. Utz, W. E. Biddison, H. F. McFarland, D. E. McFarlin, M. Flerlage and R. Martin, *Skewed T-cell receptor repertoire in genetically identical twins correlates with multiple sclerosis*. *Nature*, 1993. **364**(6434): p. 243-7. PubMed ID: 7686632.

Monozygotic twin sets who were either concordant for MS or both unaffected showed similar V alpha chain selection in response to stimulation with MBP or tetanus toxoid. Discordant twin sets, however, selected different TCRs.

- 5 A. Shanmugam, C. Copie-Bergman, G. Hashim, D. Rebibo, J. P. Jais, J. F. Bach, M. A. Bach and E. Tournier-Lasserre, *Healthy monozygous twins do not recognize identical T cell epitopes on the myelin basic protein autoantigen*. *Eur J Immunol*, 1994. **24**(10): p. 2299-303. PubMed ID: 7523133.

T cell lines specific for MBP were analyzed in eight sets of healthy monozygotic twins. Six pairs of twins carried cells that recognized shared epitopes as well as cells whose specificities were unique to each twin; two pairs exhibited a complete absence of shared epitope recognition.

- 6 D. G. Haegert, T. Cowan, T. J. Murray, V. Gadag and P. O'Connor, *Does a shift in the T-cell receptor repertoire precede the onset of MS?* *Neurology*, 1999. **53**(3): p. 485-90. PubMed ID: 10449108.

Naïve T cell repertoires in discordant MZ twins were significantly more dissimilar than those in healthy MZ twins. The authors speculate that a shift in T cell repertoire likely precedes development of MS, and that this shift, possibly caused by a factor such as a superantigen, could explain the incomplete concordance in MZ twins.

- 7 N. Joshi, K. Usuku and S. L. Hauser, *The T-cell response to myelin basic protein in familial multiple sclerosis: diversity of fine specificity, restricting elements, and T-cell receptor usage*. *Ann Neurol*, 1993. **34**(3): p. 385-93. PubMed ID: 7689820.

A study of MBP-specific T cells in three multiplex MS families showed similar frequencies of MBP-reactive T cell lines in MS subjects and healthy siblings; little overlap in the fine specificity of MBP recognition between individuals, even HLA-identical siblings; and no correlation between the use of particular DR alleles and response to particular MBP regions.

- 8 F. T. Rotteveel, I. Kokkelink, H. K. van Walbeek, C. H. Polman, J. J. van Dongen and C. L. Lucas, *Analysis of T cell receptor-gene rearrangement in T cells from the cerebrospinal fluid of patients with multiple sclerosis*. J Neuroimmunol, 1987. **15**(3): p. 243-9. PubMed ID: 3298316.

TcR beta-chain gene rearrangements were studied in 30 cloned CSF T cell lines from each of 2 MS subjects. All clones had rearrangements but none were identical. The authors conclude that if a few clones of specific T cells are involved in MS, they must be few in number compared with the total T cell repertoire in the CSF of MS subjects.

- 9 D. A. Hafler, A. D. Duby, S. J. Lee, D. Benjamin, J. G. Seidman and H. L. Weiner, *Oligoclonal T lymphocytes in the cerebrospinal fluid of patients with multiple sclerosis*. J Exp Med, 1988. **167**(4): p. 1313-22. PubMed ID: 3258624.

Of 28 CSF-derived T cell clones from one chronic progressive MS subject, 18 demonstrated common TCR gene rearrangements. Two repeated patterns were found in 26 clones derived from this subject's blood. Another MS subject demonstrated common patterns in 5 of 27 CSF-derived clones. No oligoclonal T cells were found in subjects with subacute sclerosing panencephalitis or herpes zoster meningoencephalitis, a normal control, or a subject with atypical, fatal MS.

- 10 J. R. Oksenberg, S. Stuart, A. B. Begovich, R. B. Bell, H. A. Erlich, L. Steinman and C. C. Bernard, *Limited heterogeneity of rearranged T-cell receptor V alpha transcripts in brains of multiple sclerosis patients*. Nature, 1990. **345**(6273): p. 344-6. PubMed ID: 1971424.

V alpha transcripts demonstrating sequence restrictions were found in 3 of 3 MS subject brains; no transcripts were found in control brains.

- 11 A. Ben-Nun, R. S. Liblau, L. Cohen, D. Lehmann, E. Tournier-Lasserre, A. Rosenzweig, J. W. Zhang, J. C. Raus and M. A. Bach, *Restricted T-cell receptor V beta gene usage by myelin basic protein-specific T-cell clones in multiple sclerosis: predominant genes vary in individuals*. Proc Natl Acad Sci U S A, 1991. **88**(6): p. 2466-70. PubMed ID: 1706524.

Cloned T cells from five MS subjects, characterized for their specificity to MBP epitopes and HLA-restricting molecules, showed a predominant V beta gene usage in each case. In one subject, 12 of 16 clones used the V beta 15 gene in its rearrangement; predominant rearrangements involving other genes were also found in the other subject. V beta gene usage did not correlate with specific MBP epitopes or restricting HLA molecules.

- 12 S. J. Lee, K. W. Wucherpfennig, S. A. Brod, D. Benjamin, H. L. Weiner and D. A. Hafler, *Common T-cell receptor V beta usage in oligoclonal T lymphocytes derived from cerebrospinal fluid and blood of patients with multiple sclerosis*. Ann Neurol, 1991. **29**(1): p. 33-40. PubMed ID: 1847614.

Oligoclonal T cell clones were found in the CSF and blood from five of nine MS subjects among a total of 486 clones. No oligoclonal lines were found in the blood of four normal controls or the blood or CSF of eight subjects with other neurological diseases. Four of the clones from three MS subjects demonstrated usage of the V beta 12 gene segment.

- 13 J. R. Richert, E. D. Robinson, A. H. Johnson, C. A. Bergman, L. J. Dragovic, N. L. Reinsmoen and C. K. Hurley, *Heterogeneity of the T-cell receptor beta gene rearrangements generated in myelin basic protein-specific T-cell clones isolated from a patient with multiple sclerosis*. *Ann Neurol*, 1991. **29**(3): p. 299-306. PubMed ID: 1710434.

Seventeen T cell clones from a single MS subject reactive to a particular residue of MBP showed 12 different TCR beta gene rearrangements. These clones were found to recognize MBP in conjunction with HLA DRw13.

- 14 G. Birnbaum and B. van Ness, *Quantitation of T-cell receptor V beta chain expression on lymphocytes from blood, brain, and spinal fluid in patients with multiple sclerosis and other neurological diseases*. *Ann Neurol*, 1992. **32**(1): p. 24-30. PubMed ID: 1386501.

A study of TCR V beta chains from blood, CSF and brain samples from MS subjects and controls with other neurological diseases showed a skewed expression in the blood, with overexpression of V beta genes 1 through 8. Expression of V beta 12 was increased in two MS brain samples. Patterns of expression differed among paired blood, brain and CSF samples.

- 15 G. Giegerich, M. Pette, E. Meinl, J. T. Epplen, H. Wekerle and A. Hinkkanen, *Diversity of T cell receptor alpha and beta chain genes expressed by human T cells specific for similar myelin basic protein peptide/major histocompatibility complexes*. *Eur J Immunol*, 1992. **22**(3): p. 753-8. PubMed ID: 1372258.

11 different T cell clones from MS subjects and healthy donors showed diversity in alpha and beta sequences, even in clones with similar HLA-DR context recognizing the same MBP epitope. Two clones recognizing MBP 139-153 equally well had different alpha but identical beta chains.

- 16 R. Martin, U. Utz, J. E. Coligan, J. R. Richert, M. Flerlage, E. Robinson, R. Stone, W. E. Biddison, D. E. McFarlin and H. F. McFarland, *Diversity in fine specificity and T cell receptor usage of the human CD4+ cytotoxic T cell response specific for the immunodominant myelin basic protein peptide 87-106*. *J Immunol*, 1992. **148**(5): p. 1359-66. PubMed ID: 1371525.

Heterogeneity of TCR usage was seen in 29 TCL generated against whole MBP from four MS subjects and two healthy relatives. Diversity was seen even in lines with identical peptide specificity and HLA-DR restriction.

- 17 K. W. Wucherpfennig, J. Newcombe, H. Li, C. Keddy, M. L. Cuzner and D. A. Hafler, *Gamma delta T-cell receptor repertoire in acute multiple sclerosis lesions*. *Proc Natl Acad Sci U S A*, 1992. **89**(10): p. 4588-92. PubMed ID: 1374907.

Accumulations of gamma delta T cells expressing V delta 1, V delta 2 and V gamma 2 (V gamma 9) were found in acute demyelinating MS plaques and appeared to be clonally expanded. Heat shock proteins hsp60 and hsp90 which are overexpressed in plaques, may be the target antigens for gamma delta T cells.

- 18 J. Hvas, J. R. Oksenberg, R. Fernando, L. Steinman and C. C. Bernard, *Gamma delta T cell receptor repertoire in brain lesions of patients with multiple sclerosis*. J Neuroimmunol, 1993. **46**(1-2): p. 225-34. PubMed ID: 8395544.

Only one to four rearranged TCR V gamma and V delta transcripts were detected in each of 23 brain samples obtained from 12 MS subjects (the majority expressed V gamma 2 and V delta 2 chains); whereas V gamma and V delta transcripts were found in only 1 of the 10 non-neurological control brains analyzed. Sequence analysis does not indicate an MS-specific expansion of one or more types of T cell receptor.

- 19 E. Meinl, F. Weber, K. Drexler, C. Morelle, M. Ott, G. Saruhan-Direskeneli, N. Goebels, B. Ertl, G. Jechart, G. Giegerich and et al., *Myelin basic protein-specific T lymphocyte repertoire in multiple sclerosis. Complexity of the response and dominance of nested epitopes due to recruitment of multiple T cell clones*. J Clin Invest, 1993. **92**(6): p. 2633-43. PubMed ID: 7504690.

A study of MBP-specific TCLs in 13 MS subjects and 10 healthy donors found heterogeneous anti-MBP responses in the donors. Also seen were responses in a minority of subjects that were highly restricted with respect to epitope specificity (75-87% of TCLs responding to a short MBP epitope), but heterogeneous with respect to fine specificity, TCR usage and HLA response. Evidence of persistence was seen in one MS subject. Only one of 215 TCLs expressed V beta 5.2.

- 20 J. R. Oksenberg, M. A. Panzara, A. B. Begovich, D. Mitchell, H. A. Erlich, R. S. Murray, R. Shimonkevitz, M. Sherritt, J. Rothbard, C. C. Bernard and et al., *Selection for T-cell receptor V beta-D beta-J beta gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis*. Nature, 1993. **362**(6415): p. 68-70. PubMed ID: 7680433.

Rearranged V beta 5.2 genes were detected in brain lesions of all MS subjects studied who were HLA DRB1\*1501, DQA1\*0102, DQB1\*0602, DPB1\*0401. One of the five common motifs was identical to that found in a previously described T-cell clone cytotoxic towards targets containing MBP peptide 89-106. VDJ sequences with specificity for this MBP epitope constitute 40% of the TCR V beta 5.2 N(D)N rearrangements in MS lesions.

- 21 S. Sriram, *Longitudinal study of frequency of HPRT mutant T cells in patients with multiple sclerosis*. Neurology, 1994. **44**(2): p. 311-5. PubMed ID: 8309581.

HPRT mutant frequency (mF) was found to be consistently elevated in the T cells of MS subjects of all clinical subgroups compared with controls. In subjects with chronic progressive MS, the mF increased over 3 years, appearing to correlate with disease progression.

- 22 U. Utz, J. A. Brooks, H. F. McFarland, R. Martin and W. E. Biddison, *Heterogeneity of T-cell receptor alpha-chain complementarity- determining region 3 in myelin basic protein-specific T cells increases with severity of multiple sclerosis*. Proc Natl Acad Sci U S A, 1994. **91**(12): p. 5567-71. PubMed ID: 7515505.

The CDR3 region in MBP-specific V alpha 8-positive TCRs showed marked sequence heterogeneity in individuals with severe MS, as opposed to controls and individuals with mild MS who were found to have restricted areas. Sequences from tetanus toxoid-specific V alpha 8-positive T cells were relatively homogenous within individuals regardless of disease activity and distinct from the CDR3 sequences in MBP-specific lines.

- 23 K. W. Wucherpfennig, J. Zhang, C. Witek, M. Matsui, Y. Modabber, K. Ota and D. A. Hafler, *Clonal expansion and persistence of human T cells specific for an immunodominant myelin basic protein peptide*. J Immunol, 1994. **152**(11): p. 5581-92. PubMed ID: 7514641.

Most of the MBP-specific T cell lines isolated from seven MS subjects and three normal controls were specific for MBP(84-102) and MBP(143-168) epitopes. Clonality was seen in both subjects and controls. Repeated analysis of one subject showed the persistence of clones for as long as 31 months.

- 24 L. Battistini, M. Salvetti, G. Ristori, M. Falcone, C. S. Raine and C. F. Brosnan, *Gamma delta T cell receptor analysis supports a role for HSP 70 selection of lymphocytes in multiple sclerosis lesions*. Mol Med, 1995. **1**(5): p. 554-62. PubMed ID: 8529121.

Dominant sequences were found in heat shock protein-specific gamma delta T cells in PBMC in subjects with MS and in subjects with TB. In HSP70+ T cell lines, the predominant delta chain rearrangement pattern was V delta 2-J delta 3. In four out of four MS subjects and two out of three TB subjects, the HSP70+ T cell lines were identical to one another and to a dominant sequence previously detected in MS brain lesions. Oligoclonal HSP70+ lines were also found in healthy controls but they differed from those found in both groups of subjects. In freshly isolated PBMC or in PPD+HSP70- T cell lines, rearrangement patterns were usually polyclonal.

- 25 L. Battistini, K. Selmaj, C. Kowal, J. Ohmen, R. L. Modlin, C. S. Raine and C. F. Brosnan, *Multiple sclerosis: limited diversity of the V delta 2-J delta 3 T-cell receptor in chronic active lesions*. Ann Neurol, 1995. **37**(2): p. 198-203. PubMed ID: 7847861.

A predominant gene rearrangement was found in the V delta 2-J delta 3 TCR population from plaques from nine MS subjects that was not found in CNS tissue from subjects with other neurological diseases. However, within the V delta 2-J delta 1 population, a predominant pattern was only found in one MS subject.

- 26 P. Stinissen, C. Vandevyver, R. Medaer, L. Vandegaer, J. Nies, L. Tuyls, D. A. Hafler, J. Raus and J. Zhang, *Increased frequency of gamma delta T cells in cerebrospinal fluid and peripheral blood of patients with multiple sclerosis. Reactivity, cytotoxicity, and T cell receptor V gene rearrangements*. J Immunol, 1995. **154**(9): p. 4883-94. PubMed ID: 7722338.

Higher frequencies of gamma delta T cells were found in the peripheral blood and CSF of MS subjects compared with subjects with other neurological diseases and healthy controls after stimulation with Pha; frequencies of IL-2 responsive cells were higher in controls than subjects. 13 of 20 gamma delta T cell clones from MS subjects responded

to HSP70 (but not HSP65), compared to only 2 of 30 HSP-reactive T cell clones from controls. All clones were highly reactive to bacterial superantigens regardless of gene usage. Most clones represented heterogeneous clonal origins, except for one predominant clonal origin found in one MS subject.

- 27 K. W. Wucherpfennig and D. A. Hafler, *A review of T-cell receptors in multiple sclerosis: clonal expansion and persistence of human T-cells specific for an immunodominant myelin basic protein peptide*. Ann N Y Acad Sci, 1995. **756**(p. 241-58. PubMed ID: 7544075.

Clonal T cell expansions were found to dominate the response to MBP 84-102 in two DR2+ subjects; MBP 84-102 specific T cell expansions were also found in one normal DR2+ subject.

- 28 J. R. Richert, E. D. Robinson, K. Camphausen, R. Martin, R. R. Voskuhl, M. A. Faerber, H. F. McFarland and C. K. Hurley, *Diversity of T-cell receptor V alpha, V beta, and CDR3 expression by myelin basic protein-specific human T-cell clones*. Neurology, 1995. **45**(10): p. 1919-22. PubMed ID: 7477993.

Thirteen V alpha gene segments, 18 V beta segments, 23 CDR3 alpha and 30 CDR3 beta sequences were found in 54 MBP-specific T cell clones from peripheral blood of 15 MS subjects and three normal controls. Some of the CDR3 motifs were common to several clones with the same epitope specificity; others were common to clones with different specificities.

- 29 C. Vandevyver, N. Mertens, P. van den Elsen, R. Medaer, J. Raus and J. Zhang, *Clonal expansion of myelin basic protein-reactive T cells in patients with multiple sclerosis: restricted T cell receptor V gene rearrangements and CDR3 sequence*. Eur J Immunol, 1995. **25**(4): p. 958-68. PubMed ID: 7537675.

MBP-reactive T cell clones generated from individual MS subjects revealed limited or single junctional region sequence patterns, indicating an oligoclonal or monoclonal origin. Gene usage varied among different subjects. Clones from healthy controls showed unfocused epitope specificity and gene usage.

- 30 D. A. Hafler, M. G. Saadeh, V. K. Kuchroo, E. Milford and L. Steinman, *TCR usage in human and experimental demyelinating disease*. Immunol Today, 1996. **17**(4): p. 152-9. PubMed ID: 8871344.

Meta-analysis of TCR repertoire data from 17 labs and 102 papers revealed (a) greater clonal expansion in subjects with MS and other inflammatory diseases than healthy controls, although expanded MBP-specific clones were found in healthy subjects; (b) no statistically significant differences in V beta, V alpha, or J beta gene usage among human MBP-reactive T cell clones, clones derived from MS brains, and controls; and (c) no differences in CDR3 motif frequency among human MBP-reactive T cell clones, clones derived from MS brains, and controls, although ideally this analysis would be performed on clones with identical restrictions and antigen specificities.

- 31 T. Kondo, T. Yamamura, J. Inobe, T. Ohashi, K. Takahashi and T. Tabira, *TCR repertoire to proteolipid protein (PLP) in multiple sclerosis (MS): homologies*

*between PLP-specific T cells and MS-associated T cells in TCR junctional sequences.* Int Immunol, 1996. **8**(1): p. 123-30. PubMed ID: 8671596.

Significant bias was found in V beta and J beta usage in PLP-reactive T cells isolated from the peripheral blood of three Japanese MS subjects. Overexpression of Vbeta2 and dominant usage of Jbeta2.5 were found in PLP 105-124 reactive and 95-116 reactive T cells, respectively. A majority of the T cells expressed beta-chain CDR3 motifs that were unique to MS brain infiltrates (rarely seen in control sequences from peripheral blood or tetanus toxoid-reactive T cells). Homology in betaCDR3 between PLP-reactive T cells and T cells from MS brain was found in several cases.

- 32 W. Liedtke, G. Meyer, P. M. Faustmann, H. Warnatz and C. S. Raine, *Clonal expansion and decreased occurrence of peripheral blood gamma delta T cells of the V delta 2J delta 3 lineage in multiple sclerosis patients.* Int Immunol, 1997. **9**(7): p. 1031-41. PubMed ID: 9237112.

V delta 2 J delta 3 TCR rearrangement was found in the PBLs of 27 of 28 healthy controls, 24 of 34 MS subjects and 7 of 14 autoimmune control subjects. It was also present in 5 of 11 lymphatic tissue specimens, none of which were from MS subjects. Heterogeneity of the sequenced PCR products was seen in healthy controls; oligoclonality was seen in MS and autoimmune subjects. Differences between MS subjects and controls were greater in exacerbating hospitalized patients than in clinically stable subjects. Only one sequence of 85 different sequences was shared between two MS subjects. The authors conclude that this subset of T cells might home to the CNS and thus be underrepresented in the blood of MS subjects.

- 33 P. A. Muraro, M. Vergelli, M. Kalbus, D. E. Banks, J. W. Nagle, L. R. Tranquill, G. T. Nepom, W. E. Biddison, H. F. McFarland and R. Martin, *Immunodominance of a low-affinity major histocompatibility complex-binding myelin basic protein epitope (residues 111-129) in HLA-DR4 (B1\*0401) subjects is associated with a restricted T cell receptor repertoire.* J Clin Invest, 1997. **100**(2): p. 339-49. PubMed ID: 9218510.

The majority of MBP-specific T cell lines from HLA-DR4+ MS subjects recognized MBP region 111-129. TCR rearrangements showed limited heterogeneity, which may be due to the weak binding of this epitope to DR4 and therefore requires high affinity receptors (as opposed to the diverse repertoire seen in DR15+ cells specific for MBP 81-99).

- 34 J. S. Nowak, G. Michalowska-Wender, D. Januszkiewicz and M. Wender, *Limited junctional diversity of V delta 5-J delta 1 rearrangement in multiple sclerosis patients.* Mol Chem Neuropathol, 1997. **30**(1-2): p. 95-100. PubMed ID: 9138431.

Analysis of the TCR delta gene repertoire using seminested PCR in nine MS subjects and 30 healthy controls revealed repeated V delta 5-J delta 1 nucleotide sequences in all analyzed clones from seven of nine MS subjects. The authors suggest that the clonal nature of the rearrangement may indicate an antigen-driven expansion of gamma-delta T cells, although whether this is pathogenic or secondary to damage is not known.

- 35 B. Birebent, G. Semana, A. Commeurec, G. Edan, B. Genetet and N. Genetet, *TCR repertoire and cytokine profiles of cerebrospinal fluid- and peripheral blood-*

*derived T lymphocytes from patients with multiple sclerosis.* J Neurosci Res, 1998. **51**(6): p. 759-70. PubMed ID: 9545089.

Overexpression of one of two Vbeta genes was found in 10 of 12 CSF samples from MS subjects, although the particular gene expressed varied among subjects. Comparison of CSF T cells to peripheral blood T cells showed that CSF cells produced higher levels of cytokines such as IL-4.

- 36 B. Gran, D. Gestri, A. Sottini, E. Quiros Roldan, A. Bettinardi, S. Signorini, D. Primi, C. Ballerini, R. Taiuti, L. Amaducci and L. Massacesi, *Detection of skewed T-cell receptor V-beta gene usage in the peripheral blood of patients with multiple sclerosis.* J Neuroimmunol, 1998. **85**(1): p. 22-32. PubMed ID: 9626994.

Nine of 40 MS subjects showed expansion of one or more TCRBV segments in PBMCs (six had expansion of TCRBV9 chains; three had expanded TCRBV1, TCRBV11, and TCRBV22 segments). TCRBV9 expansion was further analyzed and found to be polyclonal. Disease duration was shorter in subjects with expansion abnormalities. Observation of five subjects over time showed a regression of expanded chain expression back to normal values.

- 37 P. Lozeron, D. Chabas, B. Duprey, O. Lyon-Caen and R. Liblau, *T cell receptor V beta 5 and V beta 17 clonal diversity in cerebrospinal fluid and peripheral blood lymphocytes of multiple sclerosis patients.* Mult Scler, 1998. **4**(3): p. 154-61. PubMed ID: 9762666.

Analysis of V beta 5-J beta and V beta 17-J beta repertoire in MS subjects and controls (subjects with other neurological diseases) showed less diversity in the CSF samples than in PBLs in both groups. However, no recurrent clonal expansion was seen in the V beta 5+ T cells from MS subjects. One expanded T cell population using the same V beta 17-J beta 1.6 combination and identical CDR3 length was found in the CSF of three MS subjects but none of the controls.

- 38 G. Michalowska-Wender, J. Nowak and M. Wender, *Gamma/delta T cell receptor genes rearrangement in the blood and brain of multiple sclerosis patients. A preliminary study.* Folia Neuropathol, 1998. **36**(1): p. 1-5. PubMed ID: 9595857.

Oligoclonal rearrangements of gamma delta T cells were detected in the peripheral blood and cerebral white matter of MS subjects. Rearrangements involving V delta 1-J delta 1, V delta 2-J delta 1, V delta 3-J delta 1, and V delta 5-J delta 1 were found in an overwhelming majority of MS cases.

- 39 Y. C. Zang, M. Kozovska, I. Aebischer, S. Li, S. Boehme, P. Crowe, V. M. Rivera and J. Z. Zhang, *Restricted TCR Valpha gene rearrangements in T cells recognizing an immunodominant peptide of myelin basic protein in DR2 patients with multiple sclerosis.* Int Immunol, 1998. **10**(7): p. 991-8. PubMed ID: 9701037.

DR2- or DR4-restricted T cell clones specific for the MBP 83-99 peptide from 11 subjects with MS exhibited diverse recognition motifs, although a few clone pairs had similar motifs and sequence homology in the CDR3. DR2-restricted clones showed a biased usage of V alpha 3 and V alpha 8; V beta rearrangements were heterogeneous.

- 40 R. N. Heard, S. M. Teutsch, B. H. Bennetts, S. D. Lee, E. M. Deane and G. J. Stewart, *Lack of restriction of T cell receptor beta variable gene usage in cerebrospinal fluid lymphocytes in acute optic neuritis*. J Neurol Neurosurg Psychiatry, 1999. **67**(5): p. 585-90. PubMed ID: 10519862.  
TCRBV mRNA was found in the CSF of eight of nine optic neuritis subjects, six of six MS subjects with an acute relapse, and five of 13 controls, and was correlated with the presence of oligoclonal antibodies. Usage of a single TCRBV family was found in two of nine ON subjects and two of six MS subjects; in other subjects the number of families ranged between 5 and 15 (ON) and 4 and 17 (MS).
- 41 G. Michalowska-Wender, *The early stage of multiple sclerosis in the light of molecular and immunological studies*. Folia Neuropathol, 1999. **37**(4): p. 273-6. PubMed ID: 10705651.  
Mono- or oligoclonal V delta and J delta rearrangement (V delta 1-J delta 1, V delta 5-J delta 1 and V delta 3-J delta 1) was seen in the blood lymphocytes of MS subjects in the first two years from onset. In contrast, controls presented an overwhelming polyclonal picture. Oligoclonal bands and above-average IgG levels were detected in most of the MS subjects. TNF alpha levels in serum were also elevated in MS subjects compared with controls.
- 42 G. Michalowska-Wender, J. Nowak, J. Losy, D. Januszkiewicz and M. Wender, *Diversity of V delta-J delta gene rearrangement in peripheral blood lymphocytes and intrathecal IgG synthesis in multiple sclerosis*. Folia Neuropathol, 1999. **37**(2): p. 113-6. PubMed ID: 10464430.  
Restriction of V delta-J delta and in some cases monoclonal patterns involving V delta 1-J delta 1 or V delta 5-J delta 1 were seen in a majority of MS subjects with intrathecal IgG synthesis and oligoclonal bands. Conversely, most subjects with an IgG index below 0.75 and undetectable oligoclonal bands demonstrated oligo- or polyclonal V delta-J delta gene rearrangement.
- 43 H. Babbe, A. Roers, A. Waisman, H. Lassmann, N. Goebels, R. Hohlfeld, M. Friese, R. Schroder, M. Deckert, S. Schmidt, R. Ravid and K. Rajewsky, *Clonal expansions of CD8(+) T cells dominate the T cell infiltrate in active multiple sclerosis lesions as shown by micromanipulation and single cell polymerase chain reaction*. J Exp Med, 2000. **192**(3): p. 393-404. PubMed ID: 10934227.  
Single cell PCR in two actively demyelinating MS lesions revealed that the majority of CD8+ T cells belonged to a few clones (one clone accounted for 35% in one case). Common peptide specificities appeared to be selected in one of the cases. In both cases, the CD4+ T cell population was more heterogeneous. Most clones were present in perivascular infiltrates as well as parenchymal T cells; two CD8+ clones in one case were also detected in peripheral blood.
- 44 N. Goebels, H. Hofstetter, S. Schmidt, C. Brunner, H. Wekerle and R. Hohlfeld, *Repertoire dynamics of autoreactive T cells in multiple sclerosis patients and*

*healthy subjects: epitope spreading versus clonal persistence.* Brain, 2000. **123 Pt 3**(p. 508-18. PubMed ID: 10686174.

Analysis of MBP-specific CD4+ T-cell lines in five untreated MS subjects and four healthy controls over six years revealed that two subjects and three controls maintained a broad epitope response that fluctuated over time; two subjects initially exhibited a focused response that broadened over time; and one subject and one control had a focused response directed toward epitopes in MBP 83-102 which persisted over time. Individual clones were found to persist in the peripheral circulation for up to 7 years in the majority of both the MS subjects and controls.

- 45 D. Gestri, L. Baldacci, R. Taiuti, E. Galli, E. Maggi, M. P. Piccinni, M. Vergelli and L. Massacesi, *Oligoclonal T cell repertoire in cerebrospinal fluid of patients with inflammatory diseases of the nervous system.* J Neurol Neurosurg Psychiatry, 2001. **70**(6): p. 767-72. PubMed ID: 11385011.

Virtually complete TCRBV gene repertoires were found in CSF and PBMCs in 11 MS subjects and other subjects with other neuroinflammatory diseases (samples were collected at diagnosis). Six of 11 MS subjects and 13 of the 21 subjects overall also exhibited clonal expansion in the CSF, not segregating with any particular TCRBV gene. Expansion was seen in the periphery in four subjects, but in three of them this involved different TCRBV families than were found in the CSF. T cell expansion was not correlated with IgG oligoclonal expansion.

- 46 P. A. Muraro, L. Bonanni, B. Mazzanti, A. Pantalone, E. Traggiai, L. Massacesi, M. Vergelli and D. Gambi, *Short-term dynamics of circulating T cell receptor V beta repertoire in relapsing-remitting MS.* J Neuroimmunol, 2002. **127**(1-2): p. 149-59. PubMed ID: 12044987.

Analysis of the circulating TCRBV profile in untreated MS subjects and healthy controls revealed that TCRBV gene expansions were significantly more frequent in MS subjects than in controls ( $p < 0.001$ ), were predominantly oligoclonal (80%) and were significantly correlated with immune responses to MBP ( $p < 0.02$ ) and MRI activity ( $p < 0.05$ ). Study of expansion dynamics in MS subjects over 4-6 months showed change in the repertoire but also persistence of some expansions.

- 47 D. A. Laplaud, C. Ruiz, S. Wiertlewski, S. Brouard, L. Berthelot, M. Guillet, B. Melchior, N. Degauque, G. Edan, P. Brachet, P. Damier and J. P. Souillou, *Blood T-cell receptor beta chain transcriptome in multiple sclerosis. Characterization of the T cells with altered CDR3 length distribution.* Brain., 2004. **127**(Pt 5): p. 981-95. Epub 2004 Feb 25. PubMed ID: 14985265.

Skewed CDR3 distributions were found in subjects with clinically definite MS, with worsening RRMS, and (to a lesser extent) with subjects at a high risk of MS compared with healthy subjects. Cells from families with altered CDR3-LD expressed higher levels of IFN-gamma, IL-2, and TNF-a compared with cells from healthy individuals. Cells from four out of seven such families responded to human MBP, unlike similar cells from healthy controls.

- 48 Y. Warabi, K. Yagi, H. Hayashi and Y. Matsumoto, *Characterization of the T cell receptor repertoire in the Japanese neuromyelitis optica: T cell activity is up-*

*regulated compared to multiple sclerosis.* J Neurol Sci, 2006. **21**(p. 21. PubMed ID: 16860825.

Analysis of the T cell repertoire in NMO and MS subjects by CDR3 spectratyping found that NMO subjects had a greater number of clonally expanded Vbeta genes than MS subjects, and that both groups had more clonally expanded genes than healthy controls. Vbeta1 and Vbeta13 were activated in NMO compared with MS, and the number of clonally expanded Vbeta genes in NMO correlated with subjects' EDSS scores. In MS subjects, those with SPMS with longer disease durations and higher EDSS scores had fewer clonally expanded Vbeta genes than RRMS subjects.

- 49 J. Nowak, D. Januszkiewicz, M. Pernak, H. Hertmanowska, K. Nowicka-Kujawska, J. Rembowska, K. Lewandowski, T. Nowak and M. Wender, *Limited pattern of TCR delta chain gene rearrangement on the RNA level in multiple sclerosis.* J Appl Genet, 2001. **42**(4): p. 531-40. PubMed ID: 14564027.

Restricted patterns of Vdelta, Jdelta, and Cdelta genes were detected in the DNA and RNA of MS subjects compared with controls. A higher frequency of activated gamma/delta T cells were found in MS subjects, as well as an elevated level of CD56+ cells.

- 50 Y. Matsumoto, W. K. Yoon, Y. Jee, K. Fujihara, T. Misu, S. Sato, I. Nakashima and Y. Itoyama, *Complementarity-determining region 3 spectratyping analysis of the TCR repertoire in multiple sclerosis.* J Immunol., 2003. **170**(9): p. 4846-53. PubMed ID: 12707368.

CDR3 spectratyping in MS and healthy subjects revealed oligoclonal expansion of Vbeta5.2 in the peripheral blood of MS subjects but not healthy controls. The predominant TCR clone varied from subject to subject, but stayed consistent over time within subjects. Vbeta5.2 expansion was also detected in CSF samples of MS subjects.

- 51 D. G. Haegert, D. Galutira, T. J. Murray, P. O'Connor and V. Gadag, *Identical twins discordant for multiple sclerosis have a shift in their T-cell receptor repertoires.* Clin Exp Immunol., 2003. **134**(3): p. 532-7. PubMed ID: 14632762.

Analysis of the T-cell receptor distribution in discordant MS twin pairs and in healthy individuals showed that both MS twins affected with MS and their healthy co-twins have shifts in their CDR3 repertoire compared with healthy unrelated individuals. This indicates that CDR3 repertoire shifts precede and enhance the likelihood of developing MS, but do not by themselves cause MS.

- 52 K. W. Wucherpfennig, K. Ota, N. Endo, J. G. Seidman, A. Rosenzweig, H. L. Weiner and D. A. Hafler, *Shared human T cell receptor V beta usage to immunodominant regions of myelin basic protein.* Science, 1990. **248**(4958): p. 1016-9. PubMed ID: 1693015.

V beta 17 and V beta 12 were found in an examination of MBP-reactive T cell lines from MS subjects and healthy controls to be used in recognition of MBP 84-102. V beta 17 was found to be infrequently used in lines reactive with MBP 143-168.

- 53 B. L. Kotzin, S. Karuturi, Y. K. Chou, J. Lafferty, J. M. Forrester, M. Better, G. E. Nedwin, H. Offner and A. A. Vandenbark, *Preferential T-cell receptor beta-chain variable gene use in myelin basic protein-reactive T-cell clones from patients with multiple sclerosis*. Proc Natl Acad Sci U S A, 1991. **88**(20): p. 9161-5. PubMed ID: 1717998.

A bias for use of V beta 5.2 and V beta 6.1 was seen in MBP-specific T cell clones from the blood of MS subjects but not controls. V beta 5.2 was found in clones specific for different MBP epitopes, even from within the same individual.

- 54 Y. K. Chou, A. C. Buenafe, R. Dedrick, W. J. Morrison, D. N. Bourdette, R. Whitham, J. Atherton, J. Lane, E. Spoor, G. A. Hashim and et al., *T cell receptor V beta gene usage in the recognition of myelin basic protein by cerebrospinal fluid- and blood-derived T cells from patients with multiple sclerosis*. J Neurosci Res, 1994. **37**(2): p. 169-81. PubMed ID: 7512148.

In vitro expansion of CSF cells with IL-2/IL-4 plus accessory cells produced T cells specific for MBP that overexpressed V beta 1, V beta 2, V beta 5 or V beta 18, compared with expanded lines produced through use of other antigens. MBP-reactive T cells were found in the CSF of a relapsing-remitting MS subject during clinical activity, and the recognition pattern was also present in blood cells, even lingering after CSF reactivity dissipated during remission. Sequential analysis of MBP-reactive CSF T cells found that the V beta gene bias changed over time. Blood T cells also reflected the changed biases but retained initial bias for at least four months after disappearance from the CSF.

- 55 H. Offner and A. A. Vandenbark, *T cell receptor V genes in multiple sclerosis: increased use of TCRAV8 and TCRBV5 in MBP-specific clones*. Int Rev Immunol, 1999. **18**(1-2): p. 9-36. PubMed ID: 10614737.

Review of the data on TCR gene usage indicates more frequent usage of TCRAV8 and BV5 but not BV6 in MS subjects compared with controls in response to MBP. No differences were found between DR2+ and DR2- donors. HLA-DR alleles were found to preferentially restrict MBP responses, with higher usage of -DQ and -DP alleles by MS subjects than controls. HLA-DR2 alleles restrict only about half the MBP responses in MS subjects, less than in controls. Also, DRB1\*1501 and DRB5\*0101 subtypes are used equally to restrict MBP responses.

- 56 G. Afshar, P. A. Muraro, H. F. McFarland and R. Martin, *Lack of over-expression of T cell receptor Vbeta5.2 in myelin basic protein-specific T cell lines derived from HLA-DR2 positive multiple sclerosis patients and controls*. J Neuroimmunol, 1998. **84**(1): p. 7-13. PubMed ID: 9600703.

No overexpression of V beta 5.2 was found in analysis of 100 MBP-specific T cell lines from 17 MS subjects, one unaffected twin, and four healthy individuals.

- 57 A. Van der Aa, N. Hellings, C. C. Bernard, J. Raus and P. Stinissen, *Functional properties of myelin oligodendrocyte glycoprotein-reactive T cells in multiple sclerosis patients and controls*. J Neuroimmunol., 2003. **137**(1-2): p. 164-76. PubMed ID: 12667661.

No evidence for clonal expansions in the MOG-reactive peripheral blood T-cell repertoire were found in either MS subjects or healthy controls.

- 58 A. E. Lovett-Racke, R. Martin, H. F. McFarland, M. K. Racke and U. Utz, *Longitudinal study of myelin basic protein-specific T-cell receptors during the course of multiple sclerosis*. J Neuroimmunol, 1997. **78**(1-2): p. 162-71. PubMed ID: 9307241.

Analysis of MBP-specific TCRs in three MS subjects over three years indicated that V alpha and V beta usage remained similar over time for each subject regardless of disease activity. Lines with conserved CDR3 sequences were found in which V alpha usage remained constant over time while J alpha usage exhibited changes.

- 59 M. V. Tejada-Simon, Y. C. Zang, D. Yang, J. Hong, S. Li, R. A. Singh, E. Van den Berg-Loonen, J. M. Killian, V. M. Rivera and J. Z. Zhang, *Aberrant T cell responses to myelin antigens during clinical exacerbation in patients with multiple sclerosis*. Int Immunol, 2000. **12**(12): p. 1641-50. PubMed ID: 11099303.

T cells in 14 MS subjects experiencing exacerbation were found to undergo selective expansion and activation; during remission, their repertoires more closely resembled those in healthy controls.

- 60 M. Vergelli, B. Mazzanti, E. Traggiai, T. Biagioli, C. Ballerini, A. Parigi, A. Konse, G. Pellicano and L. Massacesi, *Short-term evolution of autoreactive T cell repertoire in multiple sclerosis*. J Neurosci Res, 2001. **66**(3): p. 517-24. PubMed ID: 11746371.

A study of the T cell repertoire in four MS subjects over six months revealed episodic fluctuations in each subject, not correlated overall with inflammatory activity.

- 61 P. A. Muraro, R. Cassiani-Ingoni, K. Chung, A. N. Packer, M. Sospedra and R. Martin, *Clonotypic analysis of cerebrospinal fluid T cells during disease exacerbation and remission in a patient with multiple sclerosis*. J Neuroimmunol., 2006. **171**(1-2): p. 177-83. Epub 2005 Nov 18. PubMed ID: 16298432.

Alteration of CDR3 length distribution in T cells obtained from CSF was observed during a MS subject's relapse.

- 62 C. Skulina, S. Schmidt, K. Dornmair, H. Babbe, A. Roers, K. Rajewsky, H. Wekerle, R. Hohlfeld and N. Goebels, *Multiple sclerosis: brain-infiltrating CD8+ T cells persist as clonal expansions in the cerebrospinal fluid and blood*. Proc Natl Acad Sci U S A., 2004. **101**(8): p. 2428-33. PubMed ID: 14983026.

Identical sets of CD8+ (but not CD4+) T cell clones were found in the brain, blood, and CSF from two MS subjects. Some of these clones were shown to have persisted for over five years in the CSF and/or blood.

- 63 T. Demoulin, G. Gachelin, D. Bequet and D. Dormont, *A biased Valpha24+ T-cell repertoire leads to circulating NKT-cell defects in a multiple sclerosis patient*

*at the onset of his disease.* Immunol Lett., 2003. **90**(2-3): p. 223-8. PubMed ID: 14687729.

Significant changes were observed in the blood Valpha24(+) T-cell repertoire of MS subjects during relapses. Most patients showed a decrease in Valpha24(+) transcript number and a decrease in the diversity of the Valpha24(+) T-cell repertoire. In one subject, these alterations led to circulating NKT cell defects.

- 64 G. P. Owens, H. Kraus, M. P. Burgoon, T. Smith-Jensen, M. E. Devlin and D. H. Gilden, *Restricted use of VH4 germline segments in an acute multiple sclerosis brain.* Ann Neurol, 1998. **43**(2): p. 236-43. PubMed ID: 9485065.

IgG VH4 sequences were found to be overexpressed in acute MS brain (60% of the VH sequences were VH4 compared to approximately 20% under normal conditions). Distinct sequence differences (clonal variants) were also found, indicating clonal expansion. Comparing VH sequences to germline counterparts revealed extensive somatic mutation and preferential accumulation of amino acid replacement mutations in complementarity determining regions.

- 65 Y. Qin, P. Duquette, Y. Zhang, P. Talbot, R. Poole and J. Antel, *Clonal expansion and somatic hypermutation of V(H) genes of B cells from cerebrospinal fluid in multiple sclerosis.* J Clin Invest, 1998. **102**(5): p. 1045-50. PubMed ID: 9727074.

Sequence analysis of the CDR3 region of rearranged VDJ genes from CSF B cells revealed an expansion of one or more dominant clones in 10 of 12 MS subjects and three of 15 controls (people with other neurological diseases). The V(H) IV family was preferentially used in clonally expanded CSF B cells. Numerous somatic mutations were found, mainly in the CDRs, with a high replacement-to-silent ratio affecting antigen receptor.

- 66 S. E. Baranzini, M. C. Jeong, C. Butunoi, R. S. Murray, C. C. Bernard and J. R. Oksenberg, *B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions.* J Immunol, 1999. **163**(9): p. 5133-44. PubMed ID: 10528220.

B cell clones in plaque tissue from 10 MS subjects compared to 4 non-MS control samples showed significant rearrangement diversity and deviation from normal Ig heavy chain repertoire; overrepresentation of VH1-69, VH4-34, VH4-39; D2, D3 and JH4 was also seen.

- 67 M. Colombo, M. Dono, P. Gazzola, S. Roncella, A. Valetto, N. Chiorazzi, G. L. Mancardi and M. Ferrarini, *Accumulation of clonally related B lymphocytes in the cerebrospinal fluid of multiple sclerosis patients.* J Immunol, 2000. **164**(5): p. 2782-9. PubMed ID: 10679121.

Oligoclonal B cell accumulations in CSF were detected in 10 of 10 MS subjects but only three of 10 subjects with other neurological disorders. VH3 and VH4 genes in the Ig V(D)J sequences in MS CSF were extensively mutated compared with germline sequences. Many clones shared the same HCDR3 gene and VH genes, but with different point mutations, indicating ongoing intraclonal diversification. VH3 gene mutation was found in a subject with viral meningitis but no intraclonal diversification was

observed. Only 1/3 of the V(D)J sequences found in the CSF of one MS subject could also be found in the PBL.

- 68 G. Mancardi, B. A. Hart, E. Capello, H. P. Brok, A. Ben-Nun, L. Roccatagliata, D. Giunti, P. Gazzola, M. Dono, N. Kerlero de Rosbo, M. Colombo and A. Uccelli, *Restricted immune responses lead to CNS demyelination and axonal damage*. J Neuroimmunol, 2000. **107**(2): p. 178-83. PubMed ID: 10854654.

Analysis of the HCDR3 length in the CSF B cells from 10 MS subjects revealed an oligoclonal accumulation of B cells; sequence analysis of the VH3 and VH4 gamma transcripts from two MS subjects showed that this accumulation was related to the expansion and diversification of limited groups of B cell clones.

- 69 T. Smith-Jensen, M. P. Burgoon, J. Anthony, H. Kraus, D. H. Gilden and G. P. Owens, *Comparison of immunoglobulin G heavy-chain sequences in MS and SSPE brains reveals an antigen-driven response*. Neurology, 2000. **54**(6): p. 1227-32. PubMed ID: 10746589.

Comparison of brain samples from three subjects with SSPE and three subjects with MS revealed similar features indicating an antigen-driven response, such as accumulation of replacement mutations in CDRs, extensive mutations from the germline segments, and uniqueness in IgG responses from sample to sample.

- 70 D. H. Gilden, M. P. Burgoon, B. K. Kleinschmidt-DeMasters, R. A. Williamson, O. Ghausi, D. R. Burton and G. P. Owens, *Molecular immunologic strategies to identify antigens and b-cell responses unique to multiple sclerosis*. Arch Neurol, 2001. **58**(1): p. 43-8. PubMed ID: 11176935.

Analysis of antibodies found in MS plaques and CSF by four laboratories indicated limited germline expression, features consistent with an antigen-driven response, and clonal expansion of B cells in MS. New methods are needed to identify the antigens targeted by the antibody response.

- 71 G. P. Owens, M. P. Burgoon, J. Anthony, B. K. Kleinschmidt-DeMasters and D. H. Gilden, *The immunoglobulin G heavy chain repertoire in multiple sclerosis plaques is distinct from the heavy chain repertoire in peripheral blood lymphocytes*. Clin Immunol, 2001. **98**(2): p. 258-63. PubMed ID: 11161983.

PCR amplification of VH sequences from lesions from a subject with acute MS showed oligoclonal and extensively mutated VH sequences with discrete intraclonal differences indicative of B cell clonal expansion. None of the VH sequences from the plaque were found in the subject's peripheral blood lymphocytes.

- 72 A. Corcione, S. Casazza, E. Ferretti, D. Giunti, E. Zappia, A. Pistorio, C. Gambini, G. L. Mancardi, A. Uccelli and V. Pistoia, *Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis*. Proc Natl Acad Sci U S A., 2004. **101**(30): p. 11064-9. Epub 2004 Jul 19. PubMed ID: 15263096.

B cell subsets found exclusively in secondary lymphoid organs were detected in the CSF but not peripheral blood of subjects with MS and other inflammatory neurological

disorders, suggesting a recapitulation of the B cell differentiation process in the CNS motivated by inflammatory conditions.

- 73 Y. Zhang, R. R. Da, L. G. Hilgenberg, W. W. Tourtellotte, R. A. Sobel, M. A. Smith, M. Olek, R. Nagra, G. Sudhir, S. van den Noort and Y. Qin, *Clonal expansion of IgA-positive plasma cells and axon-reactive antibodies in MS lesions*. J Neuroimmunol., 2005. **167**(1-2): p. 120-30. PubMed ID: 16099056.

Clonally expanded IgA-bearing plasma cells with somatic mutations and intracлонаl mutations of their V(H) genes were detected in MS lesions. IgA was found bound to axons and walls of microvessels in demyelinating and demyelinated areas, and was associated with axonal damage.

- 74 Y. Qin, P. Duquette, Y. Zhang, M. Olek, R. R. Da, J. Richardson, J. P. Antel, P. Talbot, N. R. Cashman, W. W. Tourtellotte, H. Wekerle and S. Van Den Noort, *Intrathecal B-cell clonal expansion, an early sign of humoral immunity, in the cerebrospinal fluid of patients with clinically isolated syndrome suggestive of multiple sclerosis*. Lab Invest., 2003. **83**(7): p. 1081-8. PubMed ID: 12861047.

Analysis of Ig V(H) chain genes from intrathecal B cells from subjects with a clinically isolated syndrome provided evidence of clonal expansion and somatic hypermutation of the V(H) genes. These characteristics may precede development of oligoclonal bands and the presence of multiple lesions on MRI. Subjects with clonally expanded intrathecal B cells were more likely to convert to clinically definite MS.

- 75 I. Cortese, S. Capone, S. Luchetti, L. M. Grimaldi, A. Nicosia and R. Cortese, *CSF-enriched antibodies do not share specificities among MS patients*. Mult Scler, 1998. **4**(3): p. 118-23. PubMed ID: 9762658.

Use of random peptide libraries displayed on phage identified several ligands reacting with CSF-enriched antibodies. None of these ligands were recognized by antibodies shared by any two subjects in a group of 55 MS subjects. CSF-enriched antibodies appeared to be stable over time regardless of disease progression.

- 76 H. Kohsaka, D. A. Carson, L. Z. Rassenti, W. E. Ollier, P. P. Chen, T. J. Kipps and N. Miyasaka, *The human immunoglobulin V(H) gene repertoire is genetically controlled and unaltered by chronic autoimmune stimulation*. J Clin Invest, 1996. **98**(12): p. 2794-800. PubMed ID: 8981926.

Analysis of VH gene repertoires in monozygotic twins showed a higher degree of similarity between twins than between unrelated subjects, even in twins discordant for rheumatoid arthritis.

- 77 S. Haghghi, O. Andersen, L. Rosengren, T. Bergstrom, J. Wahlstrom and S. Nilsson, *Incidence of CSF abnormalities in siblings of multiple sclerosis patients and unrelated controls*. J Neurol, 2000. **247**(8): p. 616-22. PubMed ID: 11041329.

Healthy siblings of MS subjects were more likely than unrelated controls to have CSF-enriched oligoclonal bands; siblings also had increased CSF IgG antibody titers and serum titers for measles IgG antibody compared with unrelated controls. The rate for

this partially hyperimmune condition was approximately five times the rate for clinically definite MS in siblings. These findings suggest that a genetic trait interacts with infections to cause MS.

- 78 D. Shirazian, F. Mokhtarian, B. C. Herzlich, A. E. Miller and D. Grob, *Presence of cross-reactive antibodies to HTLV-1 and absence of antigens in patients with multiple sclerosis*. J Lab Clin Med, 1993. **122**(3): p. 252-9. PubMed ID: 8409701.

Heterogeneous HTLV-1 antibodies were found to be slightly elevated in the serum of 12/34 subjects with MS as measured through ELISA and absent in serum samples from 34 normal controls. However, HTLV-1 antigens were not found in the cultured PBLs of any MS subjects.

- 79 M. Sandberg-Wollheim, J. Alumets, A. Biorklund, R. Gay and S. Gay, *Bone marrow derived cells express human T-cell lymphotropic virus type I (HTLV-I)-related antigens in patients with multiple sclerosis*. Scand J Immunol, 1988. **28**(6): p. 801-6. PubMed ID: 3068791.

Mononuclear cells from peripheral blood and CSF of seven MS subjects and lymph nodes of three MS subjects expressed antigens that reacted with antibodies specific for HTLV-I p19 and p24 gag proteins. All samples contained cells reacting with anti-p19; 3 out of 6 blood samples and 3 out of 7 CSF samples reacted with anti-p24. All lymph node samples contained cells that reacted with both antibodies. One out of 12 controls possessed blood containing reactive cells. These antibody reactions suggest the presence of a retroviral genome being expressed in MS.

- 80 P. Ferrante, E. Omodeo-Zorini, M. R. Zuffolato, R. Mancuso, R. Caldarelli-Stefano, S. Puricelli, M. Mediati, L. Losciale and D. Caputo, *Human T-cell lymphotropic virus tax and Epstein-Barr virus DNA in peripheral blood of multiple sclerosis patients during acute attack*. Acta Neurol Scand Suppl, 1997. **169**(p. 79-85. PubMed ID: 9174643.

PBMCs from 14 subjects experiencing acute MS attacks were analyzed for the DNA of herpes simplex 1 and 2, human cytomegalovirus, Epstein-Barr virus, JC virus and HTLV-1. Viral DNA was detected in all subjects. Epstein-Barr virus DNA was found for 42.8% of 14 MS subjects on the first day of an acute attack; a sharp increase of HTLV tax-rex DNA frequency (35.7%) was observed on the tenth day.

- 81 E. Wattel, M. Mariotti, J. D. Bignon, F. Agis, E. Gordien, J. Y. Muller, J. Hors and J. J. Lefrere, *No evidence of HTLV-I infection in French patients with multiple sclerosis using the polymerase chain reaction*. J Clin Pathol, 1991. **44**(10): p. 871-2. PubMed ID: 1960225.

PCR analysis was unable to detect the HTLV-I gene sequences pol, tax or env in blood samples of 54 MS subjects who were seronegative for HTLV-I/II, which suggests a lack of association between the occurrence of HTLV-I sequences and MS.

- 82 G. A. Dekaban, A. J. Hudson and G. P. Rice, *Absence of HTLV-I and HTLV-II proviral genome in the brains of patients with multiple sclerosis and amyotrophic*

*lateral sclerosis*. Can J Neurol Sci, 1992. **19**(4): p. 458-61. PubMed ID: 1423042.

Proviral DNA of HTLV-I/II was not found in the CNS tissue of subjects with MS, subjects with ALS, or controls.

- 83 H. B. Rasmussen and J. Clausen, *Search for a retrovirus in multiple sclerosis by enzymatic amplification of DNA from brain capillaries, brain tissue and mononuclear cells from peripheral blood*. Acta Neurol Scand, 1992. **86**(1): p. 87-90. PubMed ID: 1325730.

Analysis via PCR of DNA from brain capillaries, brain tissue and peripheral blood mononuclear cells from three to five MS subjects did not generate any HTLV-I hybridization signals.

- 84 A. Svenningsson, J. Lycke, B. Svennerholm, S. Gronowitz and O. Andersen, *No evidence for spumavirus or oncovirus infection in relapsing- remitting multiple sclerosis*. Ann Neurol, 1992. **32**(5): p. 711-4. PubMed ID: 1333176.

No evidence of human spumaretrovirus and oncoretroviruses (HTLV-I/II) was found in 11 subjects with RR MS in exacerbation. CSF, blood and plasma cells were cocultured with allogeneic mononuclear cells and subjected to PCR with primers for various viral proteins (all negative); no cytopathic effects were observed and supernatants were negative for reverse transcriptase activity.

- 85 G. Lisby, *Search for an HTLV-I-like retrovirus in patients with MS by enzymatic DNA amplification*. Acta Neurol Scand, 1993. **88**(6): p. 385-7. PubMed ID: 7906912.

PCR analysis using primer sets homologous to HTLV-I/II genetic sequences did not produce DNA fragments from peripheral blood mononuclear cells from 67 MS subjects and brain capillaries from 6 subjects.

- 86 K. A. Brokstad, K. H. Kalland, M. Page, H. Nyland and L. Haaheim, *Serum antibodies from MS patients do not recognize HTLV-I, HIV-1, HIV-2 and SIV*. Apmis, 1994. **102**(7): p. 514-20. PubMed ID: 7917220.

Sera from 25 MS subjects, 25 subjects with other neurological diseases, and 16 with non-neurological conditions did not react against retroviral antigens when exposed to lysates of HIV-1, HIV2, HTLV-I and SIV-infected cells. However, they did react against cellular components of the cells in which the viruses were propagated. The authors conclude that in some MS subjects, repeated seropositivity to HTLV-I may be due to reaction with host cell proteins.

- 87 K. M. Myhr, P. Frost, M. Gronning, R. Midgard, K. H. Kalland, D. E. Helland and H. I. Nyland, *Absence of HTLV-1 related sequences in MS from high prevalence areas in western Norway*. Acta Neurol Scand, 1994. **89**(1): p. 65-8. PubMed ID: 8178631.

No HTLV-I amplification products were detected in 183 MS subjects or 102 controls from Western Norway.

- 88 M. Luppi, R. Marasca, P. Barozzi, S. Ferrari, L. Ceccherini-Nelli, G. Batoni, E. Merelli and G. Torelli, *Three cases of human herpesvirus-6 latent infection: integration of viral genome in peripheral blood mononuclear cell DNA*. *J Med Virol*, 1993. **40**(1): p. 44-52. PubMed ID: 8099945.

HHV-6 DNA was found in high molecular weight fragments in the peripheral blood mononuclear cell DNA of one MS subject and two subjects with lymphoproliferative disorders, indicating possible integration with the subjects' DNA.

- 89 G. Torelli, P. Barozzi, R. Marasca, P. Cocconcelli, E. Merelli, L. Ceccherini-Nelli, S. Ferrari and M. Luppi, *Targeted integration of human herpesvirus 6 in the p arm of chromosome 17 of human peripheral blood mononuclear cells in vivo*. *J Med Virol*, 1995. **46**(3): p. 178-88. PubMed ID: 7561787.

One of 31 MS subjects was positive for HHV-6 sequences as detected by PCR analysis of PBMCs. Analysis of viral DNA by various methods produced results consistent with an in vivo integration of the complete viral sequence into the cellular genome. A hybridization site was found at the telomeric extremity of chromosome 17p.

- 90 C. Morris, M. Luppi, M. McDonald, P. Barozzi and G. Torelli, *Fine mapping of an apparently targeted latent human herpesvirus type 6 integration site in chromosome band 17p13.3*. *J Med Virol*, 1999. **58**(1): p. 69-75. PubMed ID: 10223549.

Using PBMCs of an MS subject and two lymphoma subjects, a specific target site for latent integration of the full-length HHV-6 viral genome was mapped to the distal short arm of chromosome 17. More precise mapping located the site at least 1,000 kb telomeric of the ABR oncogene, possibly near the telomeric sequences of 17p. Cytogenetic studies showed evidence of karyotype instability in the latently infected cells.

- 91 H. B. Rasmussen, C. Geny, L. Deforges, H. Perron, W. Tourtelotte, A. Heltberg and J. Clausen, *Expression of endogenous retroviruses in blood mononuclear cells and brain tissue from multiple sclerosis patients*. *Mult Scler*, 1995. **1**(2): p. 82-7. PubMed ID: 9345457.

Expression of the endogenous retroviral sequences HRES-1, HERV-K10 and ERV3 was found in most PBMC samples from both MS subjects and controls. However, composite transcripts of ERV3 and a zinc finger sequence were more frequently detected in healthy donors than in MS subjects. The retroviral element 4-1 was absent or transcribed at only low levels in PBMCs from both groups. Retroviral transcripts were found in brain samples; expression was similar in subjects compared with controls.

- 92 J. Hackett, Jr., P. Swanson, D. Leahy, E. L. Anderson, S. Sato, R. P. Roos, R. Decker and S. G. Devare, *Search for retrovirus in patients with multiple sclerosis*. *Ann Neurol*, 1996. **40**(5): p. 805-9. PubMed ID: 8957025.

No evidence was found of reverse transcriptase using a new ultrasensitive technique, Imx PERT, on 136 sera from 79 MS subjects and 128 CSF samples from 53 subjects with relapsing or chronic progressive disease. PBMCs from 19 MS subjects were also

cultured to amplify or induce expression of low-copy number or cell-associated retrovirus, but no retroviral evidence was found.

- 93 H. B. Rasmussen, C. Geny, L. Deforges, H. Perron, W. Tourtelotte, A. Heltberg and J. Clausen, *Expression of endogenous retroviruses in blood mononuclear cells and brain tissue from multiple sclerosis patients*. Acta Neurol Scand Suppl, 1997. **169**(p. 38-44. PubMed ID: 9174639.

Transcription of various endogenous retroviral sequences was detected in PBMCs and brain tissue from MS subjects as well as controls; however, a composite transcript composed of an endogenous retrovirus and a zinc finger sequence was more frequent in healthy donors than MS subjects.

- 94 H. Perron, C. Geny, A. Laurent, C. Mouriquand, J. Pellat, J. Perret and J. M. Seigneurin, *Leptomeningeal cell line from multiple sclerosis with reverse transcriptase activity and viral particles*. Res Virol, 1989. **140**(6): p. 551-61. PubMed ID: 2482522.

A leptomeningeal cell line obtained from CSF from an MS subject was found to contain retroviral particles and produce reverse transcriptase activity. No antibodies or reactivity to HTLV-I or HIV-1 or -2 was demonstrated in this subject.

- 95 H. Perron, B. Gratacap, B. Lalande, O. Genoulaz, A. Laurent, C. Geny, M. Mallaret, P. Innocenti, E. Schuller, P. Stoebner and et al., *In vitro transmission and antigenicity of a retrovirus isolated from a multiple sclerosis patient*. Res Virol, 1992. **143**(5): p. 337-50. PubMed ID: 1282730.

The novel retrovirus LM7 was shown to be transmissible to normal leptomeningeal cell cultures. Specific antibodies to LM7 were detected in MS subjects.

- 96 S. Haahr, M. Sommerlund, T. Christensen, A. W. Jensen, H. J. Hansen and A. Moller-Larsen, *A putative new retrovirus associated with multiple sclerosis and the possible involvement of Epstein-Barr virus in this disease*. Ann N Y Acad Sci, 1994. **724**(p. 148-56. PubMed ID: 7518205.

Retrovirus-like particles were found in blood and CSF cells cultured from two MS subjects; these particles tested positive in reverse transcriptase assays. The retrovirus is different from other known human retroviruses. The authors propose that because 100% of people with MS have antibodies against EBV, MS requires dual infection (delayed herpesvirus infection, such as EBV, and this new uncharacterized retrovirus).

- 97 P. Froussard, *Is LM7 a new human retrovirus or a mycoplasma virion?* Mult Scler, 1995. **1**(2): p. 88-94. PubMed ID: 9345458.

Evidence was found linking the putative retrovirus LM7 isolated from an MS subject with mycoplasma - for instance, all cells producing LM7 particles were infected with mycoplasma. The author suggests that LM7 may be related to mycoplasma or a virion associated with the bacteria, and may not be a causal agent of MS.

- 98 S. Lefebvre, B. Hubert, F. Tekaiia, M. Brahic and J. F. Bureau, *Isolation from human brain of six previously unreported cDNAs related to the reverse transcriptase of human endogenous retroviruses*. *AIDS Res Hum Retroviruses*, 1995. **11**(2): p. 231-7. PubMed ID: 7537972.

Analysis of MS plaques via PCR using reverse transcriptase primers revealed six cDNAs belonging to new members of two groups of human endogenous retroviruses. These sequences were found in all human organs tested including normal white matter.

- 99 M. Munch, A. Moller-Larsen, T. Christensen, N. Morling, H. J. Hansen and S. Haahr, *B-lymphoblastoid cell lines from multiple sclerosis patients and a healthy control producing a putative new human retrovirus and Epstein-Barr virus*. *Mult Scler*, 1995. **1**(2): p. 78-81. PubMed ID: 9345456.

B-lymphoblastoid cell lines producing retrovirus-like particles and EBV particles were established in 5 of 21 consecutive MS subjects and 1 of 13 consecutive healthy controls.

- 100 T. Christensen, A. W. Jensen, M. Munch, S. Haahr, P. D. Sorensen, H. Riemann, H. J. Hansen and A. Moller-Larsen, *Characterization of retroviruses from patients with multiple sclerosis*. *Acta Neurol Scand Suppl*, 1997. **169**(p. 49-58. PubMed ID: 9174640.

Retroviruses were analyzed from B-lymphoblastoids from six MS subjects, a subject with progressive myelopathy and a healthy control. Retroviral particles appeared to be type-C-like; Pol, Gag and Env were present; and a relation was found between disease activity and reactivity towards retroviral peptides.

- 101 H. Perron, J. A. Garson, F. Bedin, F. Beseme, G. Paranhos-Baccala, F. Komurian-Pradel, F. Mallet, P. W. Tuke, C. Voisset, J. L. Blond, B. Lalande, J. M. Seigneurin and B. Mandrand, *Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis*. *The Collaborative Research Group on Multiple Sclerosis*. *Proc Natl Acad Sci U S A*, 1997. **94**(14): p. 7583-8. PubMed ID: 9207135.

Partial characterization of a new MS-associated retrovirus (previously called LM7) yielded several regions including a pol region from RNA present at the peak of RT activity. This sequence was detected in noncellular RNA from MS plasma and in CSF fluid from untreated MS subjects. MSR/V is related to but distinct from ERV9. It is not known whether MSR/V is an exogenous virus closely related to endogenous viruses, or a replication-competent, virion-producing endogenous provirus.

- 102 P. M. Alliel, J. P. Perin, R. Pierig, J. L. Nussbaum, A. Menard and F. Rieger, *Endogenous retroviruses and multiple sclerosis. II. HERV-7q*. *C R Acad Sci III*, 1998. **321**(10): p. 857-63. PubMed ID: 9835022.

The search for new endogenous retroviral sequences homologous with MSR/V located a full length endogenous retrovirus sequence on the long arm of chromosome 7 (HERV-7q).

- 103 P. M. Alliel, J. P. Perin, R. Pierig and F. Rieger, *An endogenous retrovirus with nucleic acid sequences similar to those of the multiple sclerosis associated*

*retrovirus at the human T-cell receptor alpha, delta gene locus.* Cell Mol Biol (Noisy-le-grand), 1998. **44**(6): p. 927-31. PubMed ID: 9763196.

Another endogenous retroviral sequence homologous with MSR/V was found on chromosome 14, near the gene coding for the TCR alpha and delta chains.

- 104 T. Christensen, P. Dissing Sorensen, H. Riemann, H. J. Hansen and A. Moller-Larsen, *Expression of sequence variants of endogenous retrovirus RGH in particle form in multiple sclerosis.* Lancet, 1998. **352**(9133): p. 1033. PubMed ID: 9759750.

Sequencing of RNA from retroviral particles from PBMCs of MS subjects found homology in the gag and env elements with the human endogenous retrovirus RGH-2 in four MS cell lines. RGH-like sequences are thought to be present in about 100 copies per haploid genome but RGH particles have not yet been reported. A search for RGH sequence expression in MS subjects vs. controls showed an association with MS, and the presence of these sequences in all MS subjects with active disease.

- 105 J. A. Garson, P. W. Tuke, P. Giraud, G. Paranhos-Baccala and H. Perron, *Detection of virion-associated MSR/V-RNA in serum of patients with multiple sclerosis.* Lancet, 1998. **351**(9095): p. 33. PubMed ID: 9433428.

MSR/V RNA was found in the serum of nine of 17 MS subjects but only three of 44 controls (eight subjects with non-neurological disorders and 36 healthy adults). In the MS group, the presence of RNA was not correlated with age, sex, disease duration or MS type, but was negatively associated with treatment.

- 106 J. L. Blond, F. Beseme, L. Duret, O. Bouton, F. Bedin, H. Perron, B. Mandrand and F. Mallet, *Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family.* J Virol, 1999. **73**(2): p. 1175-85. PubMed ID: 9882319.

Characterization of HERV-W (MSR/V) revealed several genomic elements, one containing a complete HERV-W unit which was not replication competent. Certain transcripts were found to be expressed in placenta.

- 107 T. Christensen, R. R. Tonjes, J. zur Megede, K. Boller and A. Moller-Larsen, *Reverse transcriptase activity and particle production in B lymphoblastoid cell lines established from lymphocytes of patients with multiple sclerosis.* AIDS Res Hum Retroviruses, 1999. **15**(3): p. 285-91. PubMed ID: 10052759.

Long-term culturing of PBMCs from MS subjects produced retrovirus-like particles as well as Epstein-Barr virus. Detection of reverse transcriptase activity confirmed the retroviral origin of the particles.

- 108 F. Komurian-Pradel, G. Paranhos-Baccala, F. Bedin, A. Ounanian-Paraz, M. Sodoeyer, C. Ott, A. Rajoharison, E. Garcia, F. Mallet, B. Mandrand and H. Perron, *Molecular cloning and characterization of MSR/V-related sequences associated with retrovirus-like particles.* Virology, 1999. **260**(1): p. 1-9. PubMed ID: 10405350.

Characterization of MSRV sequences (HERV-W) revealed that HERV-W RNA is copackaged in extracellular particles which might be produced by replication-competent or transcomplemented HERV-W copies or by an exogenous member of the HERV-W family.

- 109 T. Christensen, P. Dissing Sorensen, H. Riemann, H. J. Hansen, M. Munch, S. Haahr and A. Moller-Larsen, *Molecular characterization of HERV-H variants associated with multiple sclerosis*. Acta Neurol Scand, 2000. **101**(4): p. 229-38. PubMed ID: 10770518.

Retroviral particles produced by MS derived B-lymphoblastoid cell lines displayed sequence variants with high homology to the potentially functional RGH subgroup from the human endogenous retrovirus RTVL-H/HERV-H. These sequences were found in the particulate fraction of MS plasma samples but not in controls. A nucleic acid binding protein was also found that corresponded to the nucleocapsid protein (Gag NC) of other retroviruses. Indications were found of the retrovirus's transmissibility to PHA-stimulated lymphocytes.

- 110 T. Christensen, L. Pedersen, P. D. Sorensen and A. Moller-Larsen, *A transmissible human endogenous retrovirus*. AIDS Res Hum Retroviruses, 2002. **18**(12): p. 861-6. PubMed ID: 12201909.

The MS-associated human endogenous retrovirus HERV-H/RGH-2 was shown to be transmissible, although at a low level, by assays for reverse transcriptase activity and assays for rescue of the retroviral vector construct.

- 111 M. Zawada, I. Liwien, M. Pernak, D. Januszkiewicz-Lewandowska, K. Nowicka-Kujawska, J. Rembowska, K. Lewandowski, H. Hertmanowska, M. Wender and J. Nowak, *MSRV pol sequence copy number as a potential marker of multiple sclerosis*. Pol J Pharmacol., 2003. **55**(5): p. 869-75. PubMed ID: 14704480.

Cytogenetic analysis using the fluorescence in situ hybridization (FISH) technique was performed on the peripheral blood cells of 16 MS subjects and 10 healthy controls. MSR pol sequence copy number was found to be significantly higher in the MS subjects. Pol sequences were found as tandem repeats on a various chromosomes.

- 112 A. Dolei, C. Serra, G. Mameli, M. Pugliatti, G. Sechi, M. C. Cirotto, G. Rosati and S. Sotgiu, *Multiple sclerosis-associated retrovirus (MSRV) in Sardinian MS patients*. Neurology., 2002. **58**(3): p. 471-3. PubMed ID: 11839854.

MSRV was detected in the CSF 50% of MS subjects at clinical onset and 40% of neurological control subjects. It was also detected in blood samples from all MS subjects, nearly all neurological controls, and very few healthy controls.

- 113 J. M. Antony, G. van Marle, W. Opii, D. A. Butterfield, F. Mallet, V. W. Yong, J. L. Wallace, R. M. Deacon, K. Warren and C. Power, *Human endogenous retrovirus glycoprotein-mediated induction of redox reactants causes oligodendrocyte death and demyelination*. Nat Neurosci., 2004. **7**(10): p. 1088-95. Epub 2004 Sep 26. PubMed ID: 15452578.

The HERV-W encoded glycoprotein syncytin was found to be upregulated in glial cells in acute lesions of MS subjects. Expression of this protein in astrocytes induced the release of redox reactants which were shown to be cytotoxic to oligodendrocytes.

- 114 H. Perron, E. Jouvin-Marche, M. Michel, A. Ounanian-Paraz, S. Camelo, A. Dumon, C. Jolivet-Reynaud, F. Marcel, Y. Souillet, E. Borel, L. Gebuhrer, L. Santoro, S. Marcel, J. M. Seigneurin, P. N. Marche and M. Lafon, *Multiple sclerosis retrovirus particles and recombinant envelope trigger an abnormal immune response in vitro, by inducing polyclonal Vbeta16 T-lymphocyte activation*. *Virology.*, 2001. **287**(2): p. 321-32. PubMed ID: 11531410.

An MSR/V envelope protein was shown to induce a superantigen-like polyclonal T-cell response in human lymphocytes with a bias in Vbeta16 chain usage.

- 115 A. Rolland, E. Jouvin-Marche, M. Saresella, P. Ferrante, R. Cavaretta, A. Creange, P. Marche and H. Perron, *Correlation between disease severity and in vitro cytokine production mediated by MSR/V (multiple sclerosis associated retroviral element) envelope protein in patients with multiple sclerosis*. *J Neuroimmunol.*, 2005. **160**(1-2): p. 195-203. Epub 2004 Dec 8. PubMed ID: 15710473.

PBMCs from MS subjects had a stronger cytokine response to the surface unit of MSR/V envelope protein (ENV-SU) than did PBMCs from healthy controls. This response correlated with EDSS scores.

- 116 E. M. Gipps and C. Kidson, *Cellular radiosensitivity: expression of an MS susceptibility gene?* *Neurology*, 1984. **34**(6): p. 808-11. PubMed ID: 6539446. Cellular radiosensitivity of T lymphocytes, B lymphoblastoid cell lines and fibroblasts was increased in 40 MS subjects compared to controls as measured by radiation-induced chromosome aberrations. The authors suggest that this radiosensitivity may have a basis in autosomal dominant inheritance (judging by patterns among first-degree relatives of MS subjects) and may be due to mutations of DNA-processing that predispose to MS.

- 117 R. Seshadri, G. R. Sutherland, E. Baker, R. Kutlaca, D. Wigmore and A. A. Morley, *SCE, X-radiation sensitivity and mutation rate in multiple sclerosis*. *Mutat Res*, 1983. **110**(1): p. 141-6. PubMed ID: 6865993.

SCE was increased in the lymphocytes of 34 MS subjects compared to controls. However, no increase was seen in X-radiation sensitivity or in 6-thioguanine-resistant mutant cells.

- 118 P. Sola, E. Merelli, P. Faglioni, D. Monti, A. Cossarizza and C. Franceschi, *DNA repair, sensitivity to gamma radiation and to heat shock in lymphocytes from acute, untreated multiple sclerosis patients*. *J Neuroimmunol*, 1989. **21**(1): p. 23-9. PubMed ID: 2908880.

No significant difference in DNA repair and survival was found between 15 MS subjects and 15 controls after exposure of PBLs to high and low gamma ray doses (2-12 Gy and 100 Gy) and high temperatures (37-45 degrees C). The authors state that genomic

instability may be of viral origin and not due to genetic defect in repair of DNA in MS subjects.

- 119 U. Flodin, B. Soderfeldt, H. Noorlind-Brage, M. Fredriksson and O. Axelson, *Multiple sclerosis, solvents, and pets. A case-referent study.* Arch Neurol., 1988. **45**(6): p. 620-3. PubMed ID: 3369968.

A case-control study involving 83 MS subjects and 467 randomized controls found an excess for MS for X-ray film examinations, but these may result from having the disease and being evaluated for its symptoms.

- 120 A. M. Landtblom, U. Flodin, M. Karlsson, S. Palhagen, O. Axelson and B. Soderfeldt, *Multiple sclerosis and exposure to solvents, ionizing radiation and animals.* Scand J Work Environ Health, 1993. **19**(6): p. 399-404. PubMed ID: 8153592.

In a Swedish study of 91 cases and 348 controls concerning environmental exposures and MS, the men with MS were found to have elevated exposures to solvents, occupational contact with dogs or cats and leisure-time contact with caged birds. Risk indicators for women included X-ray treatment and previous diseases. Risk factors for men and women combined included solvent exposure, radiological work, and previous diseases.

- 121 O. Axelson, A. M. Landtblom and U. Flodin, *Multiple sclerosis and ionizing radiation.* Neuroepidemiology, 2001. **20**(3): p. 175-8. PubMed ID: 11490163.

Comparison of 174 MS cases with 815 population controls revealed an odds ratio of 4.4 for radiological work and of 1.8 for X-ray examinations.

- 122 C. Johansen, N. Koch-Henriksen, S. Rasmussen and J. H. Olsen, *Multiple sclerosis among utility workers.* Neurology, 1999. **52**(6): p. 1279-82. PubMed ID: 10214760.

32 cases of MS developed in 31,990 employees of Danish utility companies tracked between 1900 and 1993, as compared to 23.7 expected based on national incidence rates. The authors felt that this evidence provided no support for an association between the risk of MS and occupational exposure to EM radiation.

- 123 M. Gronning, G. Albrektsen, G. Kvale, B. Moen, J. A. Aarli and H. Nyland, *Organic solvents and multiple sclerosis: a case-control study.* Acta Neurol Scand, 1993. **88**(4): p. 247-50. PubMed ID: 8256566.

No association was found between MS and exposure to organic solvents, alone or in combination with welding or other chemicals, in a study of 155 MS subjects and 200 controls in Norway.

- 124 J. T. Mortensen, H. Bronnum-Hansen and K. Rasmussen, *Multiple sclerosis and organic solvents.* Epidemiology, 1998. **9**(2): p. 168-171. PubMed ID: 9504285.

No association was found between the risk of MS and exposure to organic solvents in a study of over 200,000 solvent-exposed and -unexposed men in Denmark.

- 125 A. M. Landtblom, U. Flodin, B. Soderfeldt, C. Wolfson and O. Axelson, *Organic solvents and multiple sclerosis: a synthesis of the current evidence*. *Epidemiology*, 1996. **7**(4): p. 429-33. PubMed ID: 8793371.  
Thirteen studies were identified that included information on solvent exposure with respect to multiple sclerosis. Ten of these indicated that solvent exposure increased the risk of MS. Pooling the data from the available studies produced relative risk point estimates ranging from 1.7 to 2.6.
- 126 T. Riise, M. W. Nortvedt and A. Ascherio, *Smoking is a risk factor for multiple sclerosis*. *Neurology*, 2003. **61**(8): p. 1122-4. PubMed ID: 14581676.  
The risk of MS in individuals in a Norwegian community was found to be significantly higher in smokers than in people who had never smoked.
- 127 P. Ghadirian, B. Dadgostar, R. Azani and P. Maisonneuve, *A case-control study of the association between socio-demographic, lifestyle and medical history factors and multiple sclerosis*. *Can J Public Health*, 2001. **92**(4): p. 281-5. PubMed ID: 11965642.  
A case-control study of 200 MS cases and 202 matched controls revealed a significant association between risk of MS and cigarette smoking, as well as exposure to cats or birds, past history of trauma and eye problems, and family history of eye problems, mumps, measles, rubella, cancer and autoimmune diseases.
- 128 M. A. Hernan, M. J. Oleky and A. Ascherio, *Cigarette smoking and incidence of multiple sclerosis*. *Am J Epidemiol*, 2001. **154**(1): p. 69-74. PubMed ID: 11427406.  
Analysis of data from the Nurses' Health Study and Nurses' Health Study II revealed a relative incidence rate of MS of 1.6 for current smokers and 1.2 for past smokers, with risk of MS increasing with cumulative exposure to smoking.
- 129 M. A. Hernan, S. S. Jick, G. Logroscino, M. J. Olek, A. Ascherio and H. Jick, *Cigarette smoking and the progression of multiple sclerosis*. *Brain*, 2005. **128**(Pt 6): p. 1461-5. Epub 2005 Mar 9. PubMed ID: 15758034.  
A case control study based on the UK's General Practice Research Database revealed that cigarette smoking was associated with an increased risk of developing MS, as well as an increased risk for conversion to a secondary progressive course.
- 130 I. A. van der Mei, A. L. Ponsonby, L. Blizzard and T. Dwyer, *Regional variation in multiple sclerosis prevalence in Australia and its association with ambient ultraviolet radiation*. *Neuroepidemiology*, 2001. **20**(3): p. 168-74. PubMed ID: 11490162.  
A negative correlation was found between MS prevalence and UV radiation levels for six regions in Australia, consistent with the hypothesis that UV radiation exposure may reduce the risk of MS.

- 131 I. A. van der Mei, A. L. Ponsonby, T. Dwyer, L. Blizzard, R. Simmons, B. V. Taylor, H. Butzkueven and T. Kilpatrick, *Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study*. *Bmj.*, 2003. **327**(7410): p. 316. PubMed ID: 12907484.

A case-control study performed in Tasmania found negative associations with MS for both higher sun exposure between ages 6 and 15 and greater actinic damage of the skin.

- 132 A. D. Sadovnick, G. C. Ebers, D. A. Dyment and N. J. Risch, *Evidence for genetic basis of multiple sclerosis. The Canadian Collaborative Study Group*. *Lancet*, 1996. **347**(9017): p. 1728-30. PubMed ID: 8656905.

Imprinting is not likely to affect risk based on an observed lack of maternal influence on risk (maternal half-sibs and paternal half-sibs experienced similar risk).

- 133 J. Chataway, S. Sawcer, F. Corradu, R. Feakes, S. Broadley, H. B. Jones, D. Clayton, J. Gray, P. N. Goodfellow and A. Compston, *Evidence that allelic variants of the spinocerebellar ataxia type 2 gene influence susceptibility to multiple sclerosis*. *Neurogenetics*, 1999. **2**(2): p. 91-6. PubMed ID: 10369884.

No expansion of SCA gene CAG trinucleotide repeats was seen in 226 MS sib pair families, although excess transmission of the 22 repeat length allele of the SCA2 gene was detected in MS subjects.

- 134 M. Santos, M. do Carmo Costa, M. Edite Rio, M. Jose Sa, M. Monteiro, A. Valenca, A. Sa, J. Dinis, J. Figueiredo, L. Bigotte de Almeida, A. Valongueiro, I. Coelho, M. T. Matama, J. Pinto-Basto, J. Sequeiros and P. Maciel, *Genotypes at the APOE and SCA2 loci do not predict the course of multiple sclerosis in patients of Portuguese origin*. *Mult Scler.*, 2004. **10**(2): p. 153-7. PubMed ID: 15124760.

No significant differences were found for the SCA2 alleles between Portuguese MS subjects and healthy controls.

- 135 Y. Dai, C. Xu, M. Holmberg, A. Oturai, S. Fredrikson, M. Sandberg-Wollheim, M. Laaksonen, A. Spurkland, K. M. Myhr, L. P. Ryder, P. S. Sorensen, A. Svejgaard and J. Hillert, *Linkage analysis suggests a region of importance for multiple sclerosis in 3p14-13*. *Genes Immun.*, 2001. **2**(8): p. 451-4. PubMed ID: 11781712.

No expansions of the SCA7 gene were detected in the index cases from Swedish multiple families.

- 136 B. Mitterski, J. T. Eppelen, D. Poehlau, E. Sindern and M. Haupt, *SCA2 alleles are not general predisposition factors for multiple sclerosis*. *Neurogenetics.*, 2000. **2**(4): p. 235-6. PubMed ID: 10983720.

SCA2 alleles did not seem to differ significantly between MS cases and controls.

- 137 C. J. Willer, A. D. Sadovnick and G. C. Ebers, *Microchimerism in autoimmunity and transplantation: potential relevance to multiple sclerosis*. J Neuroimmunol, 2002. **126**(1-2): p. 126-33. PubMed ID: 12020964.

Microchimerism, which has been found to be increased in women with scleroderma and Hashimoto's thyroiditis, is suggested as a risk factor for MS.

- 138 O. Basso, R. Campi, M. Frydenberg, N. Koch-Henriksen, H. Bronnum-Hansen and J. Olsen, *Multiple sclerosis in women having children by multiple partners. A population-based study in Denmark*. Mult Scler., 2004. **10**(6): p. 621-5. PubMed ID: 15584485.

A Danish study of women with MS found that women having children with different men were not at higher risk for developing MS, indicating that microchimerism may not be a major risk factor for MS.

- 139 C. J. Willer, B. M. Herrera, K. M. Morrison, A. D. Sadovnick and G. C. Ebers, *Association between microchimerism and multiple sclerosis in Canadian twins*. J Neuroimmunol, 2006. **13**(p. 13. PubMed ID: 16843535.

A study of female twin pairs concordant or discordant for MS showed that the rate of microchimerism was significantly higher in affected versus unaffected co-twins.

- 140 S. M. Montgomery, M. Lambe, T. Olsson and A. Ekbom, *Parental age, family size, and risk of multiple sclerosis*. Epidemiology., 2004. **15**(6): p. 717-23. PubMed ID: 15475721.

An association between risk of MS and paternal age was found in an analysis of Swedish inpatient registry data; this may be due to the accumulation of gamete mutations in older men.

- 141 E. Cocco, C. Sardu, M. Lai, G. Spinicci, P. Contu and M. G. Marrosu, *Anticipation of age at onset in multiple sclerosis: a Sardinian cohort study*. Neurology., 2004. **62**(10): p. 1794-8. PubMed ID: 15159480.

Age at onset in an MS cohort from Sardinia appeared to decrease with younger generations; however, this is likely to be due to environmental, not genetic, factors.

- 142 M. G. Andreassi, *Coronary atherosclerosis and somatic mutations: an overview of the contributive factors for oxidative DNA damage*. Mutat Res, 2003. **543**(1): p. 67-86. PubMed ID: 12510018.

Evidence is presented for possible contributions of somatically acquired DNA mutations to the pathogenesis of coronary artery disease, including the examination of environmental risk factors and the potential involvement of oxidative damage to mitochondrial DNA.

### **Terms searched in conjunction with “multiple sclerosis”:**

mutagen

mosaic/ism  
imprinting  
triplet  
repeat/expansion  
somatic mutation  
somatic  
rearrangement  
retrotransposon/s  
mutation/s  
sporadic plus genetic or mutation/s  
spontaneous plus genetic or mutation/s  
retrovirus  
DNA integrate/d/ion  
LINE-1, L1, LTR  
gametogenesis  
germline  
radiation/radiation exposure  
chemical/s exposure  
carcinogen/s/ic  
cell division