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A molecular view of multiple sclerosis and experimental autoimmune encephalitis: What can we learn from the epitope data?

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Abstract

An analysis to inventory all immune epitope data related to multiple sclerosis (MS) was performed using the Immune Epitope Database (IEDB). The analysis revealed that MS related data represent >20% of all autoimmune data, and that studies of EAE predominate; only 22% of the references describe human data. To date, >5800 unique peptides, analogs, mimotopes, and/or non-protein epitopes have been reported from 861 references, including data describing myelin-containing, as well as non-myelin antigens. This work provides a reference point for the scientific community of the universe of available data for MS-related adaptive immunity in the context of EAE and human disease.

Keywords

Multiple sclerosis; Epitope; Antibody; T cell; Experimental autoimmune encephalomyelitis (EAE)

1. Introduction

Multiple sclerosis (MS) is a progressive, debilitating neurological disorder characterized by central nervous system injury. The disease is largely regarded as autoimmune, primarily associated with genetic predisposition, though environmental factors have also been implicated (Lassman, 1983; Martin et al., 1992; Hafler et al., 2005; Sospedra & Martin, 2005). The main pathological features of MS are inflammatory infiltrates, destruction of the myelin sheath and oligodendrocytes, axonal and neuronal damage as well as glial proliferation. Adaptive and immune mechanisms are at the core of disease pathogenesis. While much of the downstream immunopathology has been characterized, the initiating

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event for the development of MS has yet to be elucidated. Current treatment options for MS are based on immunomodulation/suppression, and include type I interferons, a peptidic mixture of 4 amino acids, glatiramer-acetate, a sphingosine 1 phosphate receptor agonist, fingolimod, fumaric acid, teriflunomide and monoclonal antibodies against VLA-4 and CD52 (Wagner, 2012). Antigen-specific strategies have also been proposed and tested, and while this field has seen various setbacks (Hohlfeld & Wekerle, 2004), promising data have recently been published for at least two approaches, including the administration of seven myelin peptides coupled to autologous PBMCs and skin patch application using a mixture of three myelin peptides (Lutterotti et al., 2013; Walczak et al., 2013).

A great deal of what is now understood about MS was gained through studies using experimental autoimmune encephalomyelitis (EAE). EAE is the most common animal model used to simulate the human demyelination disease, MS, as it closely parallels key features of human disease, including inflammation, demyelination and gliosis (Rivers et al., 1933; Rao & Segal, 2012). EAE models have been used extensively as surrogates for characterizing MS immunopathology and to test candidate neuroprotective and reparative strategies. These models include primarily mice and rats, but also include rabbits and non-human primates (Constantinescu et al., 2011).

Despite its many merits, EAE differs from MS in several notable aspects. In most cases, EAE is experimentally induced (as the acronym implies) using antigens (peptides and proteins) along with bacterially-derived adjuvants; spontaneous disease is achieved using transgenic mice models (Madsen et al., 1999; Quandt et al., 2012). Moreover, while both humoral (B cells, plasma cells and antibody) and cellular ($CD4^+$ and $CD8^+$) mechanisms have been shown to contribute to MS immunopathology (Lassman et al., 2007; Simmons et al., 2013 May 21), EAE represents the prototype T cell-mediated autoimmune model, whereby $CD4^+$ T cells are the predominant effector cell in the disease, which is largely driven by a pro-inflammatory Th_1/Th_{17} cytokine milieu (Kroenke et al., 2008). Therefore, while many EAE models involve well-characterized B-cell responses, relatively fewer relevant B cell epitopes have been defined.

At the antigenic level, MS and EAE share many of the same targets, including myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG) and proteolipid protein (PLP) (Sospedra & Martin, 2005). While it is known that numerous other self-antigens are involved in MS, either as initiators or bystanders, it is the myelin-containing antigens that have been most frequently associated with the autoreactivity. Furthermore, there is a remarkable overlap, at least for MBP, with respect to immunodominant regions in the context of MS-associated HLA-class II alleles and those peptides that are able to elicit EAE in various susceptible rodent strains and species and in Rhesus monkeys (Sospedra & Martin, 2005). These same antigens serve as the immunogens in EAE induction (Rao & Segal, 2012) and therefore represent ideal targets for analysis and comparison of immune reactivity patterns between EAE and MS. As it is possible that the nature of immunological differences between these two models may be manifest at the molecular level, we propose that an analysis of all “MS-specific” and EAE-specific immune epitope data may reveal valuable insights similar to the above mentioned data on MBP. The dissection of immune reactivity at this level allows for direct comparative analysis of MS and EAE

immunobiology using analogous self-antigens. Herein we provide a comprehensive analysis of all MS- and EAE-related epitope data and explore the relationship between MS and EAE as it relates to several key issues in MS immunobiology.

The goal of this analysis is to inventory all epitope data related to MS, as curated in the Immune Epitope Database (IEDB), to provide a reference point for the scientific community of the universe of available data, extract whenever possible general features of the data, and at the same time highlight areas for further research.

2. Materials and methods

2.1. IEDB queries

All queries were performed using the Immune Epitope Database and Analysis Resource (IEDB) home page search interface [www.iedb.org]. For more complex queries the advanced search interface (all fields) was utilized. Search criteria are provided in the figure legend or table notes. Results were downloaded in Excel format for detailed analysis, unless otherwise indicated. MS or EAE-specific queries were conducted using the disease finder, which selects only those data representing data generated in hosts with clinical disease. As such, these queries do not retrieve all data *associated* with disease, and therefore should be considered a subset of the entire MS-associated data captured by the IEDB, which considers clinical MS and EAE together. Antigen-specific queries include all data, regardless of the clinical state of a host. Unless otherwise indicated, antigen-specific queries were performed irrespective of disease status. Also, unless otherwise indicated, all reported data herein represent positive epitopes and/or assays only.

2.2. IEDB inclusion criteria

Our analysis includes all available data for antibody and T cell epitopes associated with MS (defined by clinical status of host and/or antigen association) in human and nonhuman (animal models) hosts. To identify MS-related data, we followed the process described by Davies et al. (2009). The data are derived from the peer-reviewed literature (PubMed), as well as data directly submitted to the IEDB. Epitope definitions (length and mass restrictions) and IEDB inclusion criteria can be found at http://tools.immuneepitope.org/wiki/index.php/Main_Page. For the purpose of this report, epitopes represent the unique molecular structures (minimal sequences, linear and discontinuous regions, as well as key residues) experimentally shown to react with a B cell or T cell receptor (no predictions). Peptidic as well as non-peptidic (*e.g.* lipids and carbohydrates) determinants are included in the IEDB.

2.3. Immunome browser

The Immunome Browser is a unique feature of the IEDB that allows the user to map the result of any query (in terms of epitopes) onto a reference genome or antigen. It does this by plotting the response frequency score (number respondents/number tested) of each epitope, by residue, along the entire length of the target protein. In this way, it is possible to visualize those regions on the antigen(s) that are more immunodominant or more frequently studied in a given population for a particular response (Ab, T, CD4, CD8, *etc.*). This provides the least

biased way to analyze the cumulative data and allows for comparisons between hosts, disease states, as well as assay type (exacerbation *versus* tolerance), as examples. The reference human antigens used to compare response patterns between MS and EAE were the following: MBP [GI: 17378805], MOG [GI: 23270927] and PLP [GI: 41393531]. In order to accommodate all defined epitopes onto a reference antigen, full-length proteins are used. For this reason, residue numbering may be different than that of certain well-established protein isoforms.

3. Results and Discussion

3.1. MS-related epitope data in the broader context

To put the MS-related epitope data into the broader context of all immune epitope data within the IEDB we first determined the relative proportion of autoimmune-specific data among all disease categories. The IEDB's categorization of all references containing epitope data from PubMed is done by disease association as previously described (Davies et al., 2009). Briefly, this categorization uses as a basis for disease association, the host's clinical status (including animal models that mimic human symptoms) and/or the epitope-derived antigens associated with disease(s). Fig. 1 shows that autoimmune (AI) diseases represent close to 30% of all epitope data housed within the IEDB, second only to infectious disease. Specific examination of the sub-categories within AI, reveals that references describing MS represent >20% of the total (Fig. 1b), making it the largest AI disease sub-category. Within this sub-category, studies of EAE predominate, with only 22% of references describing human data.

To date, there are more than 5800 unique molecular structures (peptides, analogs, mimotopes, non-peptidic molecules) reported as associated with MS (this includes all EAE studies as well) in 861 references, of which ~2400 have been found to be positive in the context of either B cell or T cell (or both) reactivity [data not shown]. Thus, MS is well covered at the molecular level by comparison to other AI disease categories. Because not all MS-related data are generated in the context of clinical disease (either MS or EAE), a secondary analysis was performed to specifically identify immune reactivity in the context of disease. Here we observed that when we filtered those data that specify MS or EAE as the disease state, there are 637 peer-reviewed papers describing a total of 1374 positive antibody/B cell and T cell epitopes, including those defined in MHC binding and/or from MHC ligand elution assays.

3.2. Analysis of the antigen composition of MS-associated epitope data for myelin-containing antigens

The main feature of MS immunopathology is antibody and T cell reactivity against self-antigens containing myelin, the chief component of white matter within the central nervous system (CNS). MS is classified into four phenotypes (I–IV); all involve T cells and macrophages, however, type II is specifically related to antibodies and complement (Lucchinetti et al., 2000). Numerous antigens derived from myelin proteins have been identified to date, and include myelin basic protein (MBP), proteolipid protein (PLP), myelin oligodendrocyte glycoprotein (MOG), myelin-associated glycoprotein (MAG),

myelin-associated oligodendrocyte basic protein (MOBP), claudin-11 (OSP), peripheral myelin protein (PMP), myelin protein P0 (MPP) and oligodendrocyte-myelin glycoprotein (OMG) (Lucchinetti et al., 2000; Hafler et al., 2005; Wucherpfennig & Sethi, 2011). To examine the full breadth of myelin-containing antigens, all MS-associated (includes MS and EAE) references captured within the IEDB were considered. For this, a list of unique antigens was generated and then organized by groups. These groups included peptidic (myelin- and non-myelin-derived) and non-peptidic (lipid and carbohydrate; see below). Table 1 provides a breakdown of all myelin-derived antigens for which epitope data have been described to date in the published literature. Of the nine myelin antigens reported to date, MBP, PLP and MOG represent 90% and 85% of assays and epitopes, within this category respectively. That these antigens are well-represented is not surprising, however, it does highlight the need for broader exploration of the involvement of additional autoantigens in the human disease as it is unlikely that these are the sole targets either as initiators or bystanders in MS immunopathology.

3.3. Analysis of non-myelin containing antigens

In addition, our analysis revealed a number of non-myelin-derived self-antigens for which epitope data have been described in EAE and patients. These include other antigens from cells within the CNS that can be broken down into three compartmental categories, those found within the nucleus, those from the cytoplasm and those that are part of the cell membrane. Table 1 lists all of these self-antigens by category and includes only those for which there were at least 10 positive assays. Again, these data represent what has been reported in the published literature to date. Here, “assay” represents individual experiments used to defined and characterize a given epitope as reported by the authors (positive outcomes only). Additional information, such as the number of different independent publications, details for the different assay methodologies used, as well as any reported negative data can also be extracted using the IEDB query interface. There are many other mammalian-derived proteins that have also been identified in the context of MS (or EAE), however, these have been identified in only 1–2 assays and therefore are considered less relevant unless further supporting evidence is generated. Those non-myelin-derived antigens with the highest number of assays/epitopes include T cell receptor (TCR), centromere-associated proteins, DNA topoisomerase, myeloperoxidase and Ro ribonucleoproteins (RNPS). Whether or not these self-antigens play a direct or by-stander role in MS/EAE pathogenesis is not fully known. A more thorough characterization of epitope reactivity of non-myelin containing self-antigens will likely be necessary for a more detailed understanding of this aspect of MS immunopathology, especially with regard to neo-determinants that arise in later disease wherein epitope spreading becomes more prominent [discussed more below]. For example, while the recently identified ATP-sensitive inward rectifying potassium channel, KIR4.1 has been of notable interest, few data have been reported to date. Indeed, only 2 antibody/B cell epitopes (defined in 3 assays) have been reported.

3.4. Non-peptidic determinants in multiple sclerosis

Next we examined the MS-associated epitope data for non-protein (NP) antigen reactivity. In addition to protein-derived determinants, the IEDB was developed to capture non-protein

structures, which include carbohydrates, lipids, drugs and other chemical entities encountered by the immune system. Myelin is primarily composed of lipids (70–80%) (Rinaldi et al., 2010), making the non-protein component of this antigen highly significant. More specifically, myelin glycolipids are the most abundant component of myelin (Shamshiev et al., 1999) and within the bilayers surrounding each nerve axon there contains components of several classes of glycolipids, including sphingolipids, glycerophospholipids, galactosyl and sterols (*e.g.* cholesterol). Of these, sphingolipids constitute the major contributor of transmembrane signaling (Merrill & Scolding, 1999). And indeed, the presence of anti-lipid antibodies, particularly anti-sphingolipid antibodies, has been established in MS patients (Kanter et al., 2006; Walter & Fassbender, 2010; Quintana et al., 2012). We therefore sought to parse all non-peptidic data related to MS to identify the relative proportion of all chemical types, in addition to glycolipids.

Fig. 2 shows the relative distribution of NP determinants (36 total unique structures) for each class of compound. Here, we see that indeed sphingolipids represent the majority of the NP determinants studied (44%), followed by glycerophospholipids (22%). Hydroxysteroids (11%) and carbohydrates (6%) were also reported, but to a lesser extent. The remaining 17% represent a disparate group of compounds with no unifying chemical component. Table 2 provides a breakdown of each group by total references and number of assays in the context of MS or EAE to show the extent to which each group has been studied to date. Of the sphingolipids, the most commonly reported determinants were gangliosides. Gangliosides have also been demonstrated to be a target of IgM auto-antibodies in MS (Marconi et al., 2006). Interestingly, while MS lipid-specific reactivity was reported (Quintana et al., 2012), EAE has not been shown to be induced using solely lipid entities (Cohen, 1981; Martin et al., 1992). However, specific treatment of mice with gangliosides has been shown to attenuate EAE (Monteiro de Castro et al., 2004). This marks another important molecular-based difference between EAE and MS and suggests the need for further exploration of the role of these non-protein moieties in patients.

3.5. Analogs and altered peptide ligands

Synthetic analogs as well as structural mimics (mimotopes) have been of interest for use in immune modulation and more specifically clinical application in immunotherapy in numerous AI diseases (Hafler et al., 2005). We therefore next sought to identify these structures, first in relation to all major antigens and, secondly in their functional context (encephalitogenic or tolerogenic). In the first analysis we performed a query using the advanced search interface to enumerate the number analogs and mimotopes by antigen. Table 3a shows the result of this query. To date, the vast majority of analogs/mimotopes have been defined for MBP (243), followed by PLP (65) and MOG (23). In most instances, these structures have been tested in the context of cellular response; however, antibody reactivity has also been reported. More than 300 MBP-related analogs have also been tested for MHC binding, representing a potential pool of synthetic structures associated with HLA-restriction.

Next, we refined our query to include those assays associated with disease alleviation (tolerance, protection, *etc.*), as well as those associated with disease exacerbation. This

includes all hosts. Table 3b list the results of this query and show that these structures include analogs of well-known epitopes, such as PLP 139–150, MBP 1–11 and MOG 35–55. Also of interest is the phenomenon of structure overlap in either context wherein the same structure can induce tolerance or exacerbate disease depending upon whether it is administered before or after disease challenge. While this provides a repeatable method to study the induction of tolerance in EAE models, the application of this has not been straightforward in addressing treatment of human disease. However, disease-exacerbating peptide variants have even been identified in humans in a clinical trial with an altered peptide ligand (Bielekova et al., 2000) arguing that a systematic understanding of peptide modifications and their disease-exacerbating or -ameliorating potential will be very useful.

3.6. Epitope-specific T cell reactivity in MS and EAE

CD4⁺ T cells have been shown to play a prominent effector role in both EAE and MS (Noseworthy et al., 2000; Markovic-Plese & McFarland, 2001). Moreover, while the role of CD8⁺ T cells has yet to be fully characterized, these cells have also been identified in the CNS lesions, suggesting their possible involvement in pathogenesis in human disease (Hayashi et al., 1988; Sobel, 1989; Babbe et al., 2000; Skulina et al., 2004).

Table 4 provides a summary of the T cell epitope data for both MS and EAE. There are currently 801 peptidic and non-peptidic T cell epitopes identified in the context of MS and EAE. A comparison of MS and EAE again shows that a greater number of epitopes have been reported in the context of human disease (468) than for EAE (383). Interestingly, nearly twice the number of assays is reported for EAE than for MS, perhaps reflecting that fact that while EAE is used extensively to study disease, a narrower repertoire of peptides are used. Looking at these data in terms of host shows that 468 T cell epitopes have been defined in humans, and for EAE the majority of T cell epitopes were reported in mice (222), followed by rats (140), NHP (Lassman, 1983), rabbits (Bronstein et al., 1999) and guinea pigs (Ascherio & Munger, 2010) [data not shown].

To investigate the relationship between T cell response patterns in humans with MS and those reported for models of EAE we used the Immunome Browser feature of the IEDB. The Immunome Browser (IB) tool allows the results of any query to be mapped onto a reference genome or antigen in order to visualize T or B cell response patterns. Each map represents the response frequency scores (respondents/number tested) [RFscore] plotted at each residue within the epitope along the length of the antigen. Plotting RFscores thereby provides a map of immunodominant or well-studied regions, as well as unreactive or untested regions. It is important to note that in order to accommodate all defined epitopes onto a reference antigen, full-length proteins are used. For this reason, residue numbering may be different than that of certain well-established protein isoforms.

Fig. 3 shows T cell response frequency scores mapped to a reference human MBP, MOG and PLP. A direct comparison of T cell responses against MBP shows fairly complete epitope mapping along the length of this antigen (here, aa135–304) for both MS (Fig. 3A) and EAE (Fig. 3B). However, there are some intriguing contrasts. First, a peak at residues ~aa135–145 (RFscores 0.55–0.65) corresponding to the well-known epitope Ac 1–11 (Rao & Segal, 2012) on the EAE map, is not reflected on the MS map. Conversely, a peak

between aa150–160 on the MS map is less pronounced for EAE. On the EAE plot, we also observe a large reactive region between aa170–240 with RFscores ranging from 0.50 to 0.70 corresponding to the well-known epitopes aa35–47 and aa84–104 (Rao & Segal, 2012). However only a small portion of this, aa220–240, is highly reactive in MS.

T cell response patterns against MOG are notably different (Fig. 3C, D). The MS plot reflects a distinct peak at the N-terminus (RF ~0.40–0.50) which is not present for EAE. Conversely, the two high peaks for EAE, representing the well-known epitopes 35–55 and 92–106 are not present in MS. While these regions were positive in MS, none achieved RF greater than 0.20. Furthermore, while data exist within the C-terminal region (aa150–250) for MS, data in the region are largely lacking in EAE.

Finally, the PLP response map for EAE narrowly reflects reactivity for five well-known epitopes used to induce disease: aa43–64, aa103–116, aa139–151, aa185–206 and aa215–232. This is in sharp contrast to the overall coverage for MS where we see complete coverage along the length of the antigen. These data show that at the residue level, ~57% of the PLP sequence reactivity is therefore unexamined or unreported in the context of EAE. While the MS-specific reactivity against PLP corresponds to some degree with the EAE data, there are several regions in MS that differ; namely, peaks at aa40–60, aa85–90 and aa240–260, which are not present in EAE. In addition, the character and magnitude of reactivity at the well-known EAE epitopes aa139–151 and aa185–206 differ substantially on the MS plot.

3.7. Differences between human and murine T cell reactivity

The results above reveal profound differences in response patterns between MS and EAE for both B cell and T cell data. While differences were expected, the amount of variance seems to support the widely acknowledged issue of translatability of EAE findings to MS immunobiology. To what extent the observed differences in overall response patterns are attributable to immunological and genetic differences, or experimental bias, cannot be directly answered in this analysis. It is likely that differences in the magnitude of observed responses (*i.e.* higher RFscores) between EAE and MS are a reflection of the inbred nature of the EAE hosts. It is also possible that the reactivity gaps observed in EAE may reflect that negative data often go unpublished, and therefore it is not known if these regions are untested or truly non-reactive (negative). Certainly the use of a narrow repertoire of specific peptides to induce EAE biases the data towards these regions. However, when the mapping reveals little or no overlap between these peptides in the human condition, it likely reflects legitimate immunological differences.

Important factors influencing these differences are immunodominance and MHC binding affinity. In some cases (Valli et al., 1993), T cell reactivity has been shown to correlate with high MHC binding affinity. However, other studies have shown that the main targets of the high avidity T cell response in MS (Bielekova et al., 2004) are epitopes that either bind poorly to the HLA alleles (*e.g.* MBP 111–129), shown (Valli et al., 1993) to be immunodominant, or bind poorly to HLA, but associated with very high affinity to the TCR of the complex (Muraro et al., 1997; Yin et al., 2011). Other peptides might not be expressed in the thymus. The prototypic example for the latter is PLP 139–154, which according to

Klein and Kyewski (Klein et al., 2000) is not expressed in the thymus. The same is true for MOG (Bruno et al., 2002). Thus, analyzing binding versus the disease-associated HLA-class II molecules provides valuable information, in the context of an autoantigen. Those epitopes that bind best may be more prone to assure stringent tolerance, while those with poorer binding are more prone to escape tolerance and therefore be immunodominant. Therefore, comparing the T cell data and the binding data has been viewed with consideration of this point. Furthermore, also in the context of HLA binding and immunodominance, since the association with the HLA-DR15 haplotypes and the two alleles in that haplotype is so strong, it is valuable to focus binding analysis to these alleles, also in comparison to others which are negatively associated with disease.

3.8. Immune modulation in MS and EAE: cytokines from helper/regulatory T cells

We first sought to compile all cytokine data reported and captured to date in the context of MS and EAE. Table 5 provides a list of all cytokine records related to these disease states and shows the total number of associated assays and epitopes. Here we see that in MS, IFN- γ , IL-4, TNF α/β , IL-2, IL-5 and IL-10 have been studied most extensively. For EAE, there has been a much broader investigation of cytokine activity. Here we observe that IFN- γ , IL-4, TNF α/β , IL-2, IL-5 and IL-10 are well studied; however IL-17, TGF β , IL-6 and IL-12 are also well represented. Surprisingly, there are few data for IL-23 generated in the EAE model. This comparison between the MS and EAE data reveals a somewhat narrow focus for human studies. Moreover, cytokines like IL-17 and IL-23 that might help elucidate epitopes targeted by important Th₁₇ cells have no data. Indeed, there is an overabundance of EAE data on Th17-related cytokines, and so far, MS data is scarce and not pointing at a major role of Th17 cells. This may still be a premature conclusion based on the above caveat (few studies to date), but more likely indicates that there are profound differences between the rodent models and humans.

Next, using these data we sought to examine the relative epitope reactivity patterns for MS using cytokines as surrogates for T helper activity (*e.g.* regulatory T cells). It is important to note that in the MS epitope literature, we do not find that authors specifically report or define epitopes recognized by regulatory T cells (Tregs), though it is acknowledged that Tregs have become a significant focus in MS immunobiology (Viglietta et al., 2004). Rather we find that studies characterized Treg activity (presence or absence) in the context of epitope-specific effector reactivity (CD4 or CD8). We therefore cannot provide a list of Treg epitopes, but can make use of the cytokine data as a surrogate for looking at the roles of different epitopes in immunomodulation (roles of different cell phenotypes). For this, we identified those cytokines with ample data: IFN- γ , TNF α , and IL-2 to approximate Th1/Th17 cell activity (inflammatory effects) and IL-10, IL-4 and IL-5 to represent Th2 cell activity (tolerizing effects). We then performed a query to identify all epitopes defined for these cytokines in the context of MS and EAE and then mapped these data to the three major antigens. We found that for MS-specific cytokine data, coverage on a per residue basis was greatest for MBP, followed by PLP and then MOG. For all three antigens, IFN- γ was most heavily studied. However, while PLP data were also generated for TNF, IL-4 and IL-10, little or no MOG specific data were available for these other cytokines. IL-5 and IL-2 data were sparse in general. Overall, we observed no discernible pattern for any cytokine that

would suggest that there is true residue-based differentiation for Th2 *versus* Th1/Th17 reactivity in the MS data. Instead we found that determinants shown to induce IFN- γ , TNF and IL-2 are often also shown to be involved in the production of IL-10, IL-5 and IL-4. This supports the idea that it is an immunoregulatory phenotype rather than individual cytokines that manifest disease (Segal et al., 1998; Codarri et al., 2010; Lovett-Racke et al., 2011).

The most notable observation was in the comparison of the MS data to the EAE data. Here, we found that whereas the response patterns for MS cytokine reactivity were distributed along the entire antigen, those observed for EAE were confined to *only* the well-known epitopes (*e.g.* MBP 1–11, MOG 35–55, PLP 139–151). Fig. 4 provides representative data to illustrate the differences observed.

The EAE data represent a narrow repertoire that reflects the well-established methodology for inducing experimental disease using encephalitogenic peptides. Notwithstanding, this overview of cytokine data underscores that in this model, only the epitopes used to induce disease are the ones “available” to manipulate the cytokine milieu or are involved in experimental disease, which is an over-simplification of the immunopathology, even for an animal model. While this model may have limits in its ability to mimic the complex immunobiology of MS at the cytokine level, EAE can nevertheless provide valuable insight for certain aspects. Applying this model, for example, to scan overlapping peptides might provide insight into the phenomenon of epitope spreading by showing the degree to which other regions/antigens are involved in IFN- γ production following MOG 35–55 EAE induction.

3.9. MHC restriction and disease susceptibility

Genetic susceptibility to MS has been linked to class II loci (de Jong et al., 2002; Sepulcre et al., 2008). Access to epitopes with defined MHC restriction is a prominent feature of the IEDB search interface. MHC restriction is defined (reported) in multiple ways; some studies report specific alleles (*e.g.* DQB1*0302), while others define restriction at the level of serotype (*e.g.* DQ8), or more generally by class (I/II) or effector cell phenotype (CD4/CD8). In the database there are three response types wherein MHC restriction data can be found: T cell response data, which include lymphoproliferation, cytotoxicity, ELISPOTS, cytokine ELISAs, ICS, and tetramer assays, *etc.*, as well as MHC binding assays and MHC ligand elution records.

We first sought to enumerate all MHC restrictions defined at the level of allele/serotype for all T cell assays reported to date for MS and EAE. Table 6 provides a listing of all human, mouse, rat and macaque alleles/serotypes by the total number of captured assays. The data show not surprisingly that the vast majority of MHC restrictions are defined as class II alleles/serotypes for both MS and EAE. Notable among these are HLA-DR15/DRB1*15:01, HLA-DR2, HLA-DRB5*01:01, HLA-DR4/DRB1*04:01, and HLA-A2/A*0201 in humans, and H-2-IAs, H-2-IAb, HLA-DR2 Tg, H-2-Db and H-2-IAu; all of these having 30 or more assays.

MHC-specific tetramers or multimers are an invaluable asset as a reagent for characterizing immune reactivity. The data within the IEDB represent a potential resource for this purpose.

Using the assay finder to filter the T cell data specifically for records defining tetramer/multimer, we found reagents validated for MBP, PLP, transaldolase 1, rhodanese-related sulfurtransferase, TMEV polyprotein and MHC class II. These included epitopes restricted by H-2-IAs, H-2-IAb, H-2-Db, and H-2Kb. Table 7 provides a list of these epitopes.

Finally, there was a large number of epitopes (>200) defined in elution assays for MS patients. These data were derived from a small number of studies targeting certain HLA molecules eluted from brain tissue (Fissolo et al., 2009) and peripheral blood (Vogt et al., 1994; Mohme et al., 2013). MHC molecules from which epitopes were eluted included, HLA-A*02:01 (3), HLA-A*11:01 (24), HLA-A1 (13), HLA-A25 (6), HLA-A3 (7), HLA-B*07:02 (2), HLA-B*44:02 (1), HLA-B*51:01 (1), HLA-B15 (1), HLA-B7 (1), HLA-B8 (3), HLA-Cw7 (2), HLA-DRB1*01:01 (44), HLA-DRB1*03:01 (60), HLA-DRB1*04:01 (12), and HLA-DRB1*15:01 (32) [data not shown]. Interestingly, few of these (~6%) were derived from a myelin-containing antigen (MBP). It is important to note that HLA molecules eluted from the CNS will likely reveal a different antigen profile than those targeted from the peripheral blood.

3.10. Epitope-specific humoral reactivity in MS and EAE

Here we provide an overview of the antibody data in the context of MS and EAE (clinical disease versus association). For this, the disease finder was used to selectively filter the cumulative data for those records wherein the host's (human or non-human model) clinical status (disease state) was reported as MS or EAE in the methods/case history.

The role of antibodies has historically been considered to be secondary. However, abnormal humoral responses in MS patients have been well documented since the 1940s (Kabat et al., 1948). Moreover, antibodies against MBP were identified in the serum (O'Connor et al., 2010), CSF (Panitch et al., 1980; Warren & Catz, 1987) and in tissue from the CNS (Warren & Catz, 1993). However, other studies have shown that these specificities may be infrequent (O'Connor et al., 2003). Antibodies against PLP, MAP, transaldolase and OSP (claudin 11) have been isolated from the CSF of MS patients (Möller et al., 1989; Banki et al., 1994; Bronstein et al., 1999). More recently papers suggesting an important role for antibodies targeting contactin-2 (TAG-1 in rats), neurofascin and KIR4.1 and have been published (Derfuss et al., 2009; Elliott et al., 2012; Srivastava et al., 2012).

MS CSF and brain from MS patient tissue do appear to harbor auto-antibodies. The MOG antigen is widely accepted by the MS B cell community as a relevant antigen at this time but MOG antibody assays work reliably when the whole protein that is properly folded is used (O'Connor et al., 2005; O'Connor et al., 2007; McLaughlin et al., 2009). This suggests that the relevant epitopes are discontinuous and thus calls any work with MOG peptides and auto-antibodies into question regarding biological relevance.

Findings related to many of the other antigens (MBP, contactin-2, GAPDH, MAG, OSP, *etc.*) have also been inconsistent, thus calling into question the relevance of these antigens to the immunopathology of the disease. Much of the peptide-based autoantibody work has not led to confirmed/validated whole protein antigens, suggesting that while these peptide epitopes are certainly real, the studies meant to use them to define a biological antigen

appear to have fallen short. Reactivity to KIR4.1 has been demonstrated in MS serum (47% of patients) and is thus a promising antigen, provided that the seminal work (Srivastava et al., 2012) can be confirmed. Likewise contactin-2 and neurofascin, as mentioned above, hold significant promise as MS-specific antigens. However, no epitopes as yet have been defined for these antigens thus highlighting a potential area for future investigation.

Table 4 provides a summary of the antibody/B cell epitope data for both MS and EAE. These data represent reactivity defined using sera, monoclonal antibodies, recombinant antibodies and CSF. There are a total of 394 B cell epitopes identified in 840 assays, including 21 non-peptidic determinants. Looking at MS and EAE separately, we found that antibody epitopes are more often defined in the context of human disease (276) than in EAE (121). This might however reflect that when EAE is induced with MOG and pertussis toxin with complete Freud's adjuvant, antibodies to MOG will be elicited regardless of the nature of the induced disease. If we evaluate these data according to host we find that 294 epitopes (peptidic and NP) have been identified in humans, and of the EAE-specific hosts, 67 epitopes were identified using mice, 48 in rats, 21 in rhesus macaques and marmosets and 11 in rabbits [data not shown].

3.11. Determinants from infectious agents in the context of MS and EAE

Much effort has been made to identify an infectious agent(s) as the initiating culprit in MS. While several candidates have been implicated, thus far no causal relationship has been established between a microbial pathogen and MS. Despite the lack of formal proof, EBV is now very well accepted and confirmed by many studies as the major environmental risk factor for MS. Low vitamin D3 and smoking are also confirmed, but probably less important. If EBV is causal or shapes the disease once it has developed is however not clear. It is suggested that immune reactivity to microbial antigens (viral, bacterial, fungal or parasitic) triggers reactivity to structurally (residues or 3D conformation) similar antigens in the CNS which then, in combination with a genetic predisposition, is perpetuated in the form of autoimmunity. Alternatively (or in addition), self-reactivity is a consequence of a by-stander damaged following the initial response to the pathogen. However manifested, this complex etiology likely involves the phenomenon of molecular mimicry (Fujinami et al., 1983; Fujinami and Oldstone 1985; Zabriskie, 1986; Wucherpfennig & Strominger, 1995), as well as epitope spreading (Lehmann et al., 1992; McRae et al., 1995; Tuohy & Kinkel, 2000), whereby the specificity of the initial immune response to a non-self-antigen (bacteria or virus) that may share homology with a myelin-containing CNS antigen (mimicry) may in certain individuals, initiate reactivity. AI disease is subsequently perpetuated when epitopes other than the initial microbial epitope(s) become recognized (epitope spreading). And indeed, evidence from the literature suggests at least the feasibility of this phenomenon. Beginning in the 1980s, several groups demonstrated autoantigen specific T cell and antibody reactivity with homologous viral peptides, as well as the ability of a viral peptide sharing sequence homology with an autoantigen to experimentally induce EAE (Fujinami et al., 1983; Fujinami & Oldstone, 1985; Wucherpfennig & Strominger, 1995). However, mimicry leading to autoreactivity has not been proven to occur in natural disease, also possibly as a result of molecular cross-reactivity also occurring between sequences with little discernible homology.

To assess the extent to which this phenomenon could be investigated at the epitope level we first surveyed the MS associated data to identify all determinants derived from microbial sources. To date, there are more than 200 epitopes derived from infectious agents captured in the IEDB in association with MS. These include B and T cell epitopes defined from viruses (348), bacteria (199), protozoa (47) and fungi (20). Many of these structures, however, may not necessarily be directly relatable to disease pathogenesis or mimicry. In order to more precisely identify microbial epitopes that have been recognized in the context of disease, again we used the Disease Finder to filter specifically for those records including subjects with “MS” or animal models of “EAE.” Here we find a total 54 viral and 8 bacterial epitopes defined in MS, and 17 viral and 14 bacterial epitopes defined in EAE.

Table 8 provides a list of the microbe-specific epitopes defined in the context of MS (Table 8a) and EAE (Table 8b). Of the 54 viral epitopes described in the context of MS, human herpesvirus 4 (EBV) represent >70% of the data, including both T cell and B cell contexts, which likely reflects the previous associated of this virus with MS (Ascherio & Munger, 2010), but does not necessarily indicate a true relationship to disease. Perhaps a more relevant investigation of this relationship would be to identify only those records in which MS patients were shown to react with a microbial-derived epitope at a frequency significantly greater than negative/normal controls. While this would not rule out MS-related immunodeficiency as the cause of this greater reactivity, it does at least provide a subset of more relevant epitope data. Of the four antibody and six T cell papers resultant from this query, only two identified statistically significant responses to microbial epitopes in MS subjects (Brookes et al., 1992; Sundström et al., 2009). The remainder demonstrated the ability of clones (mAb and T cell) to recognize microbial sequences, likely due to 3D structural similarities or TCR degeneracy, which again shows biological feasibility, but not an association. One study identified Torque Teno virus (TTV) as the main target for CSF-derived T cells that are clonally expanded during MS relapse and recognize besides TTV also many self-antigens (Sospedra et al., 2005).

Earlier investigations of this phenomenon focused solely on sequence homology between the microbial antigen and the antigen using computer analysis (algorithms) to search large sequence sets; however, this approach can be problematic. Firstly homology at the level of amino acid is not uncommon even across disparate species. Indeed a group showed that ~10,000 different peptides could conceivably satisfy the encephalitogenic requirements as mimics for the well-known MBP epitope FSWGAEGQR (Westall, 2006). However, more recently groups have taken a different approach; testing clones rather than relying on *in silico*-based approaches (Hemmer et al., 2000; Ristori et al., 2000; Markovic-Plese et al., 2005; Sospedra et al., 2005; Lünemann et al., 2006). Perhaps another relevant issue for investigation would be to look into similarities in an antigen functional and sub-cellular location as it relates to microbial and self-antigens. In addition, it may be beneficial to examine the issue of pathogen prevalence as it relates to the onset of MS. Here the question is, if highly ubiquitous viral pathogens like EBV are involved in the induction of MS, how is the prevalence of MS (which is relatively low) explained and how is this related to genetic predisposition, which would have to play a significant role given the generally high rate of microbial exposure for most humans and the relatively low rate of MS worldwide. Indeed,

many very critical aspects of this hypothesis remain unexplored and difficult to prove, and thus far the epitope data are insufficient to address it.

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Fig. 1.

A. IEDB data by categories. The categorization of all epitope-related references is by disease association and uses as a basis for disease association, the host's clinical status (including animal models that mimic human symptoms) and/or the epitope-derived antigens associated with disease(s). AI, autoimmunity; ID, infectious disease; Tranpl, transplantation; Other, papers containing epitope data that fall outside of the IEDB's scope. B. Autoimmune data distribution. Data represent the total number of references in each autoimmunity sub-category. BETAAM, beta amyloid, T1D, type 1 diabetes; DNS, disease non-specific; MS, multiple sclerosis; MG, myasthenia gravis; RA, rheumatoid arthritis.

Fig. 2. Non-protein determinants for all MS-associated references. Non-protein MS-related antigens were categorized according to the ChEBI Ontology hierarchy accessible through the Chemical Entities of Biological Interest (ChEBI) web page [www.ebi.ac.uk/chebi].

Fig. 3.

Response frequency plots for T cell reactivity in MS and EAE. The response frequency score (RFscore) is calculated using the number of respondents/number tested. The RFscore (0.0 to 1) is plotted by residues along the length of the reference antigen (MBP, MOG and PLP). Higher scores are associated with a greater number of individuals responding to a given residue. Black region indicates conservative estimates of response frequencies. Height of gray region indicates level of statistical uncertainty associated with each response frequency score. In order to accommodate all defined epitopes onto a reference antigen, full-length proteins are used. The residue numbering may therefore be different than that of certain well-established protein isoforms.

Fig. 4. Comparison of cytokine patterns using the Immunome Browser. Response frequency scores (RFscores) generated from a query designed to investigate potential differences in cytokine response patterns for MBP. Black region indicates conservative estimates of response frequencies. Height of gray region indicates level of statistical uncertainty associated with each response frequency score. It is important to note that in order to accommodate all defined epitopes onto a reference antigen, full-length proteins are used. For this reason, residue numbering may be different than that of certain well-established protein isoforms.

Table 1

MS-associated antigens. All MS-related epitope data are presented in terms of derivative antigen (myelin-containing, non-myelin-containing and infectious agents) and response type (antibody and/or T cell) and further parsed to show the total number of curated epitopes versus assays. “Assays” represent individual experiments used to define and characterize a given epitope as reported by the authors. MBP, myelin basic protein; PLP, proteolipid protein; MOG, myelin-oligodendrocyte glycoprotein; MAG, myelin-associated oligodendrocyte basic protein; PMP2/P2, peripheral myelin protein 2; PMP22, peripheral myelin protein 22; MOBP, myelin-oligodendrocyte glycoprotein; MPP/P0, myelin protein P0 precursor; OMG, oligodendrocyte myelin glycoprotein; CNPase, 2',3'-cyclic nucleotide 3-phosphodiesterase; Ro RNPs, Ro ribonucleoprotein. For non-myelin-containing antigens, sub-cellular locations were based on UniProt database assignments. These data include only positive reactivities and do not include epitopes defined in the context of MHC binding or MHC Ligand Elution.

	Antibody responses		T cell responses		Total assays	a Total epitopes
	Assays	Epitopes	Assays	Epitopes		
<i>Myelin-containing antigens</i>						
MBP	577	158	2412	376	2989	487
PLP	220	60	1641	144	1861	185
MOG	334	80	1326	203	1660	241
MAG	15	9	92	64	107	68
Claudin-11	12	7	113	38	125	39
PMP2/P2	13	7	86	9	99	13
PMP22	6	3	30	4	36	7
MOBP	6	7	103	22	109	24
MPP/P0	49	18	103	16	152	29
OMG	10	2	10	5	20	5
CNPase	5	2	85	44	89	45
<i>Non-myelin-containing antigens</i>						
Nucleus						
Centromere	150	129	0	0	150	129
DNA topoisomerase	42	35	15	14	57	48
Ro RNP's	56	43	0	0	56	43
Histone	21	20	0	0	21	20
Lupus La	18	13	0	0	18	13
Cytoplasm						

	Antibody responses		T cell responses		Total assays	a Total epitopes
	Assays	Epitopes	Assays	Epitopes		
Myeloperoxidase	54	47	0	0	54	47
Transaldolase I	41	27	3	1	44	28
S-arrestin	0	0	37	12	37	12
Alpha-crystallin	0	0	19	10	19	10
HSP/chaperonin	0	0	31	8	31	8
Cell membrane						
TCR	60	16	322	139	382	148
Myeloblastin	13	11	0	0	13	11
Aquaporin-4	0	0	14	10	14	10
MHC	8	3	6	6	14	7
<i>Antigens from infectious agents</i>						
Viruses	94	53	254	80	348	125
Bacteria	18	12	179	54	197	66
Protozoan	0	0	49	9	49	9
Fungus/Yeast	0	0	20	9	20	9
					614	209

^aTotal number of epitopes represents unique structures; thus any redundant structures found in both B and T cell assays are not duplicated here.

Table 2

Non-peptidic structures by group in MS and EAE. The total number of references and assays identified for each non-protein antigen group.

Antigen group	Total references	Total assays
Sphingolipids	14	30
Glycerophospholipids	13	25
Hydroxysteroids	6	12
Carbohydrates	1	3
Other compounds	4	8

Table 3a

Synthetic analogs/mimotopes by autoantigen. The IEDB contains data derived from synthetic analogs and mimotopes that are captured in association with a naturally-occurring antigen based either on sequence homology/structural similarity or as identified by individual authors. These data show the relative proportion of analogs/mimotopes reported for each of the well-known antigens in the context of T cell responses, B cell/antibody responses, MHC binding, and/or MHC ligand elution. Also included are structures which have been tested in the context of disease exacerbation and/or tolerization.

	Total epitopes	T cell	Antibody	Elution	MHC binding	Exacerbation	Tolerance
MBP	243	202	92	1	334	7	15
PLP	65	239	28	0	59	9	8
MOG	23	15	2	0	20	3	1

Table 3b

APL with biologically defined activity. These are the results of a query performed in Table 3a which include those assays associated with disease alleviation (tolerance, protection, *etc.*), as well as those associated with disease exacerbation (includes all hosts) showing sequences. These structures include analogs of well-known epitopes, such as PLP 139–150, MBP 1–11 and MOG 35–55. Note the phenomenon of structure overlap in either context wherein the same structure can induce tolerance or exacerbate disease depending upon whether it is administered before or after disease challenge.

T cell exacerbation MBP	T cell exacerbation MOG	T cell exacerbation PLP
YGSLPQKAQRSQDENPV	GQFGVIGPGYPICAL	EQLVKWLGLPAPI
GSLPQKSQRSQDENG	PGYPICALVGDEQED	HSLGKWLGHDPKF
YGSLPQKSQRPDQEPV	DEAELPSRISPGKNA	HSIGKWLGHDPKF
VHFFANIVTPRTP		HSLGKWIGHDPKF
VHFFKNIVTPATP		VSLGKWLGHDPKF
YGSLPQKSQRPDQDENPV		HSLGKLLGRDPKF
YGSLPQKAQRPDQDENPV		NTWTTSQSIAFPSK
		PGKVSGSNLLSISKTAEF
		RVSHSLGKWLGHDPK
T cell tolerance MBP	T cell tolerance MOG	T cell tolerance PLP
ASQYRPSQRSK + ACET(A1)	VGWYRSPASRVVHLR	HSLGKALGHDPKF
ASQYRPSQRHG + ACET(A1)		HSLGKQLGHDPKF
ASQYRPSQR + ACET(1A)		HSLGKWLGHDPKF
ASAARPSQRHG + ACET(A1)		HSLGKYLGHDPKF
ENPVVHFFANIVTPRTP		VSLGKWLGHDPKF
QKSQRSQAENPV		HSLGKWLGHDPK + GLYC(H1)
VHFFRNIVTARTP		HSLGKLLGRDPKF
YGSLPQKAQRPDQDENPV		NTWTTSQSIAFPSK
AKPVVHLFANIVTPRTP		
VHFFKNIVTPATP		
ASQYRPSQR + ACET(A1)		
VHFFGNIVTPRTP		
VHFFKNIVTARTP		
NGVGHGFGNGVGP GTGPGSG		

Table 4

Summary of epitope data. A summary is provided of all T cell and antibody data for both MS and EAE (based on clinical status of host; not mere association). Identical structures are often tested in the context of both T and B cell assays, and in the context of both MS and EAE. Thus, overlap in the values is present.

	Total epitopes	T cell epitopes	B cell epitopes	T cell assays	B cell assays	Elution assays
<i>MS</i>						
Peptidic	953	464	276	1360	413	268
Non-peptidic	21	4	18	6	32	0
T cell class I		43				
T cell class II		405				
<i>EAE</i>						
Peptidic	450	378	121	2677	371	0
Non-peptidic	21	5	16	9	24	0
T cell class I		28		144		
T cell class II		228		1385		
<i>MS and EAE</i>						
	1345	792	394	4037	784	268
	29	9	21	15	56	0

Table 5

Cytokine data by disease. Shown is the result of a query performed using advanced T cell search providing only disease state and cytokine assay type as criteria. Disease states within the “1st and 2nd *in vivo* processes,” as well as adoptive transfer were queried separately and summed for totals. Data include all antigens. EAE hosts include mice, rats, marmosets and rhesus monkeys. The total number of assays can be greater than the number of epitopes as there are multiple assays for each cytokine (*e.g.* ELISA, ELISPOT, ICS, *etc.*).

	MS		EAE	
	Assays	Epitopes	Assays	Epitopes
IFN γ	208	147	280	87
IFN β	0	0	1	1
TNF α/β	49	43	54	21
TGF β	2	2	23	13
MCP-1	0	0	4	3
MIP-1a	0	0	3	2
MIP-1b	0	0	1	1
MIP-1g	0	0	2	2
RANTES	0	0	4	4
GM-CSF	0	0	8	6
TCA-3	0	0	3	2
IL-23	0	0	4	2
IL-22	0	0	4	4
IL-21	0	0	2	2
IL-17	0 ^a	0	97	28
IL-16	0	0	1	1
IL-13	0	0	9	5
IL-12	0	0	11	6
IL-10	37	29	64	31
IL-8	0	0	1	1
IL-6	1	1	21	8
IL-5	40	22	23	6
IL-4	52	43	60	26
IL-3	0	0	2	2
IL-2	43	25	116	38
IL-1 α	0	0	2	2
IL-1 β	2	2	1	1

^a Three peptides (MBP 141–160, 156–175, 146–165) tested and found to be negative.

Table 6

MHC restriction defined in T cell assays. Data represent MS-specific and EAE-specific reactivity to all antigens, including infectious agents. In some cases, authors report only the HLA serotype and not the specific allele. Tg, transgenic mouse strain.

Allele/serotype	Total MS-specific assays	Allele/serotype	Total EAE-specific assays
DR15/DRB1*15:01	236	H-2-IAs	163
DR	92	H-2-IAb	79
DR2	45	HLA-DR2 Tg	42
DRB5*01:01	44	H-2-b class II	39
DR4/DRB1*04:01	41	H-2-Db	36
A2/A*0201	34	H-2-IAu	30
DQ1	18	H-2-s class II	28
DP	18	RT1-B	22
B7/B*0702	16	RT1-Ba	19
DR11/DRB1*11:01	15	RT1-B1	18
DQ	15	H-2-IA	13
DR1/DRB1*01:01	12	RT1-B1	12
DR3/DRB1*03:01	12	RT1-D	12
DRB1*13:02	11	DR3 Tg	11
DRB1*16:02	11	H-2-b class I	10
DRB1*04:04	10	H-2-s class I	10
A3	7	RT11 class II	9
DRB1*04:03	7	HLA-DR4/*0401 Tg	8
DRA*01:01/DRB1*15:01	6	H-2-IAg7	8
A24	6	RT1-av1 class II	8
DRB1*15:02	6	HLA-DRA*01:01/DRB1*15:01 Tg	7
B*0801	6	RT1n class II	7
DR6	5	HLA-DQ8 Tg	6
DPB1*03:01	4	HLA-DRB5*01:01 Tg	6
DR8	4	H-2-g7 class I	5
DRB1*16:01	3	H-2-u class II	4
DR5	3	H-2-Kd	4
DRA*01:01/DRB1*04:01	2	HLA-DR1 Tg	4
DQA1*01:02/DQB1*06:02	2	H-2-a class II	3
DRB1*13:01	2	HLA-DQ6/DQB1*06:02 Tg	3
DRB3*02:02	2	H-2-u class I	3
DPw5	2	RT1-Dn	3
E*01033	2	HLA-DRB1*04:04 Tg	3
DRB3*01:01	1	H-2-IAq	2
DRw4	1	Mamu-DPB1	2
DRw14	1	RT1u class II	2
DRA*01:01/DRB5*01:01	1	H-2-Qa1	2

Allele/serotype	Total MS-specific assays	Allele/serotype	Total EAE-specific assays
DRB1*09:01	1	H-2-Kb	2
DQB1*06:02	1	HLA-DR15 Tg	2
DPw2	1	RT1-Da	1
		H-2-IE	1
		HLA-DRB1*15:02 Tg	1
		RT1A class II	1

Table 7

Tetramers used in the context of disease. The results of a query performed using the advanced search interface to specifically select only those data reported using tetramers (MHC tetramer/multimer staining) and the disease state of “EAE” or “MS.” Theiler's encephalomyelitis virus, TMEV.

Epitope sequence	Antigen	MHC restriction
<i>MS</i>		
VMAPRTLVL	Class I A-2 alpha chain (3–11), human	HLA-E*01033
SLSRFSWGA	MBP (244–252), human	HLA-A*02:01
FFRDHSYQSEA	MOG analog of 98–108	DRB1*0401
FFRDHSYQEEA	MOG (98–108), human	DRB1*0401
LLFSFAQAV	Transaldolase 1 (168–176), human	HLA-A2
<i>EAE</i>		
MEVGWYRSPFSRVVHLYRNGK	MOG (33–35), mouse	H-2-Db
MEVGWYRPPFSRVVHLYRNGK	MOG (64–84), human	H-2-IAb
MEVGWYRSPFSRVVHLYRNGK	MOG (35–55), mouse	H-2-Kb
GWYRSPFSRVV	MOG (38–48), mouse	H-2-IAb
GWYRSPFSRVVH	MOG (38–49), mouse	H-2-IAb
HCLGKWLGHDPKF	PLP (140–152), mouse	H-2-IAs
HSLGKWLGHDPKF	PLP (139–151), human	H-2-IAs
YFLKWLGHPNVS	Rhodanese-related sulfurtransferase (83–95)	H-2-IAs
FHAGSLLVFM	TMEV polyprotein (268–277)	H-2-Db

Table 8

a: Microbe-specific epitopes identified in MS. A list of all epitopes and their derivative antigen originating from bacteria or viruses and found to be reactive in humans with MS.

	Epitope	Antigen name	Organism
Bacteria	ALAVLHFYDPKGAKN	N-Acetylmuramoyl-L-alanine amidase	<i>Bacillus subtilis</i>
	DFARVHFISALHGSG	GTP-binding protein EngA	<i>Haemophilus influenzae</i>
	EEFIFHFKNTRDGL	Predicted coding region HP0964	<i>Helicobacter pylori</i>
	QRCRVHFLRNVLAQV	Transposase	<i>Mycobacterium avium</i>
	QRCRVHFMRLNYTAV	Hypothetical protein	<i>Mycobacterium tuberculosis</i>
	NCWKPQVLHC	Glycogen synthase	<i>Prochlorococcus marinus</i>
	DRLLMLFAKDVVSRN	Phosphomannomutase	<i>Pseudomonas aeruginosa</i>
	VLARLHFYRNDVHKE	Pristinamycin resistance protein VgaB	<i>Staphylococcus aureus</i>
Viruses	GGRRLFFVKAHVRES	DNA polymerase	HSV-1
	GGRRLFFVKAHVR	DNA polymerase	HSV-1
	CRVLCCYVL	55 kDa immediate-early protein 1	Herpesviridae
	DFEVVTFCLKDVLPEF	Hypothetical protein	HAdV 12
	NPVMERFAAHAGDLV	Major capsid protein	HHV 1
	FRQLVHFVRDFAQLL	UL15	HHV 1
	DAYPCYFFKSACRPRAP	Helicase-primase primase subunit	HHV 2
	CLGGLLTMV	Membrane protein	HHV 4
	GGDNHGRGRGRGRGGGRPGAPG	EBNA-1	HHV 4
	GLCTLVAML	BMLF1 protein	HHV 4
	LLDFVRFMGV	EBNA3C latent protein	HHV 4
	LLWTLVLL	Membrane protein	HHV 4
	RAKFKQLL	BZLF1	HHV 4
	RPPIFIRRL	EBNA3A nuclear protein	HHV 4
	SVRDLRL	EBNA3A nuclear protein	HHV 4
	TGGVYHFVKKHVHES	DNA polymerase	HHV 4
	SQAPLPCVL	BZLF1	HHV 4
	DPGEGPSTGPRGQGDGGRRK	EBNA-1 protein	HHV 4
	EADYFEYHQEGPDGEPDVP	EBNA-1 protein	HHV 4
	GCKGTHGGTGAGAGAGAGA	EBNA-1 protein	HHV 4
	GEADYFEYHQEGPDGEPDVPPGAIEQGPAD	EBNA-1 protein	HHV 4
	GGPDGEPDVPPGAIEQGPAD	EBNA-1 protein	HHV 4
	GGPDGEPDVPPGAIEQGPADDPGEGPSTG	EBNA-1 protein	HHV 4
	GGRPGAPGSGSGPRHRDGV	EBNA-1 protein	HHV 4
	GNLGEKGDTSGPEGSGGSG	EBNA-1 protein	HHV 4
	GRRPFHPVGEADYFEYHQE	EBNA-1 protein	HHV 4
	GSGPRHRDGVRRPQKRPSCI	EBNA-1 protein	HHV 4
	GSGPRHRDGVRRPQKRPSICIGCKGTHGGTG	EBNA-1 protein	HHV 4
	MSDEGPGTGPNGLGEKGD	EBNA-1 protein	HHV 4
	PGAIEQGPADDPGEGPSTGP	EBNA-1 protein	HHV 4

a: Microbe-specific epitopes identified in MS. A list of all epitopes and their derivative antigen originating from bacteria or viruses and found to be reactive in humans with MS.

Epitope	Antigen name	Organism
PQRRGGDNHGRGRGRGRGRG	EBNA-1 protein	HHV 4
RGRGRGRGRGGGRPGAPGGS	EBNA-1 protein	HHV 4
RRPQKRPSICIGCKGTHGGTG	EBNA-1 protein	HHV 4
SGPEGSGGSGPQRRGGDNHG	EBNA-1 protein	HHV 4
SGSPRRPPPGRRPFFHPVG	EBNA-1 protein	HHV 4
SSQSSSSGSPRRPPPGRRPFFHPVGEADYFEYHQE	EBNA-1 protein	HHV 4
AGAGGGAGGAGAGGGAGGAGG	EBNA-1 protein	HHV 4
DGEPDVPPGAIEQGPADDPGEGPSTGPR	EBNA-1 protein	HHV 4
GGSGGRRGRGRERARGGSRERA	EBNA-1 protein	HHV 4
MSDEPGTGPGNGLGEKGDTSPEGSGGSGPQRRGGDNHGRGRG	EBNA-1 protein	HHV 4
PPRRPPPGRRPFFHPVGEADYFEYHQE	EBNA-1 protein	HHV 4
QGPADDPGEGPSTGPRGQGDGGRKK	EBNA-1 protein	HHV 4
RKKGGWFGKHRGQGSNPKFENIAEGLRALLARSHVERTTDEGTWVAGVF...	EBNA-1 protein	HHV 4
VEGAAAEAGDDGDDGDEGGDGEDEEGQE	EBNA-1 protein	HHV 4
GRRPFFHPVGE	EBNA-1 protein	HHV 4
VPVLAFDAARLRLLE	BOLF1	HHV 4 type 1
NLVPMTATV	HCMVUL83	HHV 5
TPRVTGGGAM	HCMVUL83	HHV 5
MDRPRTPPPSYSE	U24	HHV 6
RPRTPPPSY	U24	HHV 6
IGGRVHFFKDISPIA	Late protein	HPV type 7
PPPPSSPTHDPDSDPQIPPP	Pr gag-pro-pol	HTLV-1
YRNLVWFIKKNTRYP	Hemagglutinin precursor	Influenza A virus
MARAAFLKTVGFGG	Major core protein sigma 2	Mammalian orthoreovirus

b: Microbe-specific epitopes identified in EAE. A list of all epitopes and their derivative antigen originating from bacteria or viruses and found to be reactive in EAE models.

Epitope	Antigen name	Organism
<i>Bacteria</i>		
ALAVLHFYPDKGAKN	N-acetylmuramoyl-L-alanine amidase	<i>Bacillus subtilis</i>
RKVVTDFKNIQRI	Pseudouridine synthase	<i>B. subtilis</i>
RFPNHYGCLLPNPRTEQDN	Hypothetical protein	<i>Chlamydia pneumoniae</i>
EVLARWTGIPVS	ATP-dep protease binding subunit	<i>Escherichia coli</i>
ASMSRPVKQLK + ACET(1A)	Periplasmic beta-glucosidase	<i>E. coli</i>
EQLVKWLGLPAPI	Protease 4	<i>Haemophilus influenzae</i>
DFARVHFISALHGSG	GTP-binding protein EngA	<i>H. influenzae</i>
IAGFLTTEAVVADK	60 kDa chaperonin 2	<i>Mycobacterium</i> spp.
ASMRPNLVAL + ACET(1A)	Glycosyl hydrolase Rv2006/MT2062	<i>Mycobacterium tuberculosis</i>
QRCRVHFLRNVAQV	Transposase	<i>Mycobacterium avium</i>
QRCRVHFMRLNTAV	Hypothetical protein	<i>M. tuberculosis</i>
TFGLQLELTEGMRFDKG	60 kDa chaperonin 2	<i>Mycobacterium bovis</i>

b: Microbe-specific epitopes identified in EAE. A list of all epitopes and their derivative antigen originating from bacteria or viruses and found to be reactive in EAE models.

Epitope	Antigen name	Organism
VLARLHFYRNDVHKE	Pristinamycin resistance protein VgaB	<i>Staphylococcus aureus</i>
IIVNTWLGYPYM	Maltose transport system permease protein	<i>Salmonella enterica</i>
<i>Viruses</i>		
HEYNLWLRSPFSRYSATCPNVLH	Major capsid protein	Human herpesvirus 5
IGGRVHFFKDISPIA	Late protein	HPV type 7
IGGRVHFFKDISPIS	L2	HPV type 13
IGGRVHFFRDISPIG	Minor capsid protein L2	HPV type 40
IGSRVHFFHDISPIT	Late protein	HPV type 32
HICKGFQCFKKPRTPPPK	Large T antigen	JC polyomavirus
KVIAKWLAENVL	Replicase polyprotein 1ab	Murine hepatitis virus
AAQRRPSRPFR	Transactivator	Saimiriine herpesvirus 2
AAQRRPSR	Transactivator	Saimiriine herpesvirus 2
NFLFVFTGAAM	Capsid protein VP3	TMEV
PIYGKTISTPSDYM	Genome polyprotein	TMEV
QEAFSHIRIPLPH	Genome polyprotein	TMEV
SASVRIRYKKMKVFCPRP	Genome polyprotein	TMEV
WTTSQEAFSHIRIPLPH	Capsid protein VP2	TMEV
TGYRYDSRT	Polyprotein	TMEV
PIYGKTISTPSDY	Polyprotein	TMEV
FHAGSLLVFM	Capsid protein VP2	TMEV