

Identifying New Immunodominant Myelin Peptides in Relapsing Remitting Multiple Sclerosis Patients

Brian S. Newsom, Kathrin von Gynz Rekowski, Mitzi M. Montgomery DVM, PhD, Ed Fox MD, PhD and Jim C. Williams PhD

Opexa Therapeutics 2635 North Crescent Ridge Drive The Woodlands, TX 77381

Abstract

T-cell reactivity to peptides found to be instrumental in Multiple Sclerosis (MS) pathology and experimental animal models has centered on the major myelin proteins; Myelin Basic Protein (MBP), Myelin Proteolipid Protein (PLP), and Myelin Oligodendrocyte Glycoprotein (MOG). With recent structural studies on these proteins and discoveries of additional myelin proteins, there are questions about potential interactions between these proteins and T-cells in MS. Using synthetic peptides of 16 amino acid residues and overlaps of 12 residues, we have surveyed the resulting 163 peptides from these three proteins for their proliferative reactivity in 16 healthy subjects and 63 MS patients, including 22 from our Phase I trials of the T cell vaccine Tovaxin™. Using a T-cell Epitope Analysis Assay (EAA), peptides were screened using peripheral blood mononuclear cells and a tritiated thymidine incorporation assay. The results from this assay generated a pattern of individual, patient specific reactivity by calculating the stimulation index of T-cells in the presence of peptide antigens when compared to media controls. Each assay also included controls of non-specific or superantigenic stimuli, such as phytohemagglutinin (PHA). The results of 173 assays have allowed us to identify several peptides as being immunodominant, including some never before reported, and others as being non-reactive. The EAA can be utilized to screen additional peptides or combinations that have biological significance. The results have permitted us to reduce the number of peptides to 109, in the screening assay for Tovaxin™ vaccine production, to qualify subjects for our current Phase IIb clinical trial.

Assay Development

The manufacturing method utilized during our initial trials used immunodominant peptides of myelin proteins to find and expand autogenic T cells involved in the pathogenesis of MS. This method is limited to only detecting and utilizing a small percentage of the total autogenic clones and often does not find the clones responsible for the destruction of myelin or oligodendrocytes.

To further progress this therapy, the Epitope Analysis Assay (EAA: Figure 2) has been developed to assess the patient specific T cell repertoire reactive to the myelin proteins MBP, PLP and MOG. The peptides span the length of all three proteins and therefore allow us to monitor the individual specificity to >80% of the protein content of the myelin sheath.

EAA Results

The data from these assays has revealed unique patterns of antigen specific T cells across MS subjects. Subjects have had from 0 to 19 positive peptide mixes (out of 55 assayed) with a mean of 4.0.

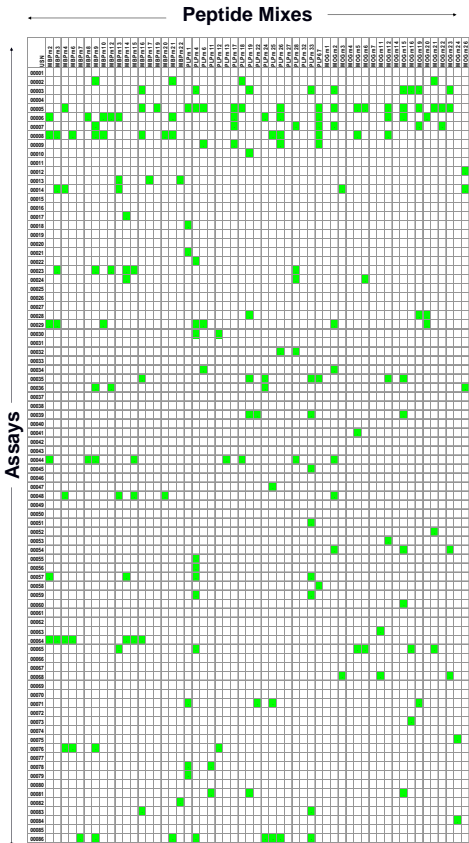


Figure 1. Summary of peptide mix reactivity in representative assays. The X axis lists the unique peptide mixes in the EAA. The Y axis shows 86 individual assays. Green squares are positive based on increased label incorporation.

Assay Summary

The EAA is used to analyze blood samples for their reactivity to peptides. The readout of the assay provides three advantages over our previous method:

1. Determination of a peptide repertoire unique to the individual
2. Ability to monitor epitope shift: the natural movement of epitope specificity over time (Figure 3)
3. The enrichment of our trial for subjects amenable to T cell vaccination

	MOGm3	MOGm4	MOGm5	MOGm6	MOGm7	MOGm11	MOGm12	MOGm14	MOGm15	Media Ctrl	
S1	0.7	0.6	0.6	0.3	0.2	0.8	0.8	0.5	2.5	1.0	
	2	7.07	4.825	6.303	6.317	4.033	6.455	5.637	3.989	24.924	6.866
	3	3.775	4.344	6.513	6.622	4.118	7.079	4.365	5.205	16.268	7.655
	4	4.605	4.423	4.731	5.945	5.572	4.316	7.249	4.602	12.920	6.461
Mean	5.124	4.331	5.516	5.664	5.208	5.950	5.759	4.512	18.031	7.686	
SD	1.624	0.959	1.798	1.441	1.041	1.449	1.445	0.918	6.183	1.362	
	11.902	4.728	4.045	3.244	4.270	17.368	7.354	5.164	223.733	6.750	
	17.126	2.778	6.404	2.988	5.119	21.965	6.577	4.813	245.602	8.185	
	27.462	1.070	5.114	5.495	6.507	21.436	2.467	4.335	259.296	6.945	
Mean	18.811	4.858	5.188	3.900	4.799	20.257	5.466	4.771	234.657	7.686	
SD	7.880	2.149	1.181	1.373	4.611	2.913	2.828	4.113	18.939	1.262	
S1	0.7	0.7	0.6	0.3	0.2	0.8	0.8	0.5	2.5	1.0	
S2	MOGm16	MOGm20	MOGm21	MOGm22	MOGm23	MOGm24	MOGm28	PHA	Media Ctrl		

Figure 2. One plate of the Epitope Analysis Assay. The EAA is used to determine the peptide reactivity profile in the peripheral blood.

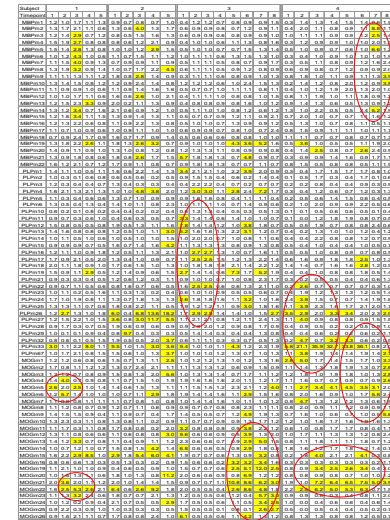


Figure 3. Epitope shift and constancy of peptide reactivity in 4 MS patients, each showing a different pattern. The change in the peptide repertoire over time is part of the natural progression of MS.

New Epitopes

Over the course of these studies we have identified peptides that appear to be immunodominant by their reactivity across a large percentage of MS subjects. Many of these peptides are not predicted to bind to known HLA alleles. Figure 4 shows the peptides that have been positive in > 10% of subjects while screening for a recent trial.

Peptide Mix	Number of Positive Subjects	Percentage of Subjects (Total of 119)
MOGm21	40	33.6%
PLPm26	28	23.5%
MOGm15	27	22.7%
PLPm4	26	21.8%
MOGm23	20	16.8%
MOGm24	15	12.6%
PLPm17	14	11.8%
PLPm32	12	10.1%

Figure 4. Peptide mixes positive in patients in the EAA while screening for a clinical trial. Using these peptides, T cell lines have been developed with repeated peptide stimulations and used for vaccines.

Summary

Analysis from our Phase I trials has shown that the T cell vaccine Tovaxin™, produced in response to myelin antigens, is effective in lowering relapse rates in RR-MS patients. Current screening techniques which use the full lengths of MBP, PLP, and MOG, have increased the specificity of the myelin peptide reactivity to allow vaccine production that could generate anti-idiotypic responses. Utilizing the EAA, we have documented patient specific epitope shifts in MS patients. Additionally, using the full length of the myelin peptides, we have documented new peptides of PLP and MOG that are immunogenic in a large percentage of MS patients. This specificity will allow us to elucidate individual anti-idiotypic responses for MS patients. The EAA screening technique has improved the production of the patient specific autologous T cell vaccine, Tovaxin™, for MS patients.

References

- Zhang JZ, Rivera VM, Tejada-Simon MV, Yang DY, Hong J, Li SF, Haykal H, Killian J and Zang YCQ. T cell vaccination in multiple sclerosis: results of a preliminary clinical trial. *J. Neurol.* 249:212-218, 2002.
- Achiron A. and Mandel M. T-cell vaccination in multiple sclerosis. *Autoimmun. Rev.* 3: 25-32, 2004.