Designing Peptide Mimetics for the Treatment of Multiple Sclerosis

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Abstract: Multiple sclerosis is a chronic inflammatory disease of the central nervous system. While the molecular basis of the disease is still unknown, research effort is currently under progress to prevent or ameliorate its effects. There are two major approaches currently in the pursuing of improved therapeutics for the treatment of multiple sclerosis. The first approach focuses on peptide or mimetic therapy and the second on immunotherapy by preventing or controlling disease through the release of appropriate cytokines.

INTRODUCTION

Peptide Mimetics as Potential Drugs in Autoimmunity

A peptidomimetic is a compound that mimics or blocks the biological effects of a peptide or a protein motif, with the potential to act as a drug molecule. No matter what is the nature of its chemical structure this is a synthetic compound, which aims to serve as a therapeutic agent for pathological conditions. It achieves this by deceiving enzyme substrates to act on a metabolic cascade or peptides to their receptor targets.

Therefore, the pharmacological success of these molecules can be correlated with the extent of their mimicry of the peptides that cause the pathological damage. In this review article, strategies will be described for designing peptide mimetics and some applications of peptide or non peptide mimetics for the treatment of Multiple Sclerosis (MS). These strategies can be applied to other major diseases such as cancer and cardiovascular diseases. A rational design is set out, which aids in the development of peptide mimics. This involves the following steps:

a. Identification of the minimal peptide amino acid sequence responsible for activity. This amino acid sequence will determine the lead compound.

b. Finding the possible bioactive conformation of the minimal peptide sequence which mimics the parent peptide or protein using cyclic or constrained analogues and a combination of NMR spectroscopy and molecular modeling as well as x-ray crystallography wherever possible to crystallize the parent compound.

c. Designing peptide and non-peptide molecular structures for medical applications.

Alternatively, random design can be applied. In random screening a collection of molecules are evaluated in a particular assay system in an attempt to search for their biological interactions.

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DISCUSSION

Autoimmunity in Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the Central Nervous System (CNS) characterized by demyelination and loss of neurologic function, local macrophage infiltrate and neuroantigen-specific $CD4^+$T cells [1,2]. Candidate autoantigens include constituents of the myelin sheath such as Myelin Basic Protein (MBP), Proteolipid Protein (PLP) and Myelin Oligodentrocyte Glycoprotein (MOG) [3-6]. Modern approaches towards the therapeutic management of MS involve the design and use of peptide analogues of disease-associated myelin epitopes to induce peripheral T cell tolerance [7-9]. Experimental Autoimmune Encephalomyelitis (E.A.E.), one of the best studied experimental animal models of MS [1], represents an invaluable *in vivo* system for the evaluation of such therapeutic approaches. E.A.E. is a Th1 (Type 1 T helper) $CD4^+$ T cell-mediated disease that can be induced by immunization with MBP, PLP or MOG proteins or peptide epitopes [10]. Extensive studies using MBP T-cell receptor (TCR) transgenic mouse has led to suggestions concerning the mechanism of recovery from E.A.E [11]. In these studies recovery from E.A.E. is associated with three major immunologic changes: (1) deletion of encephalitogenic T cells in the brain; (2) deviation of MBP-specific transgenic T cells both in the periphery and in the central nervous system and (3) deletion of transgenic T cells in the thymus through apoptosis. Thus, recovery from a classic type Th1 organ specific autoimmune disease is associated with two mechanisms of immune tolerance, deletion of autoreactive cells and immune deviation of autoreactive cells to a non-pathogenic phenotype. In another study, the frequency of MBP-reactive T cell lines was ten-fold higher in patients with acute disseminated encephalomyelitis, a postinfectious autoimmune disease of the CNS, compared to patients with encephalitis and normal subjects [12]. In lewis rats immunized with guinea pig MBP protein, encephalitogenic T cells which recognize $MBP_{74\text{-}85}$ epitope, dominate the

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immune response [13,14]. From these and other studies [15,16] in which specific immunodominant T cell epitopes of MBP may be target antigens for Major Histocompatability Complex (MHC) class II-restricted, autoreactive T cells in MS, the assumption has been that disease can be modulated with peptides that interfere with the formation of the trimolecular MHC-peptide-T cell receptor [14]. Furthermore, there is gathering evidence that analogues of disease-associated epitopes can inhibit disease through the activation of antigen specific regulatory T cells [7,15,16] and the control of cytokine secretion [17-20]. The ability to alter the cytokine secretion of autoreactive T cell lines through peptide or mimetic treatment even in longstanding autoimmune disease indicates that cytokine therapy might have therapeutic benefits by switching the function of myelin reactive T cells such that they are non-pathogenic. Extensive studies on the molecular biology and actions of cytokines have recently been reviewed [21].

Current Treatments for Multiple Sclerosis

As it is already mentioned, MS is an inflammatory demyelinating disease of the CNS. In its early stages the disease follows a relapsing and remitting course. For some patients the remitting is incomplete and enters a progressive phase during the course of the disease characterized by motor abnormalities (weakness, spasticity, brainstem involvement, internuclear ophtamoplegia, pseudo bulbar palsy) and cerebellar disturbances (ataxia, tremor). Other symptoms often encountered are fatigue, loss of bladder and bowel control and neuropsychological abnormalities.

This knowledge of the pathogenesis of the disease generated immunomodulating and immunosuppressive drugs that either treat or prevent relapse and progression and drugs that act symptomatically (antispastic, antitremor, antidepressants etc). The classified drugs tested and found to exert beneficial effects against the symptoms of MS are shown in Table **1**.

However, the above drugs have limitations (corticosteroids) or side effects. Immunosuppresant molecules, like methotrexate suggested as drug to treat chronic progressive MS, are characterized by limited efficacy and significant toxicity. Adverse effects include gastrointestinal discomfort, nausea, muscositis, headache, rash, fatigue, alopecia and infections. The immunosuppresant mitoxantrone causes lower adverse effects and has been recently approved by FDA for MS treatment. Though milder compared to other immunosuppresants, it causes the typical side effects of cancer chemotherapy:nausea, hair loss, menstrual disorders or risk of infection. Its long use has pontential for causing cardiotoxicity. The drugs against spasticity baclofen and tizanidine cause also side effects. The oral treatment with baclofen results in adverse effects, although usually transient, including neuropsychiatric (euphoria, depression, hallucination and confusion), neurological (ataxia, tremor and nystagmus) and gastrointestinal (nausea and diarrhea). The most common side effects observed by Tizanidine are dry mouths and drowsiness. Oxybutyrin is an oral anticholinergic and suffers from the side effects similar to these of all anticholinergics (dryness of the mouth, accomodation disturbances and tachycardia). The anticonvulsant carbamezapine is known to cause also dryness of the mouth. In addition, several other effects are observed such as accomodation disturbances, ataxia and diplotypia.

Table 1. Drugs Used Against MS

Immunomodulatory agents
Corticosteroids: Prednisone, Methylprednisolone
Cytokines: Interferon-1b, Interferon -1a
Cytotoxic immunosuppressants: Methotrexate, Azathioprine, Cyclophosphamide, Cyclosporine, Mitoxantrone
Other agents: Glatiramer acetate, Human immune globulin
Therapies for MS motor signs: spasticity, tremor
Antispastic agents: Baclofen, Diazepam, Dantrolene, Tizanidine, Botulium toxin
Antitremor agents: Gabapentin, Methazolamide, Propranolol, Alprazolam, Primidone
MS therapies for fatigue and depression
Antidepressants (SSRIs): Fluoxetine, paroxetine
Other CNS monoamine-modifying agents: Amantadine, Premoline, Deprenyl
Pro-vigilance agent: Modafinil
K^+-channel blocker: 4-Aminopyridine
MS therapy for sensory signs: Pain, itching
Anticonvulsants:carbazepine, gabapentin, phenytoin
Tricyclic antidepressants: **Desipramine, amitriptyline etc**
MAO inhibitor: **Moclobemide**
Antihistamine :**Hydroxyzine**
MS therapies for autonomic dysfunctions
Bladder-control problems: Oxybutynin, Propantheline, Bethanechol, Terazosin
Erection Problems: Papaverine (intracavernosal), Sidenafil
Urinary tract stasis, infection risk: **Nitrofurantoin, Trimethoprim**

Interferons (IFNs) first detected based on their antiviral properties are categorized as type I IFN (IFN-α, IFN-β, IFN-τ, IFN-ω) and type II IFN (IFN-γ). Type II IFN consistently stimulates class II MHC expression in all responsive cell lines tested, worsening disease. On the contrary Type I IFN is therapeutic in MS by inhibiting class II expression and macrophages, thereby inhibiting their activation related with pathogenesis or sustain of disease. The specific mechanism of action of these agents in multiple sclerosis are incompletely understood. Recent evidence points out that IFN-γ may act through a different mechanism inducing Human Lymphocyte Antigens (HLA) which normally are not expressed in an appreciable manner with the nervous system. Viral infection and other stresses may aggregate the disease by eliciting the secretion of γ-nterferon in the brain.

Limitations in the use of therapeutic interferons are flu-like symptoms after injections, body aching, slight fever, shelling and redness, variable in severity. In conclusion, the reported benefits from the use of interferons and copolymer-1 glaritamer acetate which is a synthetic protein comprised of the major aminoacids Glu, Ala, Lys, Tyr of MBP, are marginal [22-30].

Molecular Mimicry

Molecular mimicry has been proposed as a mechanism for the induction, or exacerbation of MS. According to this hypothesis, certain infectious agents are comprised of proteins containing peptide sequences that mimic autoantigen epitopes. Upon infection, presentation of these viral of bacterial peptides in the periphery by infected APCs inadvertently activates autoreactive T cells. In their activated state, these T cells cross the blood brain barier (BBB) and recognize the autoantigens within the CNS, initiating an inflammatory response that ultimately leads to myelin destruction. Viral and bacterial epitopes have been identified which trigger human MBP-reactive T cells obtained from MS patients. Furthermore, the discovery in recent years that there is considerable degeneracy in TCR recognition of peptide/MHC complexes and that a given TCR can react with multiple peptides, supports this theory. While the possibility of molecular mimicry as a cause of MS has been supported in vitro, only few studies have evaluated the ability of molecular mimicry to induce disease *in vivo*. In these studies human and animal viruses have been found to cause demyelination. Some of these viruses directly infect the myelin-producing oligodendrocytes, leading to cell death and demyelination. Example of virus that infects oligodendrocytes causing their death is the JC papovirus. Details of possible mechanisms of injury in MS caused by viruses can be found in the recent review by Noseworthy *et al* [30]. The success of interferon-beta in reducing clinical relapses has led some to speculate that this effect is mediated by its antiviral action which by definition is interferon's property. If MS is directly caused by a virus, then interferon-beta may be effective in controlling the virus and is consequently reducing the number of relapses. Type I IFNs may exert a therapeutic effect in MS via their antiviral properties in two ways. First, IFN may exert an inhibitory effect on an as-yet unidentified pathogen in MS tissue. This possibility is entirely speculative at present, since there is no current evidence directly implicating a viral etiology for MS. Secondly, type I IFN may reduce the frequency or severity of common viral infections in MS patients. While there is little current evidence to support the notion that type I IFN acts by reducing the frequency of clinically trivial viral infections in MS patients, it remains an attractive possibility. The identification of those myelin and viral peptides involved in the mimicry process will remain an area of significant investigation.

New Approaches in the Treatment of MS

Autoantigens

Due to adverse effects of current treatment of MS new approaches are sought. It is under clinical investigation for autoimmunity suppression the use of oral administration of auto antigens [31-33]. In this study, orally administered antigens suppress autoimmunity in animal models, including E.A.E, collagen and adjuvant-induced arthritis, uveitis and diabetes in the non-obese diabetic mouse. Low doses of oral antigen induce antigen-specific regulatory T-cells which act by releasing inhibitory cytokines such as TGF-β, IL-4, and IL-10 at the target organ. Thus, one can suppress inflammation at a target organ by orally administering an antigen derived from the side of inflammation, even if it is not the target of the autoimmune response. Initial human trials of orally administered antigen have shown positive findings in patients with MS and rheumatoid arthritis [31-33]. A double-blinded, placebo-controlled, multi-center trial of oral myelin in relapsing-remitting MS patients is in progress, as are clinical trials investigating the oral administration of type II collagen in rheumatoid arthritis, S-antigen in uveitis and insulin in type I diabetes. This promising method has the oral administration advantage over the previous methods using interferons and copolymer-1 which are intravenously administered.

Cyclic Peptide and Non-Peptide Mimetics

Other approaches presently are focusing on peptide or mimetic therapy and on the immunotherapy of MS by preventing or controlling disease through the release of appropriate cytokines.

In the first approach towards peptide mimetics the assumption has been that disease can be modulated with peptides that interfere with the formation of the trimolecular complex MHC-peptide-T cell receptor [15,16]. However, issues related to the peptide nature and cost of administered substance renders the non-peptide mimetic approach, even in its infancy, an attractive goal to pursue [34-36]. In general, peptides suffer from several disadvantages. Peptide bonds are easily hydrolyzed by proteases resulting in short duration of action and low biovailability. Furthermore, the selectivity is lost because the smaller fragments resulting from the hydrolysis cause side effects by targeting other receptors. Continuous infusions and therefore prohibitive amounts of peptides are necessary to elicit the necessary biological response. It must be clarified that autoantigens are natural proteins and do not suffer from these problems. This is because the altered peptide epitopes are treated by the body differently than natural autoantigens. To address the need for more stable molecules with the same biological activity as the original peptide or its peptide antagonist, there are two directions. One is the design and synthesis of cyclic analogues which are more stable compared to linear counterparts and which could maintain the biological function of the original peptide, yet could also be able to elicit a response in pharmacological quantities. Design of such cyclic analogues is based on Structure-Activity Studies (SAR), NMR and Molecular Dynamics studies carried out in the linear peptide and have been very effective in defining the bioactive conformation of other important peptides such as Angiotensin II [37-39], implicated in blood pressure and Thrombin Receptor Peptides [40,41], in hemostasis and angiogenesis. The cyclization of peptides has proved also to be a very valuable tool in providing analogues with increased resistance to metabolic degradation, potency, receptor

selectivity and bioavailability, all of them reflecting a better pharmacological profile. In particular, cyclic peptides have been used in several cases as synthetic immunogens, potent vaccines for diabetes, antigens for herpes simplex virus, transmembrane ion chanels, inhibitors of HIV-1 Tat-TAR interactions in human cells, of α-amylase, of pancreatic trypsin and as protein stabilizers [44]. The appropriate design of cyclic analogue by connecting the two least important residues for activity without causing drastic changes in the conformation of active peptide results in a rigid geometry of the cyclic peptide enhancing the binding affinity compared to the linear counterpart. The engineering of stable peptides is of great technological and economic importance, since the limited stability of peptides often severely restricts their medical and industrial application.

MBP Epitope 74-85 Cyclic Analogues

Structure-activity studies have shown that the MBP_{74-85} peptide (epitope of Guinea Pig MBP) induces E.A.E. in Lewis rats. Since the peptides that bind to MHC class II molecules have been determined to involve a minimum of nine amino acid residues which satisfy a particular motif, the design of a cyclic mimetic that would maintain their functional role *in vivo* is quite challenging. In a recent study [42], a head to tail intramolecular proximity between termini residues (ROESY connectivity between α Val^{12}- α Gln^{1}) was observed suggesting a cyclic conformation for linear agonist MBP_{74-85} peptide, Gln^{1}-Lys^{2}-Ser^{3}-Gln^{4}-Arg^{5}-Ser^{6}-Gln^{7}-Asp^{8}-Glu^{9}-Asn^{10}-Pro^{11}-Val^{12}-NH_2 (Fig. **1** and Fig. **2**). Using this information, a cyclic analogue was synthesized by connecting the ε amino group of Lys and γ carboxyl group of Glu at positions 2 and 9 in an attempt to synthesize more stable peptides that act against MS. However, this cyclic analogue was found to be highly potent (estimated to possess 80% encephalitogenic potency when it is compared to parent linear analogue 1) indicating that the Lys and Glu residues at positions 2 and 9 are not so important to elicit the onset of E.A.E. in Lewis rats (Fig. **1** and Fig. **2**). The choice of Lys and Glu residues at position 2 and 9 for cyclization was based on structure-activity studies carried out in linear agonist peptide [7], where single alanine substitution resulted in significant reduction of agonist activity. Single Ala substitution is an accepted method to identify pharmacophoric (or not) side chain groups of the active peptide. In our studies the potency of cyclic analogue confirmed the validity of this choice. E.A.E. induced by cyclic analogue was completely suppressed by the co-injection with the Ala^{81} MBP_{74-85} antagonist analogue. The comparable potencies of linear and cyclic analogues indicate that the encephalitogenic linear peptide participates in the trimolecular complex with a cyclic conformation in which the carboxyl group of Asp at position 81 plays an important role for activation of this complex. NMR and Molecular Dynamics studies have shown interactions between charged groups and especially between Asp carboxylate and Arg guanidino group important for activity (Fig. **2**). These investigations have been recently rewarded with the successful design and synthesis of novel quinea pig MBP_{74-85} and human MBP_{87-99} cyclic analogues with disease suppression effects in the E.A.E. system and in human peripheral blood lymphocytes [42-47]. In this study, the linear and cyclic forms of the agonist peptide MBP_{74-85} had the same effect on human T cell activation and proliferation and their effect was completely reversed by co-culturing of the cells with the linear or cyclic analogues of the antagonist peptide Ala^{81} MBP_{74-85}.

The comparable potencies of linear and cyclic analogues MBP_{74-85} and c-MBP_{74-85} as well as of analogues Ala^{81} MBP_{74-85} and c-Ala^{81} MBP_{74-85}, indicate that a cyclic conformation of the MBP_{74-85} epitope predicted using NMR and computational analysis together with a carboxyl group at position 81 and a guanidino group at position 78, are important for the function of the trimolecular complex, MHC-peptide-T cell receptor. These results constitute an example where rational drug design can lead to the development of potent molecules with improved pharmacological properties such as increased degradation resistance.

Non-peptide MBP Epitope Analogues

The other direction in drug therapy is the design and synthesis of non-peptide mimetics with the same biological activity as the parent peptide [34-36]. In this regard a new technology with the use of combinatorial chemistry, has been recently developed that generates small organic non-peptidic synthetic molecules called peptide mimetics [34-36]. Since the peptides that bind to MHC class II molecules have been determined to involve a minimum of nine amino acid residues which satisfy a particular motif [48], the design of mimetics that would require a reduced number of amino acids and still maintain their functional role in vivo is quite challenging and it is possible if there is sufficient knowledge of the conformation, of the immunophoric groups and of their distances within the parent molecule. The participation of moieties like isonipecotic acid (iNip) and aminocaproic acid (Acp), which represent lengths of approximately 1.5-2.5 alpha amino acid residues and provide flexibility to the whole peptide as it assumes all intermediate structures between folded and extended conformations, introduces parameters that have not been tested previously for their effect on the immune response [43,44]. The novel technology applied here involves incorporation of the essential functional amino acids derived from a bioactive peptide onto the arms of a "molecular hinge", which greatly facilitates the opportunity for the bioactive residues to cluster together (closed hinge) or otherwise (open hinge) in an appropriate manner [43,44]. In this work, iNip and Acp were used as spacers which allow for either a folded backbone orientation of the pharmacophoric groups or extension of the peptide chain to the desired length for maximum binding. Immunophores used were Ser, Arg, Glu, Ala, Gln and the semi-mimetic peptide synthesized with Acp as spacer but not with iNip were found to be effective in inducing the onset of E.A.E [43,44] (Fig. **3**). These findings suggest that the design and synthesis of semi-mimetic peptide molecules or non-peptide mimetic with immunomodulatory potential is possible and that eventually these molecules may form the basis for the development of novel and more effective disease-specific therapeutic agents.

Immunotherapy of Multiple Sclerosis and Clinical Perspectives

The immunotherapeutic approach towards the development of therapeutic vaccines for MS is based on the

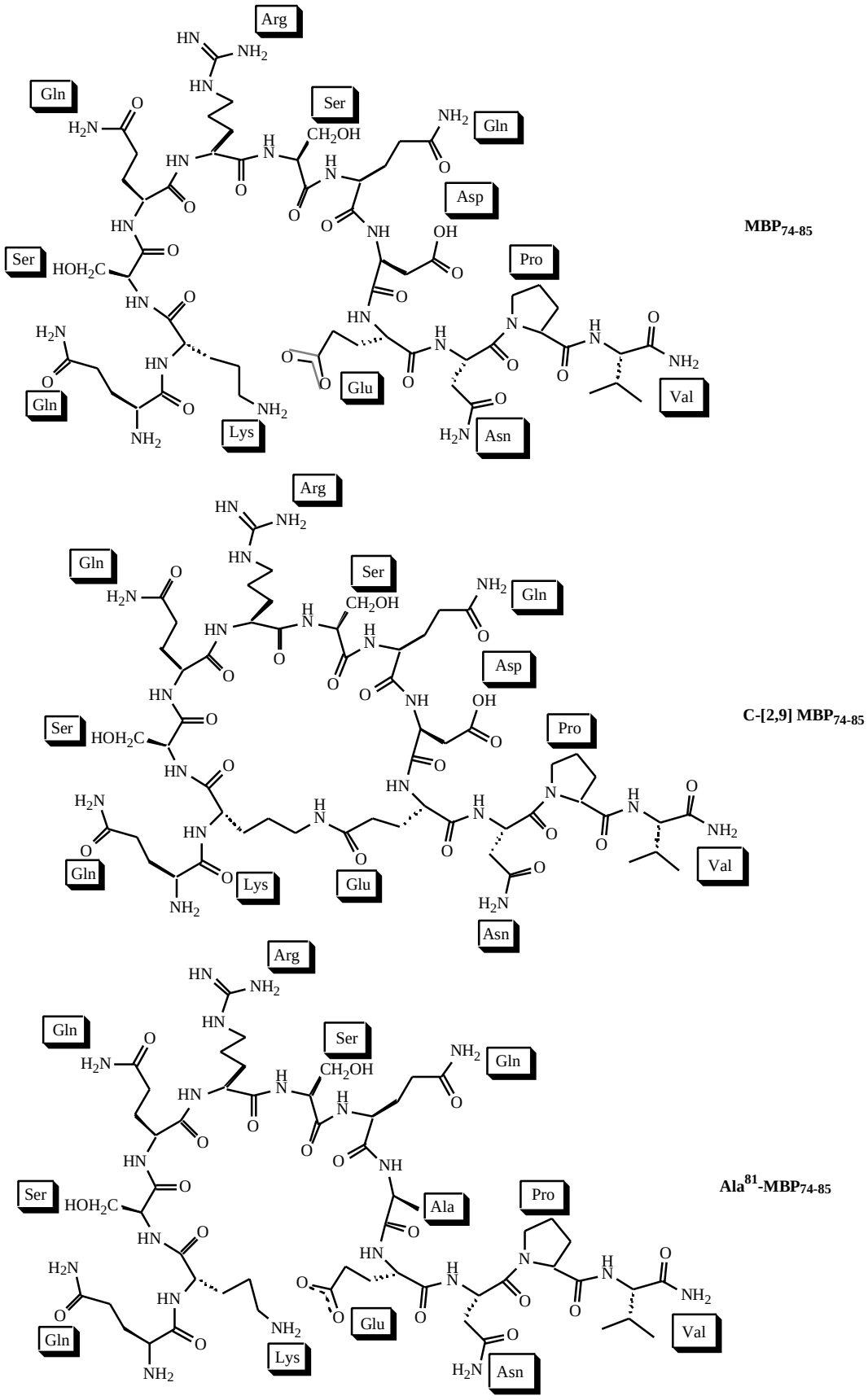
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Fig. (1).

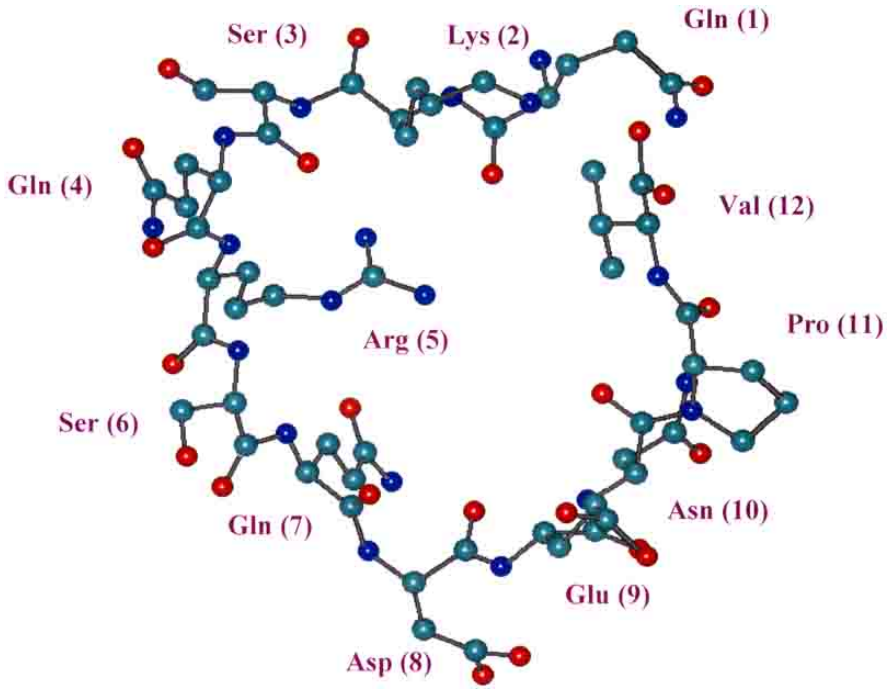
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Fig. (2a).

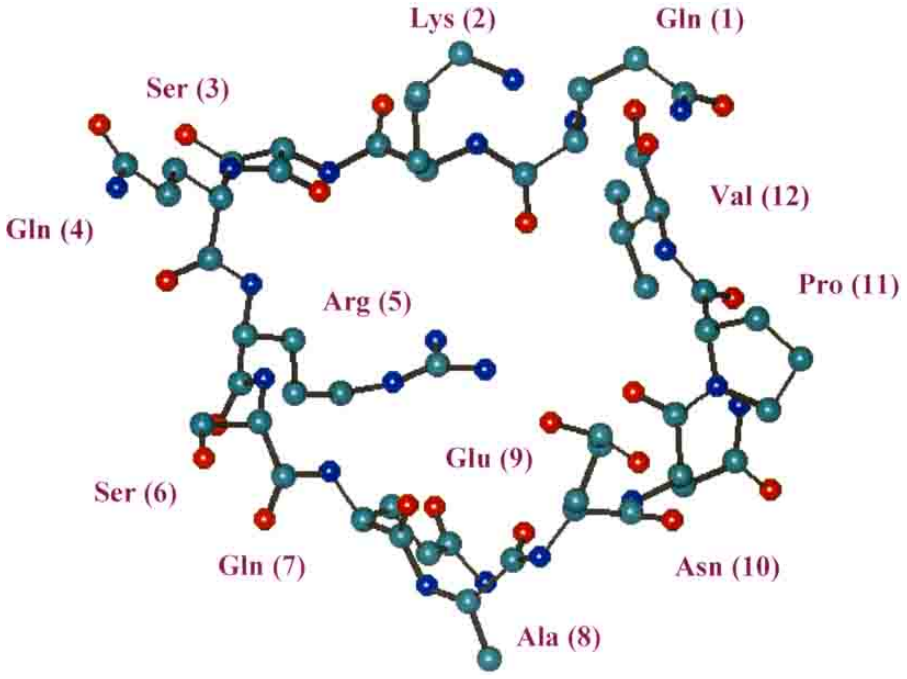
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Fig. (2b).

Fig. (2). contd….

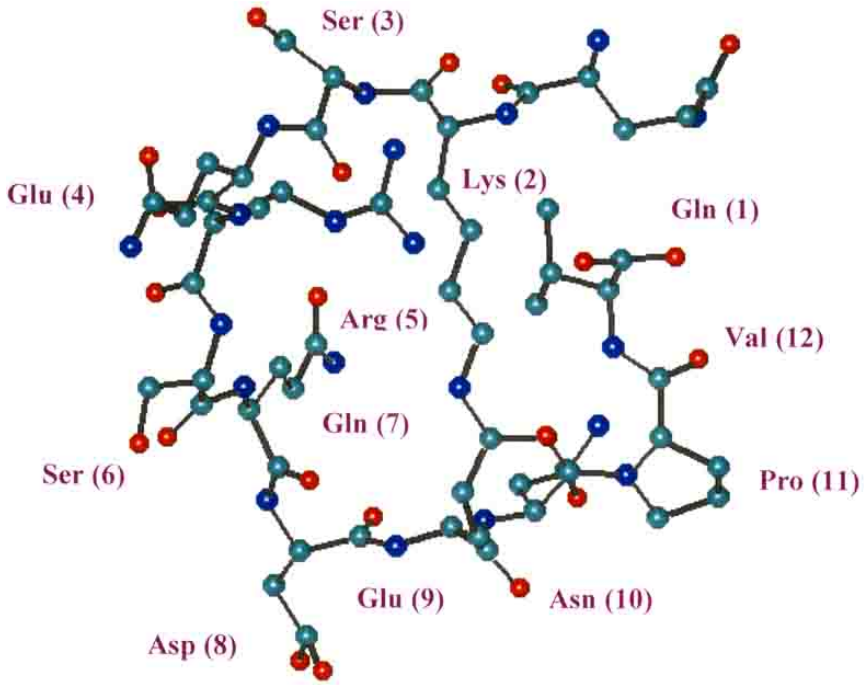
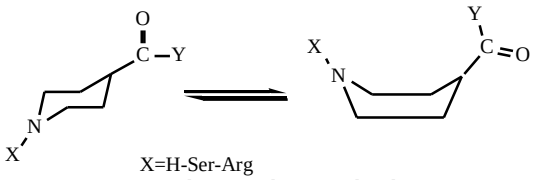


Fig. (2c).

Immunodominant MBP epitope peptide sequences

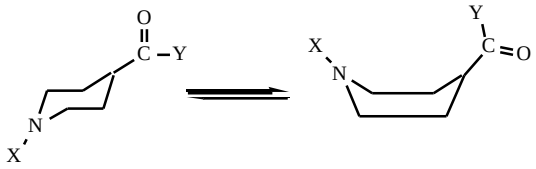
Guinea Pig MBP$_{74-85}$: Gln-Lys-Ser-Gln-Arg-Ser-Gln-Asp-Glu-Asn-Pro-Val
Human MBP$_{87-99}$: Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro

Example of Semi-mimetic peptide (chair-boat)



X=H-Ser-Arg
Y=-Glu-OH, -Gln-OH, -Ala-Gln-OH

Example of Non-peptide mimetic (chair-boat)



X,Y pharmacophoric groups

Fig. (3).

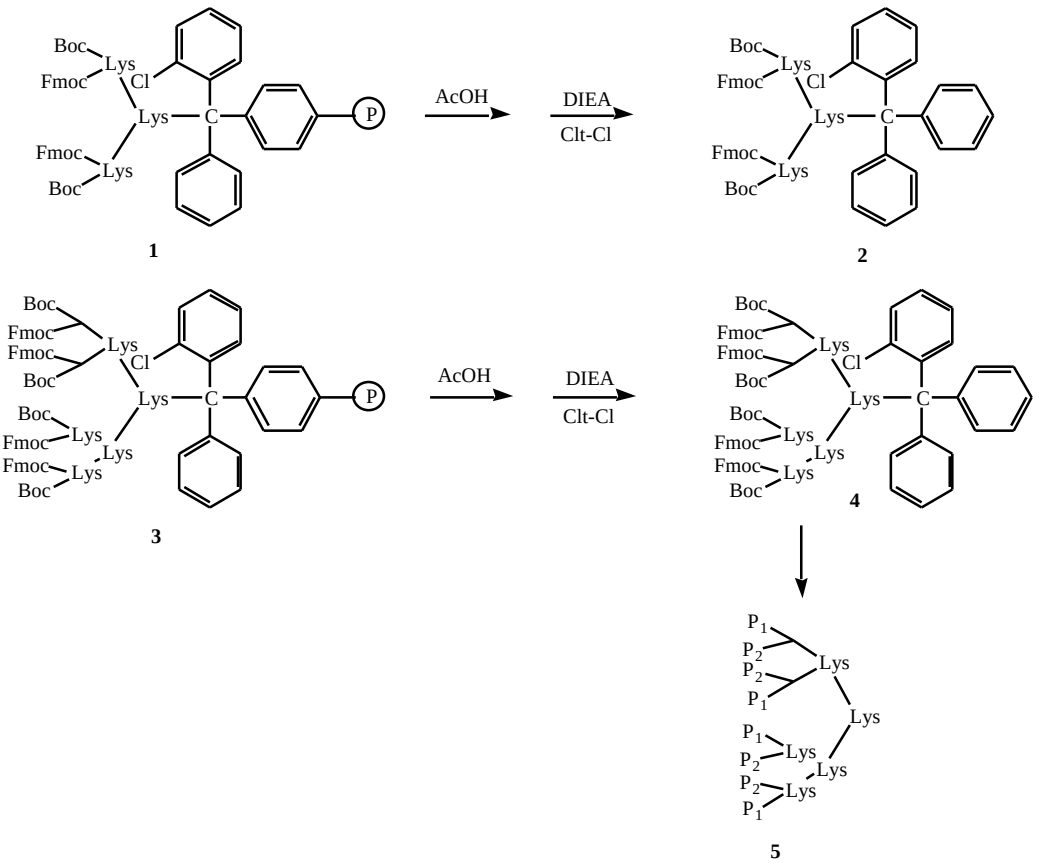
assumption that disease epitopes or their analogues can actively inhibit or prevent disease through the activation of antigen-specific regulatory T cells, or antibodies related to myelin sheath destruction [7,14-16]. The myelin sheath is constituted from three proteins the MBP, the PLP and MOG implicated in MS. Therefore, epitopes of these Myelin sheath proteins are targets for immunotherapeutic techniques. A recent study investigating the effects of antibodies on demyelination, found that in the acute areas of inflammation in MS, the active plaques, there are antibodies against a minor protein component, MOG [47,48]. MOG antibodies were related to significant myelin disruption, probably by

coating the myelin so that macrophages (destructive "big eater" cells) could engulf and destroy coated sections of myelin, blocking nerve impulses temporarily or permanently. Thus, we now know that antibodies do play a role in MS, and cooperate with white cells in advancing myelin destruction. Blocking the effects of these MOG antibodies with secondary antibodies or non-peptide mimetics might be an important avenue of future therapy.

Another direction in the immunotherapy of autoimmune diseases is the use of Multiple-Antigen Peptide (MAP) systems introduced by Tam [49-51]. This system represents a novel approach to anti-peptide antibody production. It is built on a resin which bears a core of radial branching lysine dendrites on which a number of copies of a given peptide antigen can be incorporated, (Fig **4**). Lysine derivatives have been used for the solid phase synthesis of lysine cores suitable for the assembly of antigenic peptides [49-51]. These peptides have found application in raising antibodies and in the preparation of synthetic vaccines. On a lysine core several different epitopes of a protein or of different proteins can be assembled to create the required antigenic synthetic protein. Di-epitope MAPs can be prepared by coupling two heterologous MAPs through disulphide linkages via a cysteine in the core [49-51]. Alternatively, the two epitopes can be syntesized on alternate branches of the lysine core using Boc and Fmoc chemistry. T-cell and B-cell epitopes can also be combined sequentially within a single linear sequence. Following assembly of the peptide on the MAP core, the peptide is deprotected and cleaved from the support using standard techniques, yielding a highly immunogenic macromolecular structure without the need for conjugation to a carrier protein. The MAP approach has been shown to yield higher antibody titres than using monomeric peptide-conjugates. An improvement of this method has been successful by introducing a chloro-trityl resin and a number of protecting groups for selective deprotection of blocked residues to allow incorporation of several epitopes in a single polylysine moiety [52-54].

Mannan Conjugates

Another challenging strategy in the immunotherapy of MS or other autoimmune diseases, is the use of peptides conjugated to mannan in either the oxidized or reduced form, to develop T1/T2 responses followed by release of appropriate cytokines [55-61]. The aim of this approach is the development of a therapeutic vaccine for prevention or control of the disease. We are currently investigating antigen peptide libraries of the three MBP, PLP and MOG proteins of the myelin sheath conjugated to mannan in its oxidized or reduced form. Cytokines will be measured and their effect on E.A.E in Lewis rats will be determined. Activation of human peripheral blood T cells and the secretion of cytokines will also be studied with these reagents. These studies include, the development of a recently rationally designed constrained cyclic antagonist peptide analogue c-Ala^{81}MBP$_{72\text{-}85}$ of guinea pig MBP which suppresses the development of clinical E.A.E., CNS inflammation and demyelination when co-



1 **2** **3** **4** **5**

Fig. (4).

injected with the encephalitogenic linear peptide MBP_{72-85} or its cyclic counter part c-MBP_{72-85} in Lewis rats, showing a promising pharmacological profile. Same studies are carried out with mannan conjugates of potent human MBP_{87-99} linear and cyclic analogues recently reported. The use of oxidized or reduced mannan to develop a T1 or T2 response (and the appropriate cytokines) to Myelin peptides expressed in multiple sclerosis constitutes a novel strategy for the treatment of the disease. These strategies may open new avenues in the immunotherapy not only of MS but also for other autoimmune diseases.

CONCLUSION

This review article cites current therapies for treating Multiple Sclerosis (MS) as well as new strategies in the immunotherapy of disease. Non-peptide and cyclic peptide mimetics of Myelin Basic Protein epitopes which are able to inhibit Experimental Allergic Encephalomyelitis (EAE) might be potential drugs for treating disease if the mechanisms of EAE and MS are similar. Design of peptide and non-peptide mimetics based on immunodominant sequences and using a combination of NMR Spectroscopy and Molecular Modeling can be a general method for treating autoimmune diseases.

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REFERENCES

[1] Martin, R.; McFarland, H.; McFarlin, D. *Ann. Rev. Immunol*., **1992**, *10*, 153.

[2] Steinman, L. *Cell*, **1996**, *85*, 299

[3] Pette, M.; Fujita, K.; Wilkinson, D.; Altmann, D.M.; Trowsdale, J.; Giegerich, G.; Hinkkanen, A.; Epplen, J.T.; Kappos, L.; Wekerle, H. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 7968.

[4] Ota, K.; Matsui, M.; Milford, E.L.; Mackin, G.A.; Weiner, H.L.; Hafler, D.A. *Nature, (Lond.)* **1990**, *346*, 183.

[5] Martin, R.; Jaraquemada, D.; Flerlage, M.; Richert, J.; Whitaker, J.; Long, E.O.; McFarlin, D.E.; McFarland, H.F. *J. Immunol.*, **1990**, *145*, 540.

[6] Martin, R.; McFarland, H.; McFarlin, D. *Ann. Rev. Immunol.*, **1992**, *10*, 153.

[7] Gaur, A.; Boehme, S.A.; Chalmes, D.; Crowe, P.D.; Pahuja, A.; Ling, N.; Brocke, S.; Steinman, L.; Conlon, P.J. *Jou. Neu.*, **1997**, *74*, 149.

[8] Pette, M.; Fujita, K.; Wilkinson, D.; Altmann, D.M.; Trowsdale, J.; Giegerich, G.; Hinkkanen, A.; Epplen, J.T.; Kappos, L.; Wekerle, H. *Proc. Natl. Acad. Sci. USA*, **1990**, *87*, 7968.

[9] Ausubel, L.J.; Kwan, C.K.; Sette, A.; Kuchroo, V.; Hafler, D.A. *Proc. Natl. Acad. Sci. USA*, **1996**, *93*, 15317.

[10] Martin, R.; Jaraquemada, D.; Flerlage, M.; Richert, J.; Whitaker, J.; Long, E.O.; McFarlin, D.E.; McFarland, H.F. *Science*, **1985**, *230*, 1043.

[11] Wucherpfennig, K.W.; Catz, I.; Hausmann, S.; Strominger, J.L.; Steinman, L.; Warren, K.G. *J. Clin. Invest.*, **1997**, *100*, 1114.

[12] Wauben, M.; Boog, C.; Zee, R.; Joosten, I.; Scihlief, a.; Eden, W. *J. Exp. Med.*, **1992**, *176*, 667-677.

[13] Chou, Y.; Vandenbark, A.; Jones, R.; Hashim, G.; Offiner, H. *J. Neuros. Res.*, **1989**, *22*, 181.

[14] Wucherpfenning, K.; Sette, A.; Southwood, S.; Oseroff, C.; Matsui, M.; Strominger, J.; Hafler, D.A. *J. Exp. Med.*, **1994**, *179*, 279.

[15] Vergelli, M.; Hemmer, B.; Utz, U.; Vogt, A.; Kalbus, M.; Tranquill, L.; Conlon, P.; Ling, N.; Steinman, L,; McFarland, H.; Martin R. *Eur. J. Immunol.*, **1996**, *26*, 2624.

[16] Brocke, S.; Gijbels, K.; Allegretta, M.; Ferber, I.; Piercy, C.; Blankenstein, T.; Martin, R.; Utz, U.; Karin, N.; Mitchell, D.; Verona T.; Waisman, a.; Gaur, A.; Conlon, P.; Ling, N.; Fairchild, P.; Wraith, D.; O'Carra, A.; Farhman, G.; Steinman, L. *Nature*, **1996**, *379*, 343.

[17] Windhagen, A.; Anderson, D.E.; Carrizosa, A.; Balashov, K.; Weiner, H.L.; Hafler, D.A. *J. Neuroimmunol.*, **1998**, *91*, 1.

[18] Bettelli, E.; Das, M.P.; Howard, E.D.; Weiner, H.L.; Sobel, R.A.; Kuchroo, V.K. *J. Immunol.*, **1998**, *161*, 3299.

[19] Inobe, J.; Slavin, A.J.; Komaga, Y.; Chen, Y.; Liu, L.; Weiner, H.L. *Eur. J. Immunol.*, **1998**, *28*, 2780.

[20] Ausubel, L.J.; Kkrieger, J.I.; Hafler, D.A. *J. Immunol.*, **1997** *159*, 2502.

[21] Meager, T. *Ann. Neurol.*, **1998**, *43*, 384.

[22] Wandinger, K.; Wessel, K.; Neustock, P.; Siekhaus, A.; Kirchner, H. *J. Neurol. Sci.*, **1997**, *149*, 87.

[23] Pette, M.; Pett, D.F.; Muraro, P.A.; Farnon, E.; Martin, R.; McFarlan, H. *Neurology*, **1997**, *49*, 385.

[24] European Study Group. *Lancet*, **1998**, *352*, 1491.

[25] Kappos, L.; European Study Group. *Lancet*, **1998**, *352, 1486-1487*, 1491.

[26] Stewart, V.C.; Land, J.M.; Clark, J.B.; Heales, S.J *J. Neurochem.*, **1998**, *70*, 432.

[27] Aharoni, R.; Teitelbaum. D.; Sela, M.; Arnon, R. *Proc. Antl. Acad. Sci. USA*, **1997**, 94, 10821.

[28] Udaltsov A.V. *Biochemistry*, **1999**, 64, 795.

[29] Weiner, H.L. *Annu. Rev. Med.*, **1997**, 48, 341.

[30] (a) Marvin, D., Drugs Topics, **2000**, 93. (b) Noseworthy, J.H., Lucchinetti, C.; Rodriguez, M.; Weinshenker, B.G. *New Eng. J. Med.*, **2000**, *343*,13.

[31] Hafler, D.A.; Kent, S.C.; Pietrusewicz, M.J.; Khoury, S.J.; Weiner, H.L.; Fukaura, H. *Ann. N.Y. Acad. Sci.*, **1997**, *835*, 120.

[32] Kent, S.C.; Fukaura, H.; Piertusewicz, M.J.; Khoury, S.J.; Weiner, H.L.; Hafler, D.A. *Ann. N.Y. Acad. Sci.*, **1997**, *815*, 412.

[33] European Network on Biomedical and Health Research. *"Bioactive Peptide in Drug Discovery and Design: Medical Aspects"*, J. Matsoukas and T. Mavromoustakos (Eds.) Biomedical and Health Research Series, Vol. 22, IOS Press, 1999.

[34] Moore, G.; Smith, J.; Baylis, B.; Matsoukas. J. *Adv. Pharmcol.*, **1995**, *33*, 91.

[35] Giannis, A.; Rubsam, F. Peptidomimetics in Drug Design. *Adv. Drug Res.*, **1997**, *29*, 1-78.

[36] Matsoukas, J.; Hondrelis, J.; Keramida, M.; Mavromoustakos, T.; Makriyannis, A.; Yamdagni, R.; Wu, Q.; Moore, G. *J. Biol. Chem.*, **1994**, *269*, 5303.

[37] Matsoukas, J.; Hondrelis, J.; Agelis, G.; Barlos, K.; Gatos, D.; Ganter, R.; Moore, D.; Moore, G. *J. Med. Chem.*, **1994**, *37*, 2958.

[38] Mavromoustakos, T.; Kolokouris, A.; Zervou, M.; Roumelioti, P.; Matsoukas, J.; Weisemann, R. *J. Med. Chem.*, **1999**, *42*, 1714.

[39] Matsoukas, J.; Ancas, J.; Mavromoustakos, T.; Kolocouris, A.; Roumelioti, P.; Vlahakos, D.; Yamdagni, R.; Wu, Q.; Moore, G. *Bioorg. Med. Chem.*, **2000**, *8*, 1.

[40] Matsoukas, J.; Panagiotopoulos, D.; Keramida, M.; Mavromoustakos, T.; Yamdagni, R.; Wu, Q.; Moore,G.; Saifeddine, M.; Hollenberg, M. *J. Med. Chem.*, **1996**, *39*, 3385.

[41] Alexopoulos, K.; Fatseas, P.; Melissari, E.; Vlahakos, D.; Smith, J.; Mavromoustakos, T.; Saifeddine, M.; Moore, G.; Hollenberg, M.; Matsoukas, J. *Bioorg. Med. Chem.*, **1999**, *7*, 1033.

[42] a. Tselios, T.; Probert, D.; Matsoukas, E.; Troganis, A.; Gerothanassis, P.; Mavromoustakos, T.; Moore, J.;Matsoukas, J. *J. Med. Chem.*, **1999**, *42*, 1170. b. Tselios, T.; Probert, L.; Kollias, G.; Matsoukas, E.; Roumelioti, P.; Alexopoulos, K.; Moore, G.; Matsoukas, J. *Amino Acids*, **1998**, *14*, 333.

[43] a. Tselios, T.; Daliani. I.; Probert, L.; Deraos, S.; Matsoukas, E.; Roy, S.; Pires, J.; Moore, G.; Matsoukas, J. *Bioorg. Med. Chem.*, **2000**, *8*, 1903-1909. b. Tselios, T.; Daliani, I.; Matsoukas, E.; Roumelioti, P.; Alexopoulos, K.; Probert. L.; Pires, J.; Moore, G.; Matsoukas, J. Semi-mimetic peptides with Immunomodulatory activity based on Myelin Basic Protein (74-85): Synthesis and Conformational Properties, in "*Bioactive Peptides in Drug Discovery and Design: Medical Aspects"*, J. Matsoukas and T. Mavromoustakos (Eds.). Biomedical and Health Research Series, Vol. 22, IOS Press, 1999.

[44] Tselios, T.; Daliani, I.; Deraos, S.; Thymianou, S.; Matsoukas, E.; Troganis, A.; Gerothanassis, I.; Mouzaki, A.; Mavromoustakos, T.; Probert, L.; Matsoukas, J. *Bioorg. Med. Chem. Lett*., **2000**, *18*, 2713-1717.

[45] a. Pires, J.; Matsoukas, J.; Moore, G. *Proc. West. Pharmac. Soc.*, **1998**, *41*, 194. b. Pires, J.; Tselios, T.; Matsoukas, J.; Moore, G.; *Drug Devel. Res.*, **1999**, *48*, 1-5, 15.

[46] Moore, G.; Matsoukas, J. Charge Transfer Interactions in Peptide Hormones in Hellenic Forum on Bioactive Peptides., Ed. P. Cordopatis) Crete University Press, **1997**, 161.

[47] Mazzucco, S.; Nardi, E.; Chelli, M.; Ginanneschi, M.; Rapi, G.; Papini, A.; Vergelli, M.; Bazzanti, B.; Massacesi, L.; Amaducci, L. *Lett. in Pept. Sci.*, **1999**, *6*, 51.

[48] Mazzucco, S. Mata, S.; Vergelli, M.; Fioresi, R.; Nardi, E.; Mazzanti, B.; Chelli, M.; Lolli, F.; Ginanneschi, M. Pinto, F.; Massacesi, L.; Papini, A.M. *Bioorg. & Med. Chem. Lett.*, **1999**, *9*, 167.

[49] Tam, J.P.; Lu, V.A. *Proc. Natl. Acad. Sci. U.S.A.*, **1989**, *86*, 9084.

[50] Tam, J.P. *Proc. Natl. Acad. Sci. U.S.A.*, **1988**, *85*, 5409.

[51] Tam, JP.; Clavijo, P.; Lu, Y.; Nussenzweig, R.S.; Nussenzweig, B.; Zavala, F. *J. Exp. Med.*, **1990**, *171*, 299.

[52] Barlos, K.; Gatos, D.; Schafer, W. *Angew. Chem., Int. Ed. Engl.*, **1991,** *30*, 590.

[53] Aletras, A.; Barlos, K.; Gatos, D.; Koutsogianni, S.; Mamos, P. *Int. J. Pept. Prot. Res.*, **1995**, *45*, 488.

[54] Krambovitis, E.; Hatzidakis, G.; Barlos, K. *J. Biol. Chem.*, **1998**, 273, 10874.

[55] Apostolopoulos, V.; Pietersz, G.A.; Loveland, B.E.; Sandrin, M.S.; McKenzie, I.F.C. *Proc. Natl. Acad. Sci. USA*, **1995,** *92*, 10128.

[56] Apostolopoulos, V.; Pietersz, GA.; McKenzie, I. *Vaccine*, **1996**, *14*, 930.

[57] Lofthouse, S.A.; Apostolopoulos, V.; Pietersz, G.A.; McKenzie, I.F.C. *Vaccine*, **1997,** *15*, 1586.

[58] Apostolopoulos, V.; Lofthouse, S.; Popovski, V.; Chelvanayagam G.; Sandrin, M.; McKenzie I. *Nature Biot.*, **1998**, *16*, 276.

[59] Pietersz, G.A.; Wenjun, L.; Popovski, V.; Caruana, J-A.; Apostolopoulos, V.; McKenzie, I.F.C. *Cancer Immunol. Immunother*., **1998,** *45*, 321.

[60] Lees, C.J.; Apostolopoulos, V.; McKenzie, I.F.C. *J. Interferon Cytokine Res.*, **1999,** *19*, 1373.

[61] Apostolopoulos, V.; Pietersz, G.A.; Gordon, S.; Martinez-Pomares, L.; McKenzie, I.F.C. *Eur. J. Immunol.*, **2000,** *30*, 1714.