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(54) Title: SYNTHETIC PEPTIDES FOR THE TREATMENT OF MYASTHENIA GRAVIS

(57) Abstract

Peptides having at least nine amino acid residues each including an amino acid sequence which corresponds to position p200-208 or p262-266 of the human acetylcholine receptor α-subunit, but differing therefrom by one or more amino acid substitutions, are disclosed. These peptides inhibit the proliferative response of human peripheral blood lymphocytes to the myasthogenic peptides p195-212 and p259-271 and are suitable for treatment of subjects afflicted with myasthenia gravis.

SYNTHETIC PEPTIDES FOR THE TREATMENT OF MYASTHENIA GRAVIS

Field of the Invention

The present invention relates to synthetic peptides useful for the treatment of myasthenia gravis (MG) patients, and to pharmaceutical compositions comprising these peptides by themselves, in a polymerized form or attached to a macromolecular carrier.

Description of the Background Art

Autoimmune diseases are characterized by immune responses that are directed against self antigens. These responses are maintained by the persistent activation of self-reactive T lymphocytes. T lymphocytes are specifically activated upon recognition of foreign and/or self antigens as a complex with self Major Histocompatibility Complex (MHC) gene products on the surface of antigen-presenting cells (APC).

Myasthenia gravis (MG) is an autoimmune disorder, the symptoms of which are caused by an antibody-mediated autoimmune attack on the acetylcholine receptor (AChR) of the post-synaptic membrane of the neuromuscular junction. This antibody attack results in loss of acetylcholine receptors and jeopardizes normal neuromuscular transmission, leading to episodic muscle weakness, chiefly in muscles innervated by cranial nerves, and to fatigability.

T lymphocytes are considered to play a central role in the autoreactive process, but the specific immunoregulatory mechanisms by which the T cells exert their regulatory role leading to the induction and various clinical manifestations of MG are poorly understood, and no specific cure is available for the treatment of myasthenic patients. Presently, treatment is with cholinesterase inhibitors, such as pyridostigmine and neostigmine, thymectomy, corticosteroids, and immuno-suppressive agents and plasmapheresis.

MG is a well defined autoimmune disorder mediated by antibodies specific to determinants of the AChR. Specific genes of the human MHC, the HLA system, were shown to be significantly associated with the disease. The high frequency of certain histocompatibility antigens (HLA - B8, DR3) in MG patients suggests a defect of immunoregulation that might be expressed on the level of T cells.

In previous studies we found that two peptides representing sequences of the human AChR α-subunit (p195-212 and p257-269) significantly stimulated peripheral blood lymphocytes (PBL) from MG patients in comparison to healthy controls (Brocke, S. et al. (1988), J. Clin. Invest. 82:1894-1900). In addition, a correlation was demonstrated between the HLA-DR types of the MG patients and their responses to these peptides. Thus, all patients that expressed HLA-DR3 responded to p257-269 and 83% of patients who expressed HLA-DR5 responded to p195-212.

Extension of this research using inbred mouse strains led to the identification of high, intermediate and low responder strains to the sequences p195-212 and p259-271 of the human AChR α-subunit. Furthermore, lymph node cells, from Torpedo-derived AChR immunized SJL and BALB/c mice, proliferated in response to p195-212 and p259-271, respectively, even better than to the immunizing antigen (Brocke, S. et al. (1990), Immunol. 69:495). These results indicate that peptides p195-212 and p259-271 are immunodominant murine T cell epitopes.

Long-term T cell lines and clones of C3H.SW origin specific to synthetic immunogenic peptides p195-212 and p259-271 were established in our laboratory and described by Brocke, S. et al. (1990a), Internat. Immunol. 2:735-742.

Using these cell lines and clones, it is possible to characterize the T cell recognition process of myasthenic epitopes. Using this method, T cell lines and clones specific to p195-212 were established from lymph node cells of low (C3H.SW) and high (SJL) responder mouse strains, and T cell lines and clones specific to p259-271 were developed

from lymph node cells of low (C3H.SW) and high (BALB/c) responder mouse strains.

European patent publication no. 432,691 describes an assay for the measurement of direct binding of a peptide that is a T-cell epitope to gene products of the major histocompatibility complex (MHC), classes I and II, on the surface of intact living antigen-presenting cells (APC). The assay comprises incubating the labelled peptide with the APC and monitoring the extent of binding by the addition of a probe that reacts with the ligand used to label the peptide. The assay is suitable for autoimmune diseases and other immunological disorders. With this assay it was demonstrated that p195-212 binds directly to MHC class II molecules on living APC from several different mouse strains. This observed binding capacity for the peptide was shown to correlate with the proliferative potential of the different mouse strains and was inhibited by the relevant anti-I-A antibodies. In addition, it was shown that APC from MG patients and healthy controls, who responded by proliferation to peptides p195-212 and/or p259-271, also bound the same peptides, labelled with biotin. The ability to screen peptides by their direct binding to MHC products and by their stimulatory capacity to T cells might shed light on the role of MG-related epitopes in the pathogenesis of the disease.

It has been suggested that peptide analogs obtained by amino acid substitutions in a stretch of the sequence of a peptidic antigen relevant to an autoimmune disease might lead to peptides that bind to MHC gene products but that do not stimulate specific helper T cells. Such peptides can be used to inhibit competitively T cell reactivity *in vitro* and *in vivo* and thus for treatment of the corresponding autoimmune disease (Sakai, K. et al. (1989), PNAS 86:9470; Urban, J.L. et al. (1989), Cell 59:257; European patent publication no. 432,691, and PCT publication no. WO 92/04049. However, none of the references provide any information or guidance on the amino acids of the pathogenic peptide that can be substituted and which amino acids can serve as suitable substituents, in

order to obtain a peptide analog that will successfully compete with the pathogenic peptide and thus be useful for preventing or treating autoimmume diseases in general, and myasthenia gravis in particular.

Summary of the Invention

It is an object of the present invention to provide synthetic peptides that are analogs of myasthogenic T cell epitopes which represent sequences of the human AchR subunit, and are designed for specific treatment of myasthenia gravis. These synthetic peptides bind to MHC Class II gene products but do not activate the T cells involved in MG-related autoimmune responses.

The invention thus provides a response-inhibiting peptide of at least nine amino acid residues capable of inhibiting the proliferative response of T lymphocytes from a myasthenia gravis patient to a myasthogenic peptide corresponding to a sequence of the human acetylcholine receptor α-subunit selected from the group consisting of peptide p195-212 having the formula of SEQ ID NO:1:

```
195                     200                     205
Asp-Thr-Pro-Tyr-Leu-Asp-Ile-Thr-Tyr-His-Phe-

                    210
    Val-Met-Gln-Arg-Leu-Pro-Leu
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and the peptide p259-271 having the formula of SEQ ID NO:2:

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    260                     265                     270
Val-Ile-Val-Glu-Leu-Ile-Pro-Ser-Thr-Ser-Ser-Ala-Val
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said response-inhibiting peptide comprising an amino acid sequence which includes at least amino acid residues 200-208 of SEQ ID NO:1 or amino acid residues 262-266 of SEQ ID NO:2, but differing therefrom by one to three amino acid substitutions, said amino acid substitutions being selected from those which cumulatively do not substantially change

the volume, hydrophobic-hydrophilic pattern and charge of the corresponding portion of the unsubstituted myasthogenic peptide wherein 201 Ile, 205 Phe, 206 Val or/and 207 Met of the amino acid residues 200-208 of SEQ ID NO:1, or 263 Leu, 264 Ile or/and 265 Pro of amino acid residues 262-266 of SEQ ID NO:2 is/are substituted by a different one of any of the amino acids Met, Gly, Ala, Phe, Val, Leu, Ile, Pro, or Trp;

wherein 202 Thr, 203 Tyr or/and 208 Gln of the amino acid residues 200-208 of SEQ ID NO:1, or 266 Ser of the amino acid residues 262-266 of SEQ ID NO:2 is/are substituted by a different one of any of the amino acids Gln, Ser, Thr, Tyr, Arg, Lys, His, Asn, Asp, or Glu;

wherein 200 Asp or/and 204 His of the amino acid residues 200-208 of SEQ ID NO:1 or 262 Glu of the amino acid residues 262-266 of SEQ ID NO:2 is/are substituted by a different one of any of the amino acids Ser, Thr, Gln, Tyr, Arg, Lys, His, Asn, Glu or Asp;

wherein a hydrophilic amino acid residue of the amino acid residues 200-208 of SEQ ID NO:1 or of 262-266 of SEQ ID NO:2 is substituted with a hydrophobic amino acid; or/and

wherein a hydrophobic amino acid residue of the amino acid residues 200-208 of SEQ ID NO:1 or of 262-266 of SEQ ID NO:2 is substituted with a hydrophilic amino acid residue.

The invention further relates to pharmaceutical compositions and to a method for treatment of myasthenia gravis by administration of the peptides of the invention by themselves, in a polymerized form or attached to a macromolecular carrier.

The invention also provides a method for selecting response-inhibiting peptides capable of inhibiting the proliferative response of T lymphocytes from a myasthenia gravis patient, comprising:

synthesizing a peptide of at least nine amino acid residues which includes at least amino acid residues 200-208 of SEQ ID NO:1 or amino acid residues 262-266 of SEQ ID NO:2, but different therefrom by one to three amino acid substitutions, said substitutions being selected from those

which cumulatively do not substantially change the volume, hydrophobic-hydrophilic pattern and charge of the corresponding portion of the unsubstituted myasthogenic peptide, said substitutions being as defined in claim 1;

testing said peptide for its ability to inhibit the proliferative response of T cells from a myasthenia gravis patient, or a T cell line or clone which is specific to a myasthogenic peptide of SEQ ID NO:1 or SEQ ID NO:2 from which said peptide is derived, to the corresponding peptide of SEQ ID NO:1 or SEQ ID NO:2 to which the T cells are specific; and

selecting and producing said peptide only if it is capable of inhibiting said proliferative response.

Brief Description of the Drawings

Figure 1 is a graph showing proliferative responses of the T cell line, TCBALB/c259-271, to various doses of the myasthogenic peptide p259-271 and its analogs p305, p306 and p307.

Figure 2 is a graph showing inhibition of the p259-271 specific proliferative response of the T cell line TCBALB/c259-271, with various doses of the inhibitory peptides p305, p306, p307 and p195-212.

Figure 3 is a graph showing specificity of the helper activity of the T cell line, TCBALB/c259-271, with various doses of the peptides (antigen = Ag) p259-271, p305, p306 and p307.

Figure 4 is a graph showing inhibition of the helper activity of the T cell line, TCBALB/c259-271, with various doses of the peptide p259-271 alone or in combination with each of the analogs p305, p306 and p307.

Figure 5 is a graph showing proliferative responses of lymph node cells of BALB/c mice immunized with p259-271, to various doses of p259-271, p305, p306 and p307.

Figure 6 is a graph showing inhibition of the proliferative responses of lymph node cells of BALB/c mice immunized with p259-271, to various doses of p259-271 alone or in combination with p306.

Figure 7 is a graph showing inhibition of the p195-212 specific proliferative response of the T cell line, TCSJL195-212, with two different doses of mixtures of p195-212 with its analog p455.

Detailed Description of Preferred Embodiments

The present invention relates to analogs of myasthogenic peptides p259-271 (SEQ ID NO:1) and p195-212 (SEQ ID NO:2) which will bind with high affinity to the appropriate MHC Class II molecules but will not lead to further activation of T cells. Examples of such analogs are provided, as is a procedure which may be followed by anyone of ordinary skill in the art in order to identify additional peptides which will

also accomplish this function. The invention is based on the design and synthesis of peptides with amino acid substitutions at different positions that are based on parent peptides p195-212 and p259-271. By means of appropriate substitutions, analogs can be identified which are antagonists to the action of the myasthogenic epitopes in the course of myasthenia gravis. An analog which will bind with high affinity to the appropriate MHC Class II molecules but will not lead to further activation of T cells will compete with the myasthogenic peptides which must bind to the same MHC Class II molecules in order to cause activation of the T cells which, in turn, lead to the production of the antibodies which cause myasthenia gravis. By competing with the myasthogenic peptides which cause T cell proliferation, the adverse effects of myasthenia gravis can be ameliorated.

It has been discovered that the portions of the myasthogenic epitopes p259-271 and p195-212 which are most important for their function as a T cell epitope are amino acid residues 200-208 of SEQ ID NO:1 and 262-266 of SEQ ID NO:2. Thus, the changes are preferably made in these core areas. It is expected that the amino acid residues outside of these core areas are not as important to the function of binding to the appropriate MHC Class II molecules and thus it is not expected that changes in these amino acid residues will prevent further activation of the T cells. Accordingly, there is no need to change them, but if substitutions are made in the non-core amino acid residues, they should be selected to fulfill the guidelines discussed herein as to the cumulative effects of the substitutions.

Amino acids may be divided along the lines of volume, hydrophobic-hydrophilic pattern and charge. With respect to volume, those of ordinary skill in the art understand that the amino acids with the largest volume are Trp, Tyr, Phe, Arg, Lys, Ile, Leu, Met and His, while those with the smallest volumes are Gly, Ala, Ser, Asp, Thr and Pro, with others being in between.

With respect to hydrophobic-hydrophilic pattern, it is will known that the amino acids Gly, Ala, Phe, Val, Leu, Ile, Pro, Met and Trp are hydrophobic, whereas all of the remaining amino acids are hydrophilic. Among the hydrophilic amino acids, Ser, Thr, Gln and Tyr have no charge, while Arg, Lys, His and Asn have a positive charge and Asp and Glu have negative charges.

What is important in selecting peptides to be tested for their potential in inhibiting the proliferative response of T lymphocytes from a myasthenia gravis patient to the myasthogenic peptide SEQ ID NO:1 or SEQ ID NO:2 to which it corresponds, is that the substitutions be selected from those which cumulatively do not substantially change the volume, hydrophobic-hydrophilic pattern and charge of the corresponding portion of the unsubstituted myasthogenic peptide. Thus, a hydrophobic residue may be substituted with a hydrophilic residue, or vice-versa, as long as the total effect does not substantially change the volume, hydrophobic-hydrophilic pattern and charge of the corresponding unsubstituted myasthogenic peptide. As indicated above, these substitutions are preferably made in the important epitopic core areas of residues 200-208 of SEQ ID NO:1 and 262-266 of SEQ ID NO:2.

It is preferred that the synthesized analog of the present invention have a length of at least nine amino acids and preferably from 9-12 amino acids. Each of the analogs preferably has from 1-3 substitutions in the core area.

In a preferred embodiment of the present invention, the substitution or substitutions are selected so as to substitute a hydrophobic amino acid residue in the core area with another hydrophobic amino acid residue selected so as not to cumulatively substantially change the volume of the unsubstituted peptide. For example, in p200-208, the hydrophobic amino acids are 201 Ile, 205 Phe, 206 Val and 207 Met. Each of these may be substituted with any of the other hydrophobic residues in this preferred embodiment. Thus, for example, 207 Met may be substituted with Gly, Ala, Phe, Val, Leu, Ile, Pro or Trp. It has been established that

substitution with Ala provides particularly good results. Similarly, in p262-266, 262 Leu, 264 Ile and 265 Pro are all hydrophobic. Thus, from one to three of these can be substituted with another hydrophobic residue so long as the cumulative effect on the volume is not substantial. Thus, for example, the 265 Pro may be substituted by Gly, Ala, Phe, Val, Leu, Ile, Met or Trp. It has been found that substituting the 265 Pro with Phe gives particularly good results.

In a second embodiment, a hydrophilic non-charged residue may be replaced by a charged or non-charged hydrophilic residue. Hydrophilic non-charged residues in the p200-208 peptide include 202 Thr, 203 Tyr and 208 Gln. In the p262-266 peptide, 266 Ser is a non-charged hydrophilic residue. Thus, for example, 208 Gln may be changed to any of Ser, Thr, Tyr, Arg, Lys, His, Asn, Asp or Glu and 266 Ser may be changed to any Thr, Gln, Tyr, Arg, Lys, His, Asn, Asp or Glu. Again, the change must be selected such that the cumulative effect of all changes does not substantially change the volume, hydrophobic-hydrophilic pattern and charge of the corresponding portion of the unsubstituted myasthogenic peptide. Specific examples of changes within this embodiment are the substitution of 208 Gln with Asn or Asp and the substitution of 266 Ser with Lys or Asp.

In another embodiment of the present invention, charged hydrophilic residues are substituted by charged (same or opposite charge) or non-charged hydrophilic residues. In the p200-208 peptide, 200 Asp is a negatively-charged hydrophilic residue and 204 His is a positively-charged hydrophilic residue. In p262-266, 262 Glu is a negatively-charged hydrophilic residue. Thus, for example, 200 Asp may be substituted with Ser, Thr, Gln, Tyr, Arg, Lys, His, Asn or Glu and 262 Glu may be substituted with Ser, Thr, Gln, Tyr, Arg, Lys, His, Asn or Asp. Specific examples may be substituting 200 Asp with Lys (opposite charge) or 262 Glu with Ser (charged to non-charged). Again, whatever substitution is made must be selected so as to not cumulatively substantially change the volume, hydrophobic-hydrophilic pattern and charge of the

corresponding portion of the unsubstituted myasthogenic peptide.

Finally, in a further embodiment, it is also possible to replace a hydrophilic residue with a hydrophobic residue or vice-versa, as long as the cumulative effect is within the guidelines. Examples of such substitutions are the substitution of 204 His with Gly or 203 Tyr with Phe or the substitution of 262 Glu with Ala.

Those of ordinary skill in the art of peptide chemistry will readily recognize, by strictly theoretical considerations, what the cumulative effect will be on the charge, hydrophobic-hydrophilic pattern and volume of a given peptide as short as nine amino acids. Thus, it would not take undue experimentation to determine which substitutions should be tried in order to identify analogs in accordance with the present invention which have the function of competing with the native myasthogenic peptides for binding the appropriate MHC Class II molecules but which will not lead to further activation of T cells.

It should be understood that the amino acids which are used for substituting into the native peptide may include modified peptides such as norleucine, hydroxyproline, hydroxylysine, gamma-carboxyglutamic acid, etc.

Some specific substitutions, any 1-3 of which which otherwise comply with the guidelines presented herein, may be present in an analog according to the present invention, include the following: 200, Asp-->Lys; 203, Tyr-->Phe 204, His-->Gly; 204, Tyr-Phe; 207, Met-->NLeu; 207, Met-->Ala; 208, Gln-->Asp; 208, Glu-->Asn; 262, Glu-->Asp; 262, Glu-->Lys; 262, Glu-->Ser; 262, Glu--Ala; 265, Pro-->Leu; 265, Pro-->Phe; 266, Ser-->Lys; and 266, Ser-->Asp.

It is expected that the substitution of hydrophilic amino acids, that are mainly involved in T cell receptor interactions, will be effective in blocking T cell activation. Substitutions in the hydrophobic residues may contribute to higher stability of the analog-MHC complexes.

It should be understood that other modifications of the myasthogenic peptides are also contemplated by the present invention. Thus, the peptide of the present invention is intended to include a "chemical derivative" thereof which retains at least a portion of the function of the peptide which permits its utility in preventing or inhibiting T cell proliferative responses and autoimmune disease.

A "chemical derivative" of a peptide of the present invention contains additional chemical moieties not normally a part of the peptide. Covalent modifications of the peptide are included within the scope of this invention. Such modifications may be introduced into the molecule by reacting targeted amino acid residues of the peptide with an organic derivatizing agent that is capable of reacting with selected side chains or terminal residues. Many such chemical derivatives and methods for making them are well known in the art.

Also included in the scope of the invention are salts of the peptides of the invention. As used herein, the term "salts" refers to both salts of carboxyl groups and to acid addition salts of amino groups of the peptide molecule. Salts of a carboxyl group may be formed by means known in the art and include inorganic salts, for example, sodium, calcium, ammonium, ferric or zinc salts, and the like, and salts with organic bases such as those formed for example, with amines, such as triethanolamine, arginine, or lysine, piperidine, procaine, and the like. Acid addition salts include, for example, salts with mineral acids such as, for example, hydrochloric acid or sulfuric acid, and salts with organic acids, such as, for example, acetic acid or oxalic acid. Such chemical derivations would preferably be used to modify the pharmaceutical properties of the peptide insofar as stability, solubility, etc., are concerned.

Once an analog in accordance with the present invention is produced, its ability to inhibit the proliferative response of T lymphocytes to the corresponding myasthogenic peptides may be readily determined by those of ordinary skill

in the art without undue experimentation using tests such as those described herein. One test which may be readily conducted is for the ability of substituted peptides to inhibit *in vitro* the proliferative responses of certain T cell lines and clones to the original peptide. The T cell lines and clones are those which have been produced which are specific to the myasthogenic epitopes of SEQ ID NO:1 or SEQ ID NO:2. Another test which can be conducted in order to select analogs having the desired activity is to test for the ability of the substituted peptides to inhibit the ability of the T cell lines and clones to provide help to peptide-specific B cells in the presence of the parent peptide. The substituted peptides may also be tested for their ability to bind directly, following biotinylation, to MHC Class II products on antigen-presenting cells of the relevant strains and to inhibit the binding of the parent myasthogenic epitopes.

Substituted peptides which test positive in one or more of these *in vitro* tests will provide a reasonable expectation of *in vivo* activity. However, *in vivo* tests can also be conducted without undue experimentation. One such *in vivo* animal test would be to immunize naive mice with the myasthogenic peptide and co-inject the mice with the analogs by various routes and dose schedules. Lymph node cells can then be analyzed for their proliferative potential to the myasthogenic peptides. In addition, titers of anti-AChR antibodies in the sera of these mice can be measured to record the effect of the analogs on the *in vivo* helper cell activity. The advantages of these *in vivo* assays is that, although they are not aimed at assessing the effects of the analogs on disease induction, they allow *in vivo* screening of all analogs in a relatively short time.

As final proof of therapeutic activity, such activity may be directly measured in a murine model *in vivo*. It has previously been shown that some T cell lines and clones specific to the myasthogenic T cell epitopes are capable of inducing MG-related autoimmune manifestations in mice (Yaffe, D. et al. (1977), Nature 270:725-727; and Inestrosa, N.C. et

al. (1983), Exp. Cell. Res. 147:393-405). Therefore, naive mice can be injected with such clones and treated with the selected substitute peptides in order either to prevent or to remit the autoimmune responses. The peptides can be injected into the mice by different routes at different dosages and at different time schedules. In order to determine the pharmacokinetic parameters of the analogs, including volume of distribution, uptake into antigen preventing cells and clearance, one can use biotinylated derivatives of the analogs. The concentration of the soluble fraction of the analogs in the various body fluids can be determined by ELISA, using avidin-coated plates and specific anti-peptide antibodies. Cell bound analogs can be analyzed by FACS, using fluorochrome-conjugated avidin or streptavidin. Furthermore, the treated mice can be tested periodically in order to determine the effect of the peptides on the autoimmune responses and on disease manifestations elicited in the mice by the T cell clones.

It can thus be seen that, besides the preferred embodiments which have been shown to be operable in the examples herein, those of ordinary skill in the art will be able to determine additional analogs which will also be operable following the guidelines presented herein without undue experimentation.

A relatively simple *in vitro* test can also be conducted in order to assay for the expected therapeutic efficacy of any given substituted peptide on any given myasthenia gravis patient. In order to assess the ultimate goal of producing peptides that will bind with high affinity to the appropriate MHC Class II molecules but will not lead to further activation of T cells and will therefore have a therapeutic effect on MG patients, the peptides may be assayed, following biotinylation, for their ability to bind directly to HLA Class II products on antigen-presenting cells in the peripheral blood lymphocytes of the myasthenia gravis patients and to inhibit the binding of the parent myasthogenic epitopes. Healthy control donors and control peptides may be used in such assays to verify their specificity.

A preferred form of the therapeutic agent in accordance with the present invention is the form of a multi-epitope single peptide. Thus, in a preferred embodiment, analogs of each of the two myasthogenic peptides are covalently linked to one another, such as by a short stretch of alanine residues or by a putative site for proteolysis by cathepsin. See, for example, U.S. Patent 5,126,249 and European Patent 495,049 with respect to such sites. This will induce site-specific proteolysis of the preferred form into the two desired analogs. Alternatively, a number of the same or different substituted peptides of the present invention may be formed into a peptide polymer such as, for example, polymerization of the peptides with a suitable polymerization agent, such as 0.1% glutaraldehyde (Audibert et al. (1981), Nature 289:593). The polymer will preferably contain from 5 to 20 peptide residues. Such peptide polymers may also be formed by cross-linking the peptides or attaching multiple peptides to macromolecular carriers. Furthermore, the formulation may simply be a mixture of different peptides in accordance with the present invention.

Suitable macromolecular carriers are, for example, proteins, such as tetanus toxoid, and linear or branched copolymers of amino acids, such as a linear copolymer of L-alanine, L-glutamic acid and L-lysine and a branched copolymer of L-tyrosine, L-glutamic acid, L-alanine and L-lysine (T,G)-A--L, or multichain poly-DL-alanine (M. Sela et al. (1955), J. Am. Chem. Soc. 77:6175). The conjugates with the carriers are obtained, for example, by first coupling the peptide with a water-soluble carbodiimide, such as 1-ethyl-3 (3'-dimethylaminopropyl) carbodiimide hydrochloride, and then performing the conjugation with the macromolecular carrier as described by Muller, G.M. et al. (1982), Proc. Natl. Acad. Sci. USA 79:569. The contents of the coupled peptide in each conjugate are determined by amino acid analysis, in comparison to the composition of the carrier alone.

According to a preferred embodiment of the present invention, one or more active peptides may be attached to a

suitable macromolecular carrier or may be polymerized in the presence of glutaraldehyde.

The peptides, polymers thereof or their conjugates with suitable macromolecular carriers, will be given to patients in a form that insures their bioavailability, making them suitable for treatment. If more than one peptide analog is found to have significant inhibitory activity, these analogs will be given to patients in a formulation containing a mixture of the peptides. If an individual patient responds to both pathogenic MG related peptides, namely p195-212 and p259-271, the ultimate treatment will contain the appropriate inhibitory analogs of both peptides in a suitable form.

The invention further includes pharmaceutical compositions comprising at least one synthetic peptide according to the invention, a conjugate thereof with a suitable macromolecular carrier or a polymer thereof optionally with a pharmaceutically acceptable carrier.

The route of administration may include oral, intravenous, subcutaneous, intraarticular, intramuscular, by inhalation, intraperitoneal, intranasal, intrathecal, intradermal, transdermal or other known routes, including the enteral route.

The dose ranges for the administration of the compositions of the present invention are those large enough to produce the desired effect, whereby, for example, an immune response to the myasthogenic peptide, as measured by T cell proliferation *in vitro*, is substantially prevented or inhibited, and further, where the disease is significantly treated. The doses should not be so large as to cause adverse side effects, such as unwanted cross reactions, generalized immunosuppression, anaphylactic reactions and the like.

Effective doses of the peptides of this invention for use in treating an immune-related disease are in the range of about 1 μg to 100 mg/kg body weight. The dosage administered will be dependent upon the age, sex, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The synthetic analogs of sequences of the human AChR are aimed at inhibiting or suppressing specific antigen responses of MG patients, without harming other immune responses. This approach is of utmost importance since the currently accepted treatment for MG involves administration of immunosuppressive agents that are both non-specific and have multiple adverse side effects.

The invention will now be illustrated by the following non-limiting examples.

EXAMPLES

Example 1. Preparation of the peptides

The synthetic peptides p195-212 and p259-271 and their analogs p455 (207, Met-->Ala); (200, Asp-->Lys); (203, Tyr-->Phe); (204, His-->Gly); (208, Gln-->Asp); p305 (266, Ser-->Asp); p306 (262, Glu-->Lys); p307 (262, Glu-->Asp); (262, Glu-->Ser); and (262, Glu-->Ala) are synthesized by the Merrifield solid phase technique (Merrifield et al. (1963), J. Am. Chem. Soc. 85:2149), with a peptide synthesizer, using commercially available side-chain protected amino acids. Amino acids are added at each step with at least 99% efficiency. The protecting groups are removed and the peptides are cleared from the resin with anhydrous HF.

The peptides are purified by extraction with ethyl acetate or isopropyl acetate and by HPLC. The purity of the peptides is verified by HPLC and by amino acid analysis.

While all of the above peptides have only one substitution each, other peptides having two or three substitutions and retaining the goals of the guidelines presented herein may be synthesized in the same manner.

Example 2. Inhibition of proliferative responses *in vitro* of T cell clones to peptide p259-271

T cell lines and clones specific to p259-271 were developed from lymph node cells of high responder BALB/c mice according to the method described by Brocke et al. (1990a),

supra, and designated TCBALB/c259-271 (Mozes, E. et al. (1991), "Abstracts, 15th International Congress of Biochemistry, Jerusalem, p. 20).

The proliferative response of the T cell clones was assessed by measuring ^{3}H-thymidine incorporation into cells following incubation for the final 16 hours of culture. Cells (10^4 cells/well) of the T cell line TCBALB/c259-271 were incubated in the presence of irradiated (3000 rad) syngeneic spleen cells from BALB/c mice (0.5 x 10^6 cells/well) as antigen-presenting cells, in the presence of various concentrations (1, 5, 10, 20 μg/well) of the peptides p195-212, p259-271, p305, p306 and p307. Cultures were set in 0.2 ml RPMI 1640 medium supplemented with 2mM glutamine, 1mM sodium pyruvate, non-essential amino acids, 100 U/ml penicillin, 100 μg/ml streptomycin, 0.25 μg/ml fungizone, 5 x 10^{-5} M 2-mercaptoethanol, 10mM HEPES buffer (enriched medium) and 10% fetal calf serum (FCS), for 48 hr, followed by an overnight pulse with ^{3}H-thymidine (0.5 μCi of 5 Ci/mmol). Following incorporation of the isotope, cells were harvested 16 hr later onto a filter paper and radioactivity was determined. The results are shown in Fig. 1 (expressed as mean CPM of triplicates ± SD). Peptides p305 and p307 triggered low proliferative responses of the T cell line at all doses tested (up to 12% and 34.3% respectively, of the response obtained with p259-271), whereas p306 did not stimulate the TCBALB/c259-271 cell line to proliferate. It is not surprising that peptide p195-212 is also shown to competitively inhibit proliferation of T cells specific for peptide p259-271, as p195-212 is directed to the same MHC-determinant as p259-271. Brocke et al. (1990a), *supra*, reports that p195-212 inhibits the proliferative response of the TCSW 259-271 T cell line and p259-271 inhibits the specific proliferative response of the TCSW 195-212 line.

Inhibition of the proliferative responses was performed by addition of increasing doses of the tested substituted peptides (25, 50, 75, 100 μg/well) into the *in vitro* proliferative cultures. Cells (10^4 cells/well) of the T

cell line TCBALB/c259-271, were incubated in the presence of irradiated syngeneic spleen cells (0.5 x 10^6 cells/well), p259-271 (1 μg/well) and the various doses of the inhibitory peptides p305, p306, p307 and of p195-212, for 48 h. Thereafter, ^{3}H-thymidine (0.5 μCi of 5Ci/mmol) was added, and 16 h later plates were harvested onto a filter paper. Results are expressed as % of inhibition of the p259-271 specific proliferative response. As shown in Figure 2, p306 inhibited up to 93% of the proliferative response of the TCBALB/c259-271 line to p259-271.

Example 3. ***In vitro* Antibody Production Assay**

The synthetic peptides p305, p306 and p307 were examined for their ability to stimulate the TCBALB/c259-271 T cell line to collaborate with peptide-specific B cells in an antibody production assay. B cells, purified from spleens of BALB/c mice previously immunized with 20 μg of p259-271, were cultured (0.5 x 10^6 cells/well) with normal syngeneic spleen cells (0.5 x 10^6 cells/well), different doses of p305, p306 and p307 (0.001, 0.01, 0.1, 1 and 5 μg/well) and cells of the TCBALB/c259-271 line (10^4 cells/well), in 96-well microtiter plates. After 3 days incubation period, medium (enriched RPMI supplemented with 7.5% FCS) was exchanged with fresh medium without antigen, and 4 days later, supernatants were harvested and tested (by solid phase RIA) for the presence of specific anti-p259-271 antibodies. Results are expressed as mean CPM of triplicates ± SD.

As can be seen in Figure 3, low antibody titers were obtained in the presence of p305, whereas the helper activity stimulated by p307 was as efficient as that observed in the presence of the parent peptide. In contrast, no antibody activity could be detected when p306 was added to the culture.

Inhibition of the T cell helper activity was performed by addition of increasing doses of the tested substituted peptides into the *in vitro* culture mixtures. B cells, purified from spleens of BALB/c mice previously immunized with 20 μg of p259-271, were cultured (0.5 x 10^6

cells/well) with normal syngeneic spleen cells (0.5 x 10^6 cells/well), different doses of p259-271 alone (0.001, 0.01, 0.1, 1 and 5 μg/well) or together with p305, p306 or p307 (20μg of each) and cells of the TCBALB/c259-271 line (10^4 cells/well), in 96-well microtiter plates. After 3 days incubation period, medium as above was exchanged with fresh medium, and 4 days later, supernatants were harvested and tested (by solid phase RIA) for the presence of specific anti-p259-271 antibodies. Results are expressed as mean CPM of triplicates ± SD. As shown in Figure 4, p259-271 specific antibody production was inhibited up to 80% in the presence of p306.

Example 4. Antigen-Specific Proliferative Response of Mouse Lymph Node Cells After Immunization with p259-271

In order to find out whether the peptides p305, p306 and p307 will either stimulate or inhibit the proliferative response of a more heterogeneous T cell population, namely lymph nodes, the following experiments were performed.

The peptides p305, p306 and p307 were examined for their ability to stimulate lymph node cells of p259-271 immunized BALB/c mice to proliferate, in comparison to the parent peptide p259-271. Lymph node cells (0.5 x 10^6 cells/well) obtained from BALB/c mice immunized with p259-271, were incubated in enriched medium containing 1% normal mouse serum, in the presence of various concentrations of the peptides (10, 20, 50, 100 μg/well) for 96 h. Thereafter, ^{3}H-thymidine (0.5 μCi of 5 Ci/mmol) was added and 16 h later plates were harvested onto a filter paper. Results are expressed as mean CPM of triplicates. As shown in Fig. 5, peptides p305 and p307 triggered low proliferative responses of the cells (up to 53.2% and 32.3% respectively, of that obtained by using p259-271) whereas p306 did not stimulate the lymph node cells to proliferate.

Inhibition of the proliferative responses of lymph node cells of BALB/c mice immunized with p259-271 was done by adding the peptide analog p306 into the incubation mixture at

the same time as the pathogenic peptide p259-271. Lymph node cells (0.5 x 10^6 cells/well) obtained from BALB/c mice immunized with p259-271, were incubated in the presence of various concentrations of p259-271 (1, 5, 10, 20 μg/well) and 100 μg/well of p306 for 96 h. Thereafter, ^{3}H-thymidine (0.5 μCi of 5 Ci/mmol) was added and 16 h later plates were harvested onto a filter paper. Results are expressed as mean CPM of triplicates. As shown in Fig. 6, the peptide p306 inhibited up to 64.7% of the proliferative response of the lymph node cells to p259-271.

Example 5. Inhibition of Proliferative Responses *in vitro* of T Cell Clones to Peptide p195-212

T cell lines and clones specific to p195-212 were established from lymph node cells of high (SJL) responder mouse strain by the method described by Brocke et al. (1990a), *supra*, and designated TCSJL195-212 line (Mozes, E. et al. (1991), *supra*).

The proliferative response of the T cell clones was assessed as in the protocol Example 2 which resulted in Fig. 2.

The p195-212 peptide analog, p455, did not stimulate cells of the TCSJL195-212 line to proliferate. Moreover, as shown in Figure 7, p455 inhibited more than 99% of the proliferative response of the TCSJL195-212 line to p195-212. This represents very substantial inhibition.

Example 6. Inhibition of Proliferation of Human T Cells to the Pathogenic Peptides of Myasthenia Gravis

The action of the pathogenic peptide p259-271 and its analogs p305, p306 and p307, and of p195-212 and its analog p455, on proliferative responses of human T cells was assessed.

Peripheral blood lymphocytes (PBL) of myasthenia gravis patients and of the appropriate control donors (2 x 10^5 cells/well) were assayed in microtiter plates in 0.2 ml enriched medium containing 10% autologous serum, in the

presence of different doses of the peptides p195-212 and the analog p455 for 96h, followed by an overnight pulse with 0.5 μCi of ^{3}H-thymidine. Cells were harvested and radioactivity determined. Inhibition of the proliferative responses was done by adding different doses of p455 into the incubation mixture at the same time as the pathogenic peptide p195-212. Results are reported in Table 1 as (SI) stimulation indices (col. 2 and 3), % inhibition (col. 4).

The PBL of MG patients were tested for their ability to proliferate in the presence of p195-212 and the peptide analog p455. Further, the ability of the analog p455 to inhibit p195-212 specific proliferative responses of the PBL was tested. Table 1 summarizes the responses of three MG patients in these tests. As can be seen in this table, PBL from patients E.K., C.G. and M.R. proliferated in response to p195-212 (SI=6.3, 4.3 and 3.6, respectively), whereas no proliferative responses to the analog were detected at all concentrations (10, 25, 50, 100 μM, corresponding to 20, 50, 100, 200 μg/well) tested. In addition, the analog was able to inhibit the proliferative responses of the PBL from all three patients by 77-100% at an Inhibitor:Stimulator (I:S) ratio of 1:1.

TABLE 1

Peripheral Blood Lymphocyte Proliferative Responses of Myasthenia Gravis Patients to p195-212 and its Analog p455

Patient	p195-212	Analog SI	% Inhibition
	SI* (μc/well)!	μg/well)	p455:p195-212)
E.K.	6.3 (50)	1	77 (1:1)
C.G.	4.3 (25)	1	100 (1:1)
M.R.	3.6 (25)	1	100 (1:1)

* Stimulation Index = response/background

! Concentration of peptide at SI

The PBL of MG patients were assayed as above for their ability to proliferate in the presence of different doses of p259-271 and its analogs p305, p306 and p307. In addition, the ability of different doses of the analogs to inhibit p259-271 specific proliferative responses of the PBL was tested. Table 2 summarizes the responses of three MG patients in these tests. As can be seen in this table, PBL from patients I.T., E.K. and C.G. proliferated in response to p259-271 (SI=4.1, 3.7 and 2.7, respectively). Moreover, for each patient, at least one analog was able to inhibit the p259-271 induced proliferative response of the PBL.

TABLE 2

Peripheral Blood Lymphocyte Response of Myasthenia Gravis Patients to p259-271 and its Analogs

Patient	p259-271 SI* (μM)!	p305 % Inhibition (I:S)**	p306 % Inhibition (I:S)	p307 % Inhibition (I:S)
I.T.	4.1 (25)	34.3 (4:1)	0	78 (1:1)
E.K.	3.7 (100)	82.7 (1:1)	100 (1:1)	70 (1:1)
C.G.	2.7 (100)	85.6 (1:1)	71.6 (1:1)	65 (1:1)

* Stimulation Index = response/background

! Concentration of peptide at SI

** Inhibitory peptide:Stimulating peptide

Example 7. Induction and Treatment of Experimental MG in Mice

Naive syngeneic mice are inoculated (i.v. in PBS 14 times) with activated peptide specific T cells of the lines TCSJLp195-212 and TCBALB/c259-271 or their derived clones (5-10 x 10^6 cells). Autoimmune response is determined by serological (e.g., anti murine AChR antibodies), histological and electrophysiological parameters.

An autoimmune response was induced in the inoculated mice as demonstrated by the ability of their sera to stain cells of the C_2 line, a murine muscle cell line that expresses

AChR upon differentiation (described in Yaffe, D. and Saxel, O. (1977), *supra*; and Inestrosa, N.C. et al., (1983), *supra*). Furthermore, electromyography of the inoculated BALB/c mice revealed a typical myasthenic decrement of the compound muscle action potential (CMAP), that was not observed in the control groups.

In order to prevent or cure the induced experimental MG, peptide analogs are administered a) before inoculation of the peptide specific T cells; b) concomitant with inoculation of the peptide specific T cells; c) after inoculation with the peptide specific T cells but before the appearance of autoimmune responses; d) after the appearance of experimental MG. Reduction in the severity of the autoimmune response indicates suitability of treatment with the peptide.

Reference to known method steps, conventional method steps, known methods or conventional methods is not in any way an admission that any aspect, description or embodiment of the present invention is disclosed, taught or suggested in the relevant art.

The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art (including the contents of the references cited herein), readily modify and/or adapt for various applications such specific embodiments, without undue experimentation, without departing from the general concept of the present invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented

herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance presented herein, in combination with the knowledge of one of ordinary skill in the art.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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MOZES, EDNA

(ii) TITLE OF INVENTION: SYNTHETIC PEPTIDES FOR THE TREATMENT OF MYASTHENIA GRAVIS

(iii) NUMBER OF SEQUENCES: 2

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/900,393
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO 1:

Asp Thr Pro Tyr Leu Asp Ile Thr Tyr His Phe Val Met
1 5 10

Gln Arg Leu Pro Leu
15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Val Ile Val Glu Leu Ile Pro Ser Thr Ser Ser Ala Val
1 5 10

CLAIMS:

1. A response-inhibiting peptide of at least nine amino acid residues capable of inhibiting the proliferative response of T lymphocytes from a myasthenia gravis patient to a myasthogenic peptide corresponding to a sequence of the human acetylcholine receptor α-subunit selected from the group consisting of peptide p195-212 having the formula of SEQ ID NO:1:

```
195                     200                     205
Asp-Thr-Pro-Tyr-Leu-Asp-Ile-Thr-Tyr-His-Phe-

                    210
    Val-Met-Gln-Arg-Leu-Pro-Leu
```

and the peptide p259-271 having the formula of SEQ ID NO:2:

```
260                 265                 270
Val-Ile-Val-Glu-Leu-Ile-Pro-Ser-Thr-Ser-Ser-Ala-Val
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said response-inhibiting peptide comprising an amino acid sequence which includes at least amino acid residues 200-208 of SEQ ID NO:1 or amino acid residues 262-266 of SEQ ID NO:2, but differing therefrom by one to three amino acid substitutions, said amino acid substitutions being selected from those which cumulatively do not substantially change the volume, hydrophobic-hydrophilic pattern and charge of the corresponding portion of the unsubstituted myasthogenic peptide wherein 201 Ile, 205 Phe, 206 Val or 207 Met of the amino acid residues 200-208 of SEQ ID NO:1, or 263 Leu, 264 Ile or 265 Pro of amino acid residues 262-266 of SEQ ID NO:2 is/are substituted by a different one of any of the amino acids Met, Gly, Ala, Phe, Val, Leu, Ile, Pro, or Trp;

wherein 202 Thr, 203 Tyr or 208 Gln of the amino acid residues 200-208 of SEQ ID NO:1, or 266 Ser of the amino acid residues 262-266 of SEQ ID NO:2 is/are

substituted by a different one of any of the amino acids Gln, Ser, Thr, Tyr, Arg, Lys, His, Asn, Asp, or Glu;

wherein 200 Asp or 204 His of the amino acid residues 200-208 of SEQ ID NO:1 or 262 Glu of the amino acid residues 262-266 of SEQ ID NO:2 is/are substituted by a different one of any of the amino acids Ser, Thr, Gln, Tyr, Arg, Lys, His, Asn, Glu or Asp;

wherein a hydrophilic amino acid residue of the amino acid residues 200-208 of SEQ ID NO:1 or of 262-266 of SEQ ID NO:2 is substituted with a hydrophobic amino acid; or

wherein a hydrophobic amino acid residue of the amino acid residues 200-208 of SEQ ID NO:1 or of 262-266 of SEQ ID NO:2 is substituted with a hydrophilic amino acid residue.

2. A response-inhibiting peptide according to claim 1, conjugated to a macromolecular carrier.

3. A response-inhibiting peptide according to claim 2, wherein the macromolecular carrier is a protein or a copolymer of amino acids.

4. A response-inhibiting peptide according to claim 3, wherein the protein carrier is tetanus toxoid.

5. A response-inhibiting peptide in accordance with any one of claims 1 to 4 having only a single said amino acid substitution.

6. A response-inhibiting peptide in accordance with claim 5, wherein said amino acid substitution is at amino acid 200, 203, 204, 207, 208, 262, 265 or 266.

7. A response-inhibiting peptide in accordance with claim 6, wherein said myasthogenic peptide is peptide p195-212 and said amino acid substitution is at amino acid 200, 203, 204, 207 or 208.

8. A response-inhibiting peptide in accordance with claim 6, wherein said myasthogenic peptide is peptide p259-271 and said amino acid substitution is at amino acid 262 or 266.

9. A response-inhibiting peptide in accordance with any one of claims 1 to 8, wherein said substitutions are selected from the group consisting of:

200, Asp→Lys; 203, Tyr→Phe; 204, His→Gly; 207, Met→NLeu; 207, Met→Ala; 208, Gln→Asp; 208, Gln→Asn; 262, Glu→Asp; 262, Glu→Lys; 262, Glu→Ser; 262, Glu→Ala; 265, Pro→Leu; 265, Pro→Phe; 266, Ser→Lys; 262, Glu→Ala; 265, Pro→Leu; 265, Pro→Phe; 266, Ser→Lys; and 266, Ser→Asp.

10. A response-inhibiting peptide in accordance with any of the preceding claims comprising a first amino acid sequence wherein said myasthogenic peptide is p195-212, and a second amino acid sequence wherein said myasthogenic peptide is p259-271, wherein said first and second sequences are linked together.

11. A response-inhibiting peptide in accordance with claim 10, wherein said first and second sequences are linked together by a short stretch of alanine residues.

12. A response-inhibiting peptide in accordance with claim 10, wherein said first and second sequences are linked together by a site for proteolysis by cathepsin.

13. A response-inhibiting peptide in accordance with claim 1, having 9-12 amino acid residues.

14. A peptide polymer capable of inhibiting the proliferative response of T lymphocytes from a myasthenia gravis patient to a myasthogenic peptide corresponding to a sequence of the human acetylcholine receptor α-subunit

selected from the group consisting of peptide p195-212 having the formula of SEQ ID NO:1:

195 200 205
Asp-Thr-Pro-Tyr-Leu-Asp-Ile-Thr-Tyr-His-Phe-

210
Val-Met-Gln-Arg-Leu-Pro-Leu

and the peptide of p259-271 having the formula of SEQ ID NO:2:

260 265 270
Val-Ile-Val-Glu-Leu-Ile-Pro-Ser-Thr-Ser-Ser-Ala-Val

said peptide polymer including an amino acid sequence of at least nine amino acid residues which includes at least amino acid residues 200-208 of SEQ ID NO:1 or amino acid residues 262-266 of SEQ ID NO:2, but differing therefrom by one to three amino acid substitutions, said amino acid substitutions being selected from those which cumulatively do not substantially change the volume, hydrophobic-hydrophilic pattern and charge of the corresponding portion of the unsubstituted myasthogenic peptide, said substitutions being as defined in claim 1, said amino acid sequence appearing in said peptide polymer a plurality of times.

15. A peptide polymer in accordance with claim 14, containing from 5 to 20 of said amino acid sequences.

16. A peptide polymer in accordance with claim 14 or 15, wherein said amino acid sequences are linked together by cross-linking.

17. A peptide polymer in accordance with claim 14, containing at least one first amino acid sequence wherein said myasthogenic peptide is p195-212, and at least one

second amino acid sequence wherein said myasthogenic peptide is p259-271.

18. A pharmaceutical composition for the treatment of myasthenia gravis comprising an effective amount of a response-inhibiting peptide in accordance with any one of claims 1 to 13 and a pharmaceutically acceptable excipient.

19. A pharmaceutical composition for the treatment of myasthenia gravis comprising an effective amount of a mixture of at least two different peptides in accordance with any one of claims 1 to 13.

20. A pharmaceutical composition for the treatment of myasthenia gravis comprising an effective amount of a peptide polymer in accordance with claim 14 and a pharmaceutically acceptable excipient.

21. A method for selecting response-inhibiting peptides capable of inhibiting the proliferative response of T lymphocytes from a myasthenia gravis patient, comprising:

synthesizing a peptide of at least nine amino acid residues which includes at least amino acid residues 200-208 of SEQ ID NO:1 or amino acid residues 262-266 of SEQ ID NO:2, but different therefrom by one to three amino acid substitutions, said substitutions being selected from those which cumulatively do not substantially change the volume, hydrophobic-hydrophilic pattern and charge of the corresponding portion of the unsubstituted myasthogenic peptide, said substitutions being as defined in claim 1;

testing said peptide for its ability to inhibit the proliferative response of T cells from a myasthenia gravis patient, or a T cell line or clone which is specific to a myasthogenic peptide of SEQ ID NO:1 or SEQ ID NO:2 from which said peptide is derived, to the corresponding peptide of SEQ ID NO:1 or SEQ ID NO:2 to which the T cells are specific; and

selecting and producing said peptide only if it is capable of inhibiting said proliferative response.

22. A process for the preparation of the peptide of any one of claims 1 to 13 which comprises synthesizing the peptide on a resin by a solid phase technique using the appropriate side-chain protected amino acids, removing protecting groups, clearing the peptide from the resin and purifying the peptide.

23. A process for the preparation of the peptide polymer of any one of claims 14 to 17 which comprises synthesizing a peptide of any one of claims 1 to 13 on a resin by a solid phase technique using appropriate side-chain protected amino acids, removing protecting groups, clearing the peptide from the resin, purifying and polymerizing the peptide to yield said peptide polymer.

24. Process for the preparation of the pharmaceutical composition of claims 18 to 20 which comprises combining the peptide(s) of claims 1 to 13 or the peptide polymers of claims 14 to 17 with a pharmaceutically acceptable excipient.

FIG. 1

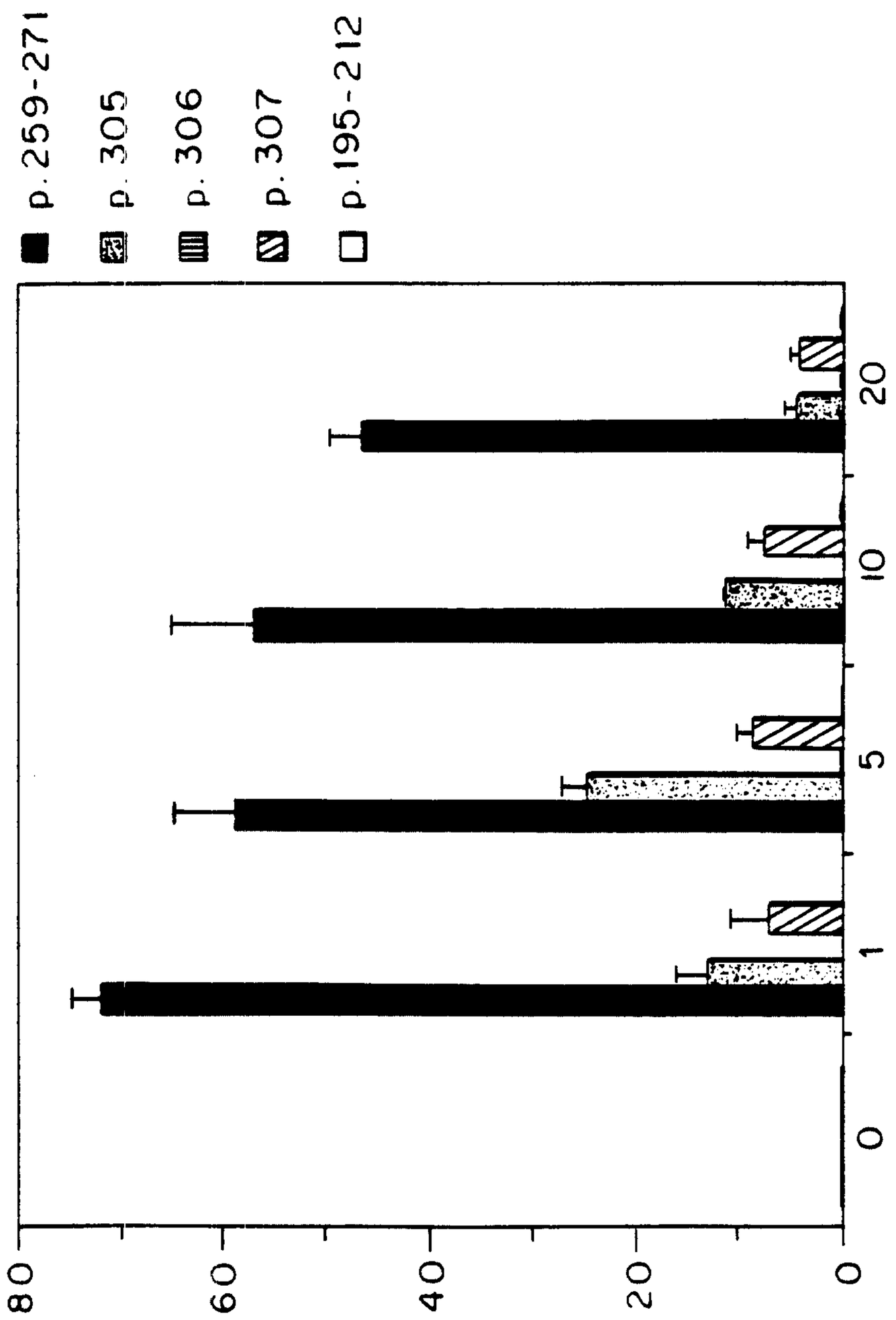

p.259-271
p.305
p.306
p.307
p.195-212
0
1
5
10
20
PEPTIDE DOSE (ug/well)
0
20
40
60
80
CPM (x10-3)

FIG. 2

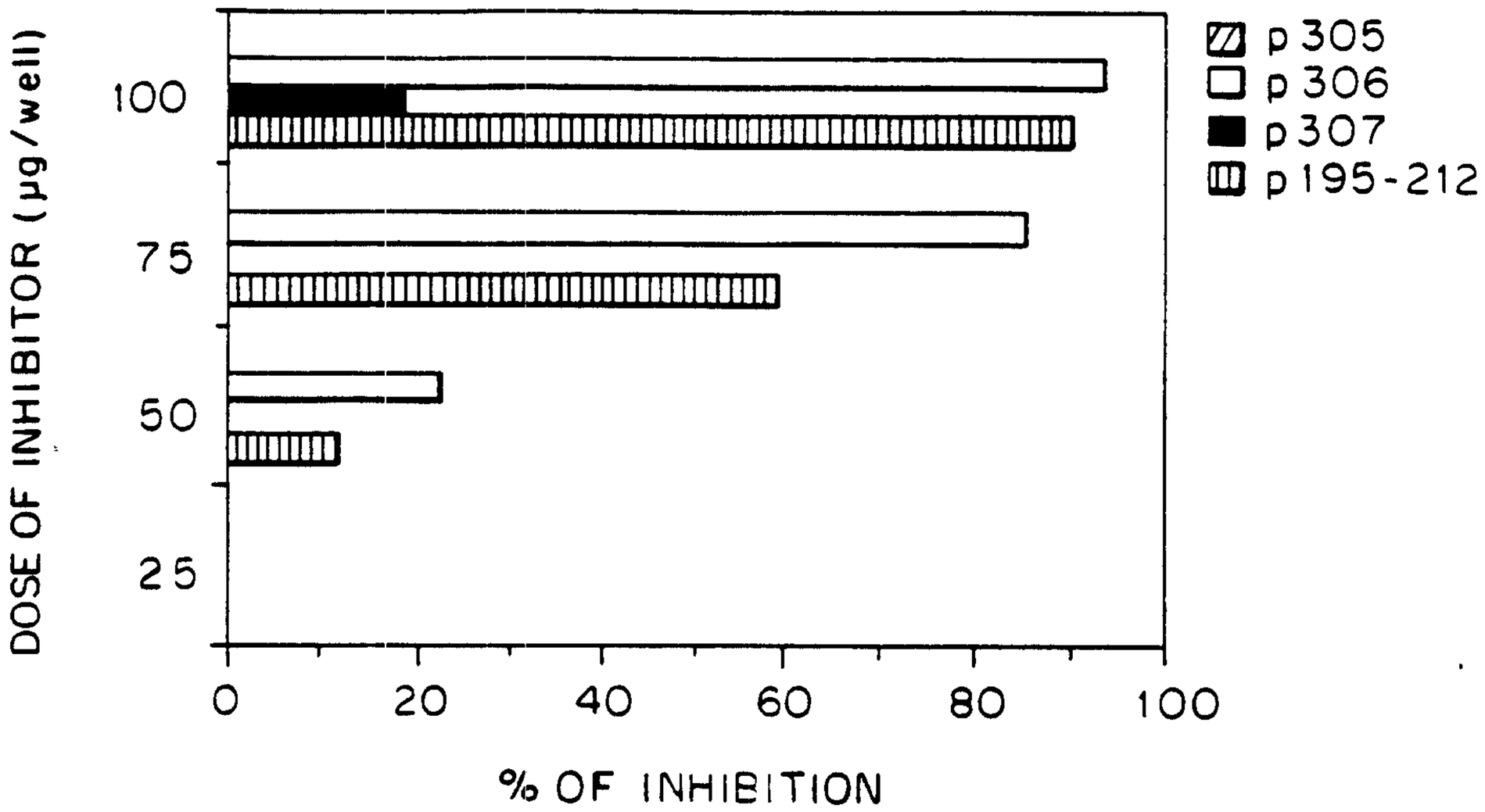

DOSE OF INHIBITOR (µg/well)
100
75
50
25
0
20
40
60
80
100
% OF INHIBITION
p 305
p 306
p 307
p 195-212

FIG. 3

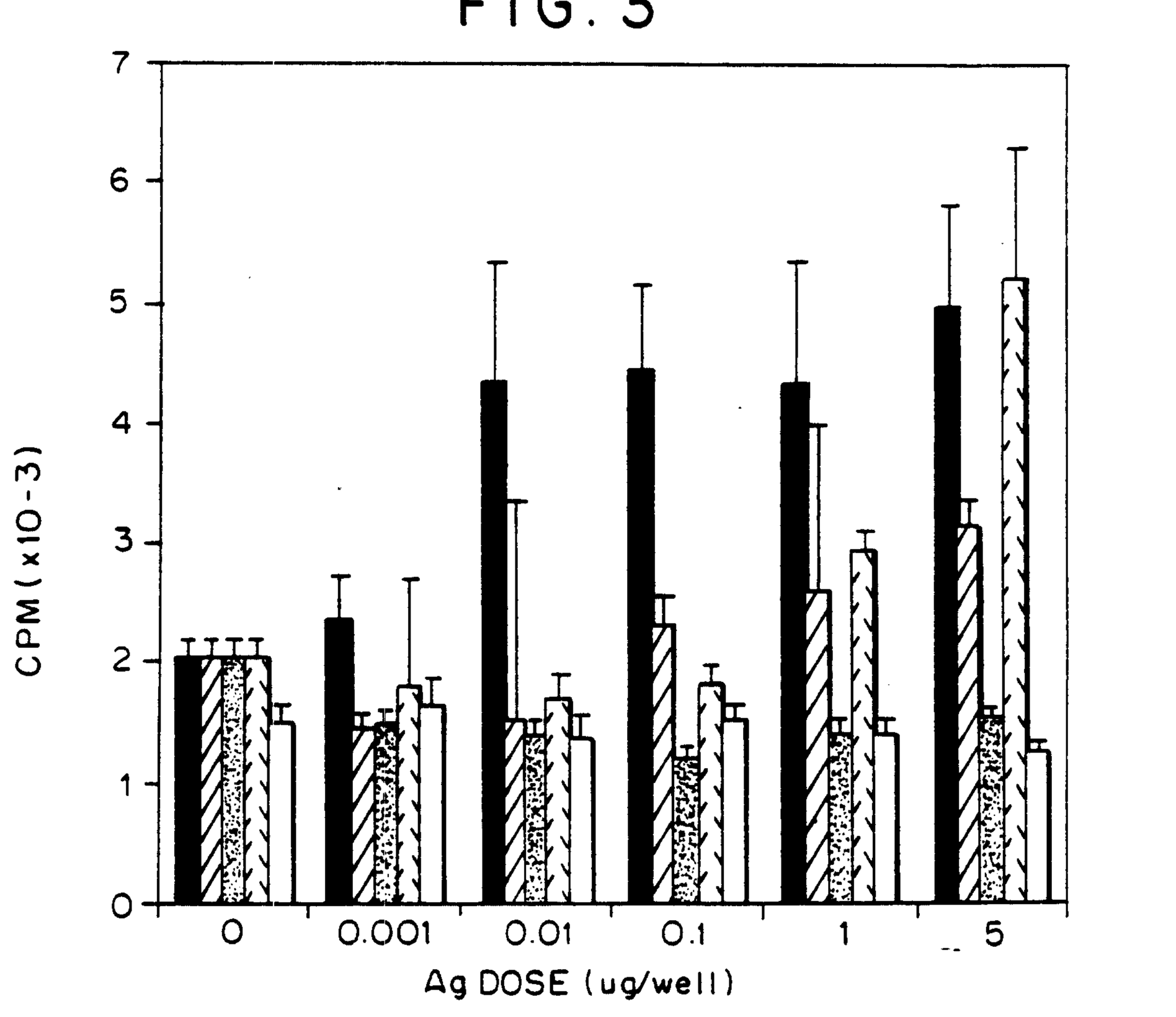
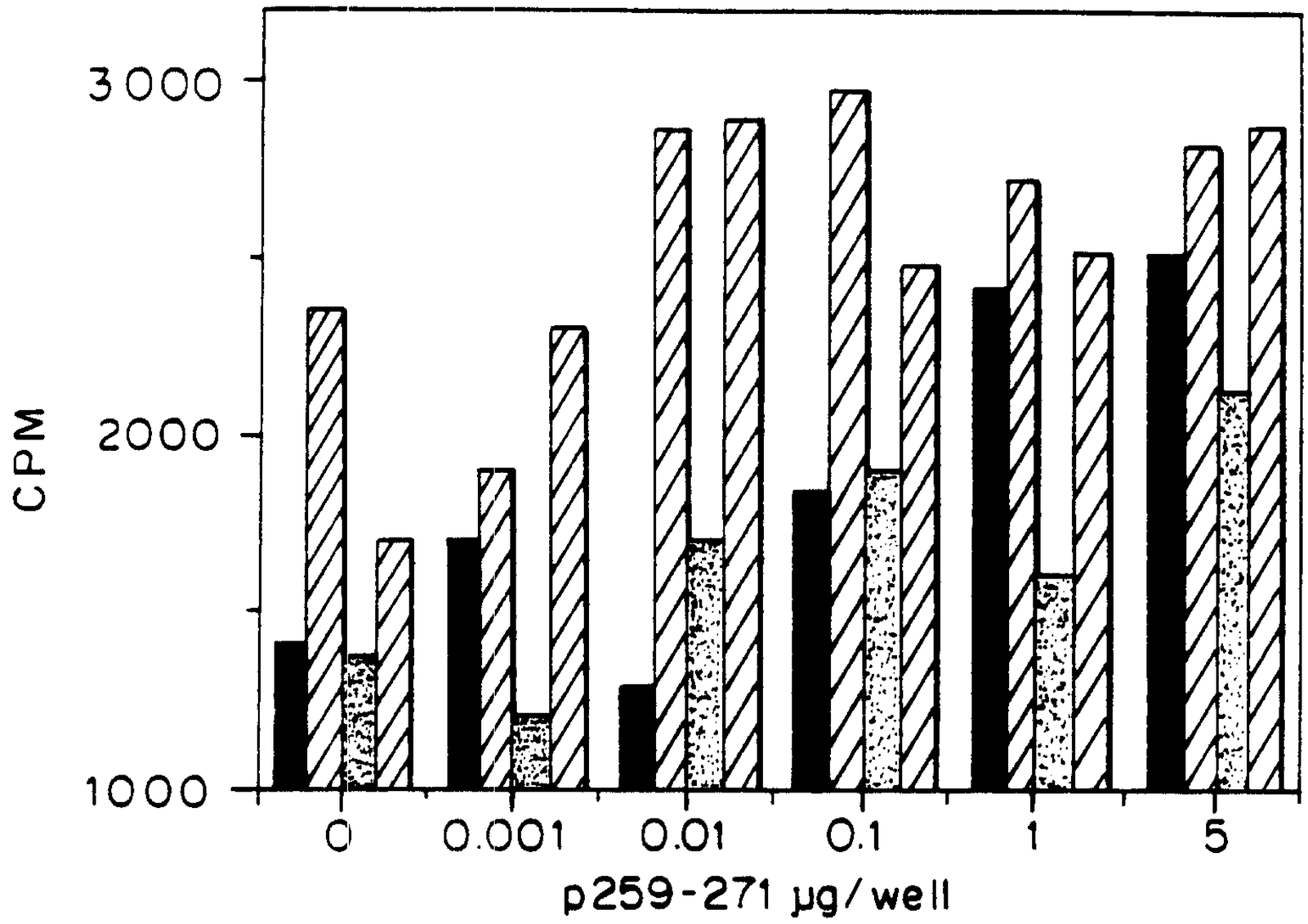

CPM (x10-3)
7
6
5
4
3
2
1
0
0
0.001
0.01
0.1
1
5
Ag DOSE (ug/well)
p 259-271
p 305
p 306
p 307
NOT CELLS

FIG. 4



■ p259-271
▨ p259-271 + p305 20µg
▣ p259-271 + p306 20 µg
▨ p259-271 + p307 20 µg

FIG. 5

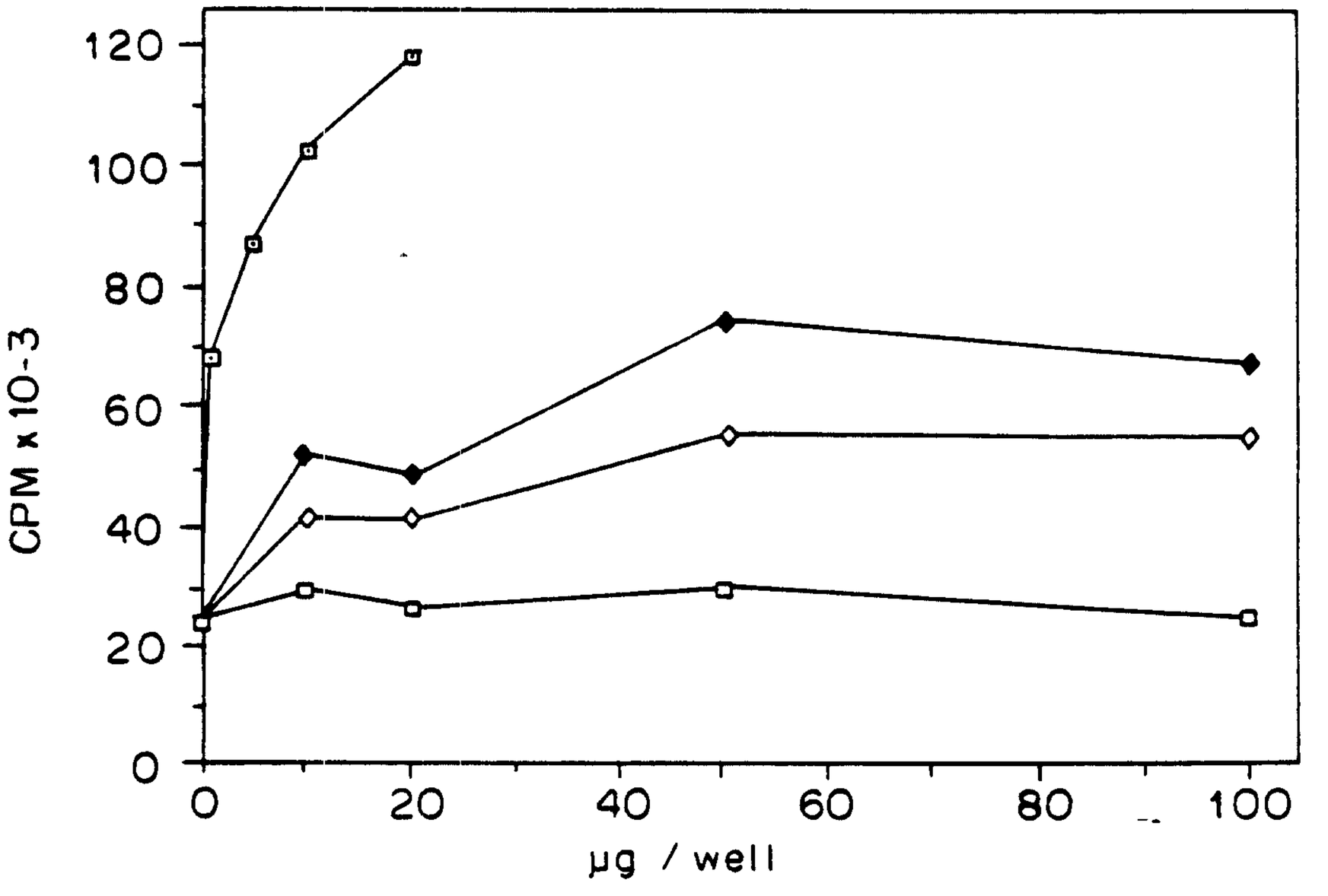


FIG. 6

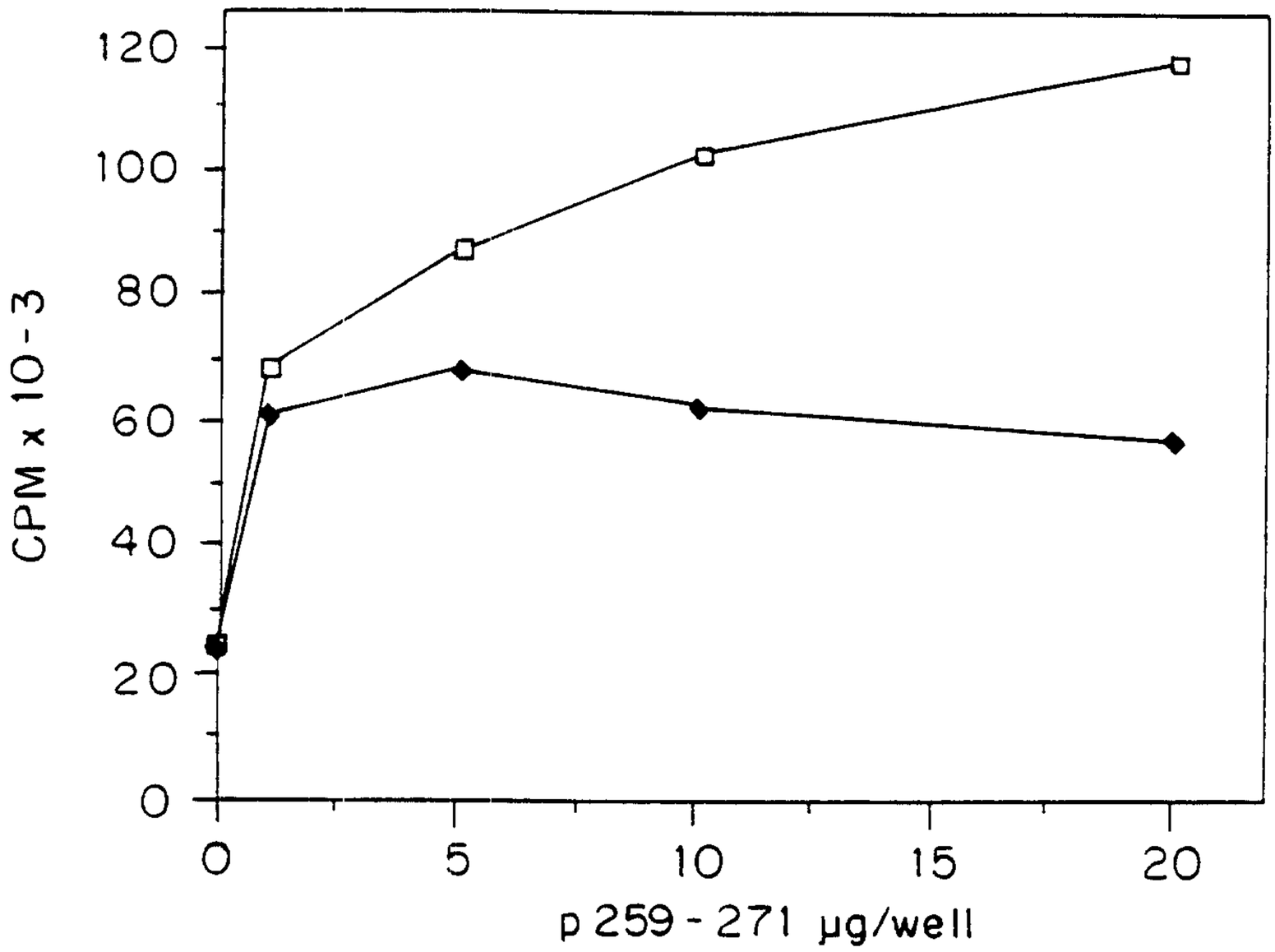
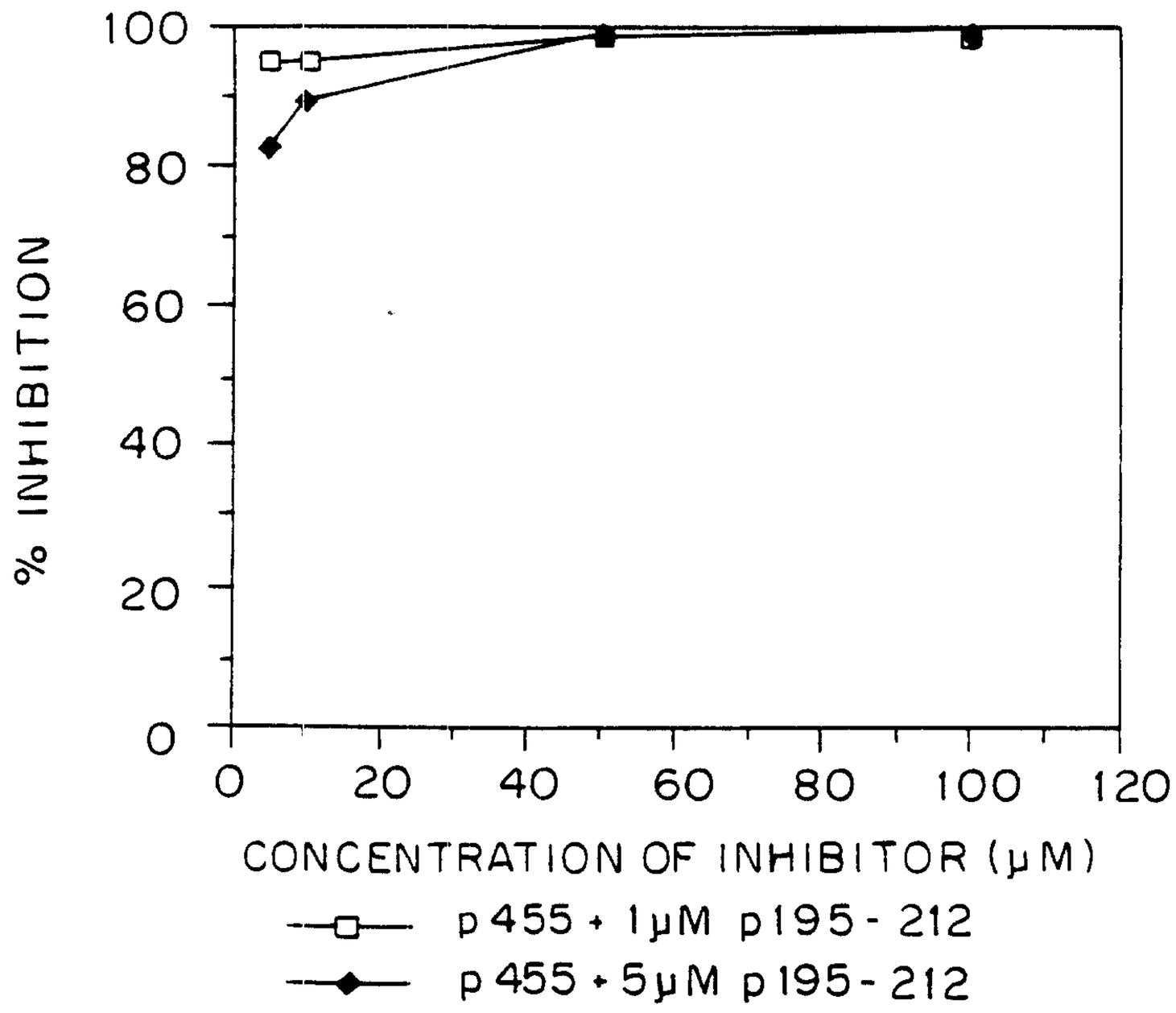

120
100
80
60
40
20
0
CPM x 10-3
0
5
10
15
20
p 259 - 271 µg/well
p259-271
+p 306 100 µg/ well

FIG. 7


100
80
60
40
20
0
% INHIBITION
0
20
40
60
80
100
120
CONCENTRATION OF INHIBITOR (µM)
p 455 + 1µM p195 - 212
p 455 + 5µM p195 - 212