but at the upper field location he could not (50% correct, $n = 180$). However, when the size of the stimulus was increased to 2° on a side, discrimination became possible at both locations. This reveals a pattern of visual abilities that contrasts sharply with that found at the island of sparing.

Because CLT's preserved visual function is limited to an isolated region embedded in his scotoma, it cannot be attributed to a general retinotectal system. The preserved function could be attributed to either a corresponding island of partially preserved striate cortex or an isolated region of partially functioning extrastriate cortex accessed by geniculo-extrastriate projections. Both explanations are viable, but we regard the former as more likely, because a magnetic resonance–based flat-map reconstruction (*19*) of CLT's right hemisphere shows considerable sparing of extrastriate areas but only a minimal region of remaining striate tissue (Fig. 2).

The existence of a small island of sparing embedded within the blind field raises the possibility that similar islands could mediate blindsight in other patients. Visual field mapping that we conducted with one additional patient is commensurate with this possibility. The mapping indicates that regions shown by standard perimetry to have uniformly degraded vision can, with stabilized perimetry, resolve into a mosaic of blind and seeing regions. Patchy distributions of residual vision within scotomas may therefore be more common than previously realized. Of course, the fact that cortical sparing is capable of mediating blindsight does not exclude the possibility that a retinotectal pathway mediates blindsight in some cases. Visual capacities have been reported in the blind field of some hemispherectomy patients (*8, 20*), although these capacities could be attributed to adaptive cortical rearrangements. The evaluation of the role of cortical sparing in blindsight will require detailed visual-field mapping with additional patients. However, our results underscore the fact that manifestations of blindsight must be interpreted with considerable caution. Only when one can rule out the possibility that preserved visual functions are a consequence of preserved cortex can an interpretation based on a retinotectal system be regarded with confidence. Our findings indicate that small regions of preserved visual cortex may be difficult or impossible to detect with conventional perimetric methods.

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# Amelioration of Autoimmune Encephalomyelitis by Myelin Basic Protein Synthetic Peptide–Induced Anergy


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**Experimental autoimmune encephalomyelitis (EAE), a demyelinating disease of the central nervous system that can be induced in susceptible strains of mice by immunization with myelin basic protein (MBP) or its immunodominant T cell determinants, serves as a model of human multiple sclerosis. Tolerance to MBP in adult mice was induced by intraperitoneal injection of synthetic peptides of immunodominant determinants of MBP and prevented MBP-induced EAE. Furthermore, tolerance-inducing regimens of peptides administered to mice after the disease had begun (10 days after induction with MBP) blocked the progression and decreased the severity of EAE. Peptide-induced tolerance resulted from the induction of anergy in proliferative, antigen-specific T cells.**


Over the past decade, it has become apparent that synthetic peptides corresponding to the major immunodominant T cell determinants of native protein antigens can induce unresponsiveness both to themselves and to the native protein antigen in neonatal and adult mice (*1–4*). The use of peptide-specific tolerance as a means to treat human autoimmune disease is a consequence of these studies. Murine EAE is a model of human multiple sclerosis (MS) (*5*) and is caused by an immune response to MBP. We have now induced tolerance to synthetic peptides corresponding to the major immunodominant determinants of MBP (peptides Ac 1–11 and 35–47) or to intact MBP by intraperitoneal injection of an emulsion of the peptide or protein in incomplete Freund's adjuvant (IFA), as has been described for tolerance induction to other proteins or peptides (*3*). Two weeks after administration of tolerogen, mice were injected subcutaneously at the base of the tail with the same peptide antigen or the intact protein emulsified in complete Freund's adjuvant (CFA), the usual route of immunization. T cell proliferative assays performed on regional draining lymph node cells 10 days later revealed that the mice had been made tolerant to the peptide or protein administered as tolerogen. Both peptides Ac 1–11 and 35–47 induced tolerance to recall challenge by themselves in PL/J mice (Fig. 1A) (*6*).

In a separate experiment, PL/J mice were injected intraperitoneally with Ac


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1–11, 35–47, intact MBP, or a mixture of the two synthetic peptides in IFA. Control mice were simply given IFA or irrelevant peptides intraperitoneally at the same time that tolerogen was administered to the experimental groups. Two weeks later, all mice were challenged with MBP (Fig. 1B). MBP was a strong tolerogen, diminishing the T cell proliferative response of regional draining lymph node cells after challenge with MBP. The mixture of the two major immunodominant determinants was almost as effective as intact MBP in inducing tolerance to a subsequent injection of MBP. The relative extent of tolerance induced by the single peptides (35–47 was less effective than Ac 1–11) corresponded to the hierarchy of immunodominance of these peptides, as assayed after priming of PL/J mice with intact MBP (Fig. 1C). In MBP peptide responses, irrelevant peptide was indistinguishable from IFA as a control (6).

We next investigated whether peptide-induced tolerance might have an impact on disease. Groups of (PL/J × SJL)$F_1$ mice were injected intraperitoneally with Ac 1–11, 35–37, a mixture of the two peptides, or IFA as a control 15 days before an encephalitogenic challenge with MBP (4, 7). Intraperitoneal injection with the weakly tolerogenic (Fig. 1B) and weakly immunogenic (Fig. 1C) peptide 35–47 resulted in a modest decrease in mean clinical score and a relapsing disease (Fig. 2). The severity of EAE was least affected by 35–47. The more immunodominant determinant Ac 1–11 was more efficient in ameliorating disease, but, as with tolerance induction, the mixture of the two peptides was most efficient in preventing disease (Fig. 2). The efficiency of the mixture of peptides in the prevention of EAE was better demonstrated in the comparison of mean maximum severity (MMS) scores; MMS is the mean of the highest clinical score of each animal that became sick in the group. The MMS score for the tolerance-inducing injection of the mixture of peptides was 0.52 as compared with the MMS score of 1.05 for the Ac 1–11 regimen and 3.15 for the 35–47 regimen. Control animals who were not injected with peptide had an MMS score of 3.65.

In addition to reducing the mean clinical score, the peptide mixture administered in a tolerance-inducing regimen reduced the incidence of disease in another group of (PL/J × SJL)$F_1$ mice (Fig. 3). Whereas 100% of control animals, who received IFA alone 15 days before encephalitogenic challenge with MBP, had developed severe and relapsing EAE by 14 days after challenge, only four of the ten mice that had been exposed to the peptide mixture showed any disease symptoms, and they quickly recovered.

Although prevention of disease in adult animals by exposure to the MBP peptide antigens was effective and suggested an approach for the prevention of autoimmune disease in humans, the development of immunotherapeutic approaches that target individuals with clinical disease is also desired. Therefore, we subjected 13 (PL/J × SJL)$F_1$ mice to the disease-provoking regimen of MBP (4, 7). When this group of mice showed the first clinical signs of overt disease (day 10, when one of thirteen had a clinical score of 1, indicative of a weak tail), seven of the mice were injected intraperitoneally with the MBP peptide mixture in IFA. The remaining six mice, one of which was the animal showing the signs of disease, received no additional treatment. Only one of the seven mice that received the peptide mixture developed disease (Fig. 4). In contrast, all six of the control mice developed severe EAE (three died within 30 days of disease induction; all were dead by day 40). No disease relapse was apparent in the ensuing 90 days of observation in the

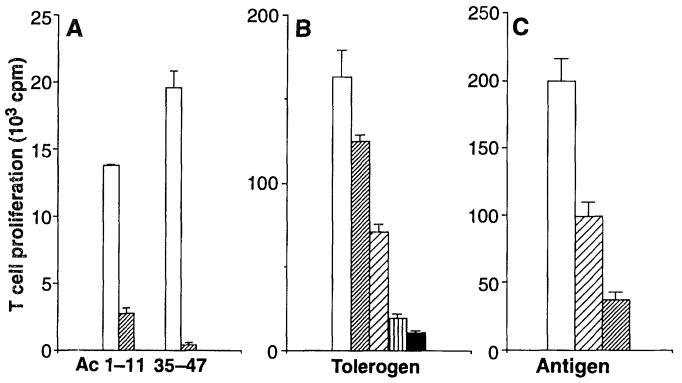


**Fig. 1.** (**A**) Effect of intraperitoneal injection of MBP peptides on tolerance to challenge with the same peptide used later as immunogen. Groups of five PL/J mice were injected intraperitoneally with either IFA alone (open bars) or 300 μg of Ac 1–11 or 35–47 in IFA, as indicated (striped bars). Two weeks later, experimental animals were injected with the same peptide (100 μg in CFA at the base of the tail) used as tolerogen or, in IFA controls, with either Ac 1–11 or 35–47. Ten days later, pooled lymph node cells were analyzed for response to the immunizing antigen. Cells ($0.5 \times 10^6$) were cultured for 4 days and pulsed with 1 μCi of [$^3$H]-labeled thymidine 18 hours before harvest. Results are the means ± SEM of triplicate culture; cell-associated radioactivity was measured. (**B**) Induction of partial tolerance to MBP in PL/J (*H-2$^u$*) mice by synthetic peptides or MBP. Groups of three to five mice were injected intraperitoneally with IFA alone (open bar) or with the following peptides emulsified in IFA: 300 μg of 35–47 (thin diagonal striped bar), 300 μg of Ac 1–11 (thick diagonal striped bar), a mixture of 300 μg each of Ac 1–11 and 35–47 (vertical-striped bar), or .300 μg of MBP (solid bar). Two weeks later, the mice were injected subcutaneously with MBP in CFA, and after a further 10 days the proliferative responses of pooled mouse lymph node cells to MBP were measured as described in (A). Results are means ± SEM ($n$ = 3). (**C**) Maximum proliferative responses of inguinal lymph node cells in an antigen recall assay. Adult PL/J mice were immunized with 100 μg of MBP in CFA at the base of the tail 10 days before the assay. The lymph node cell proliferation assay was performed as described in (A) with an added antigen titration. Results represent the maximum proliferation seen on each titration curve for the peptides or MBP: 1 μM MBP (open bar), 125 μM Ac 1–11 (thick diagonal striped bar), or 125 μM 35–47 (thin diagonal striped bar). Results are means ± SEM ($n$ = 3).

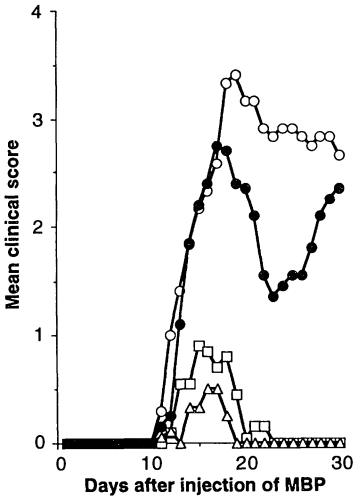


**Fig. 2.** Prevention of MBP-induced EAE in mice with different peptides used as tolerogens. Groups of ten adult (PL/J × SJL)$F_1$ mice were injected intraperitoneally with IFA alone (open circles) or with the following peptides emulsified in IFA: 300 μg of 35–47 (closed circles), 300 μg of Ac 1–11 (open squares), or a mixture of 300 μg each of Ac 1–11 and 35–47 (open triangles). Fifteen days later, EAE was induced by immunization with 200 μg of guinea pig MBP in CFA that contained 500 μg of a heat-killed H37Ra strain of *Mycobacterium tuberculosis*. Pertussis toxin (200 ng) was administered intravenously twice on the day of immunization and also 48 hours later. Mice were monitored daily for disease symptoms and were scored for disease severity on the basis of the following scale: 0, no signs of disease; 1, limp tail; 2, weakness in the hind limbs; 3, hind limb paralysis; 4, moribundity; and 5, death. Data are expressed as the mean clinical score on each day for all the animals in the group. Mice were observed for 30 days. If a mouse died of EAE, a score of 5 was included for that animal in calculations for all subsequent days of analysis.

treated group of mice.

Finally, we investigated the mechanism that underlies this disease treatment model. Regional draining lymph node cells were isolated 24 hours after intraperitoneal injection of the MBP peptide mixture in IFA, which was administered 10 days after primary immunization with MBP in CFA. T cell proliferative responses to MBP were diminished in lymph node cells from treated mice as compared with controls (Fig. 5). In the presence of recombinant interleukin-2 (IL-2), the antigen-specific proliferative responses of lymph node cells from the treated mice increased to amounts comparable to controls. These data support the hypothesis that the intraperitoneal administration of MBP peptides in IFA renders lymph node T cells anergic.

EAE in mice is a well-studied model of autoimmune disease in animals (*4, 5, 7–11*). The immunogenetics of susceptibility are well characterized. MBP has been analyzed for pathogenic and immunodominant T cell determinants, and at least three different regions in MBP have been identified as disease-inducing determinants in susceptible (*H-2*$^u$ and *H-2*$^s$) strains of mice (*12*). Analysis of the T cell response to these determinants in susceptible strains of mice revealed a limited use of T cell receptor (TCR) $V_\beta$ chain by responding T cells (*13*). These data formed the basis for a disease prevention and disease therapy strategy in which researchers used monoclonal antibodies to TCR $V_\beta$8.2 to eliminate cells capable of responding to peptide Ac 1–11 (*14, 15*). It was possible both to prevent and to treat disease by the removal of those T cells capable of responding to the disease-provoking encephalitogenic determinant. The success of such treatment was, however, not complete. In general, the incidence, but not the severity, of disease was diminished (*16*). The enthusiasm that greeted TCR-specific immunotherapy, either by the use of monoclonal antibodies in mice (*14–16*) or by TCR $V_\beta$ peptide vaccination in rats (*17, 18*), has been somewhat tempered by the demonstration of multiple TCR use in response to MBP determinants in patients with MS (*19–21*).

Previous studies suggested that the use of peptides to treat EAE might be successful. Attempts at preventing EAE that used poorly defined antigen preparations yielded mixed results (*22*). Neonatally induced tolerance to Ac 1–11 successfully prevented disease in adult mice when they were challenged with Ac 1–11 (*4*). However, such neonatally tolerant mice developed severe EAE when challenged with MBP. Synthetic peptide analogs of Ac 1–11 with selective amino acid substitutions have been shown to be effective in both the prevention and the treatment of peptide-induced disease (*7, 23*). Such peptide analogs might be antagonists for the specific TCR, as suggested by a study on the mechanism of analog effects (*24*). None of these experiments, however, successfully blocked disease induced by MBP.

The development of antigen-specific tolerance as a preventative or curative therapy may finally yield specificity of treatment for autoimmune diseases. Our data support the concept that dominance in immune response induction potential of synthetic peptides of MBP is directly correlated with their tolerance induction potential. Further, we showed that tolerance induction with peptides before challenge with MBP blocked

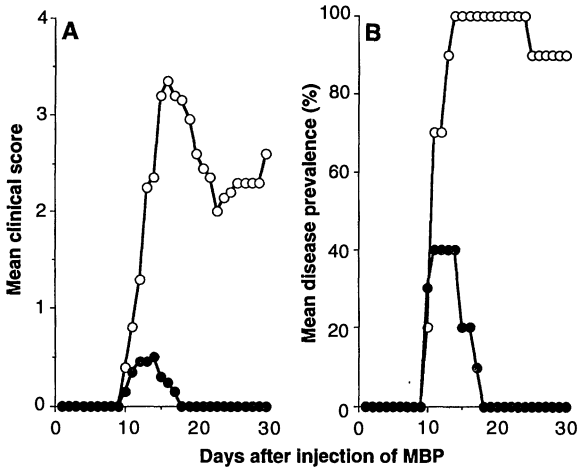


**Fig. 3.** Disease severity and prevalence in mice after administration of MBP peptides as tolerogens. Groups of ten adult (PL/J × SJL)$F_1$ mice were treated intraperitoneally with IFA alone (open circles) or with 300 μg each of 35–47 and Ac 1–11 (closed circles) in IFA. Fifteen days later, EAE was induced with guinea pig MBP. Both disease severity (**A**), monitored as described in Fig. 2, and mean disease prevalence (**B**) were measured. For the latter, the number of sick mice each day of the assay was expressed as a percentage of the total number of mice in the group.

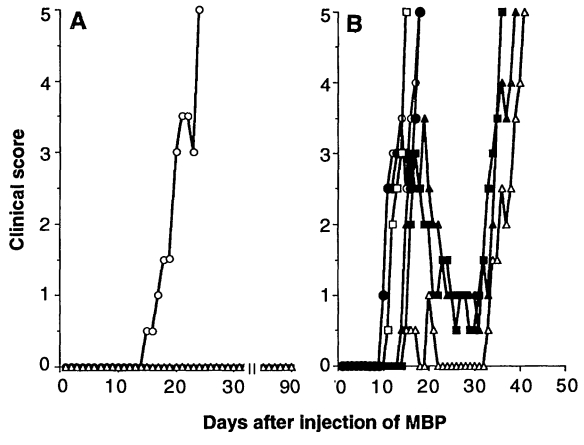


**Fig. 4.** Effect of MBP peptides on ongoing EAE. We immunized 13 adult (PL/J × SJL)$F_1$ mice with guinea pig MBP to induce EAE, as described in Fig. 2. On day 10, when the first clinical signs of disease became evident, one group of seven mice was injected intraperitoneally with 300 μg each of Ac 1–11 and 35–47 emulsified in IFA (**A**). The remaining six mice were treated with IFA alone (**B**). Mice were monitored daily and scored for disease severity as described in Fig. 2. Clinical scores of each mouse are shown.

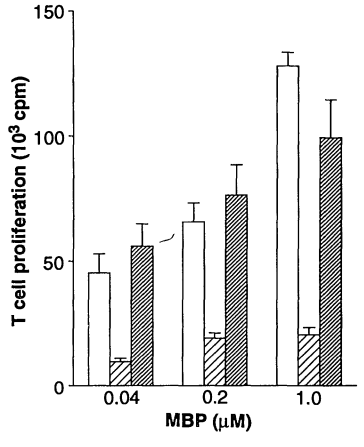


**Fig. 5.** MBP-specific proliferative response of lymph node cells from mice injected intraperitoneally with Ac 1–11 and 35–47 10 days after immunization with MBP. Two groups of three adult (PL/J × SJL)$F_1$ mice were immunized with 100 μg of guinea pig MBP in CFA subcutaneously at the base of the tail. Ten days later, the experimental group received intraperitoneally a mixture of 300 μg each of peptides Ac 1–11 and 35–47 emulsified with IFA. The control group received IFA alone. One day later $0.5 \times 10^6$ pooled inguinal lymph node cells from each group were cultured for 4 days with different doses of MBP, as indicated. Cells from peptide-treated mice were cultured in the absence (thick diagonal striped bar) or presence (thin diagonal striped bar) of recombinant human IL-2 (5 unit/ml); cells from control animals (open bars) were incubated in the absence of IL-2. [$^3$H]Thymidine (1 μCi) was added for the last 18 hours. Results are presented as means ± SEM of triplicate cultures after subtraction of background radioactivity, which was 34,456 ± 166 cpm with IL-2 and 3210 ± 481 cpm without IL-2 for control mouse cells and 38,336 ± 4,238 cpm and 665 ± 201 cpm for peptide-treated mouse cells cultured in the presence and absence of IL-2, respectively. Background counts were obtained by culturing cells in the absence of MBP.

disease induction in a similar hierarchy. Finally, a mixture of the immunodominant determinants of MBP (Ac 1–11 and 35–47) delivered in a tolerogenic manner successfully treated ongoing disease by inducing anergy in the antigen-specific T cells.

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# Intrinsic Quantal Variability Due to Stochastic Properties of Receptor-Transmitter Interactions


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**Synaptic events at the neuromuscular junction are integer multiples of a quantum, the postsynaptic response to transmitter released from one presynaptic vesicle. At central synapses where quanta are small, it has been suggested they are invariant due to occupation of all postsynaptic receptors, a concept neglecting inherent fluctuations in channel behavior. If this did occur, the quantal release model would not apply there and could not be used to localize sites of synaptic modification. Monte Carlo simulations of quanta include transmitter diffusion and interactions with postsynaptic receptors that are treated probabilistically. These models suggest that when there are few postsynaptic channels available at a synapse, their stochastic behavior produces significant intrinsic variance in response amplitude and kinetics, and saturation does not occur. These results were confirmed by analysis of inhibitory quanta in embryonic and adult Mauthner cells involving a small and large number of channels, respectively. The findings apply to excitatory synapses as well.**


Stochastic properties of ionic channels have not been incorporated in schemes of central synaptic transduction. Rather, it has been assumed that the size and shape of neuronal responses can be accounted for by laws of mass (average) action. Responses are generally modeled by a series of coupled differential equations (*1*, *2*) that do not take intrinsic variations into account. Such computations seem to reinforce the conclusion that quantal events in the central nervous system involving few channels exhibit minimal variance (*3*), particularly when a synaptic vesicle contains a large number of transmitter molecules (*4*). Apparently, quantal size is "saturated" (i) even if not all channels are opened at the response peak (*3*, *5*) or (ii) because the transmitter released always opens all channels (100% saturation) (*6*, but see *7*). If so, quantization of synaptic amplitudes in the central nervous system would not reflect the release process, such as at the neuromuscular junction, but would be a postsynaptic phenomenon (*5*). Also, neurotransmission would be different at peripheral and central junctions.

The quantal parameters used in statistical analyses of synaptic transmission and its modifications would have new meanings. The variables $n$, the number of available release units or active zones, and $P$, the probability of release of a quantal packet (*7*, *8*), would be compounded by the number of receptor clusters at a synapse and the likelihood that they are functional. Furthermore, if quantal size, $q$, were fixed, the 10 to 50% coefficient of variation (CV) of spontaneous events observed in some studies (*9–11*) would arise from activation of different receptor aggregates. Resolution of this issue, which requires characterization of quanta generated at one site, is important for further investigations of the cellular basis of processes underlying synaptic plasticity, such as long-term potentiation. Although quantal analysis may reveal which parameter is modified (*12–14*), a clear model is important because interpretations differ according to the initial assumptions (*7*, *15*).

We have combined experiments in situ with Monte Carlo simulations of quantal events to identify sources of fluctuations at single synapses. The model keeps track of the state of each molecule and computes transitions individually. Its validity was confirmed by electrophysiological verification of testable predictions.

In our model of a central synapse there is one presynaptic release site, a 20-nm cleft, and an apposed postsynaptic membrane with a circular receptor aggregate surrounded by a receptor-free zone (*1*), with a combined diameter of ≥1 μm (*16*). The cleft is subdivided into four layers of more than 100,000 rectangular solids (Fig. 1A). This division defines the volume in Cartesian coordinates ($x,y,z$), with the center of the release site as the origin. A fifth layer represents the postsynaptic membrane. At time zero, a quantal packet of T transmitter molecules (A) is released, and each is followed in space and time with steps ($\Delta t$) of 1 μs. Each molecule's diffusion path (Fig. 1B) is calculated by random selection of an incremental distance and direction along each axis from a distribution function relating the average distance moved to the


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