tation of protein antigens processed from the cytosol requires the transport of newly synthesized class I molecules from the ER, whereas presentation of exogenous synthetic peptides does not.

The dependence of protein antigen presentation on the transport of class I molecules from the ER indicates that antigen association occurs either within the ER or in a post-ER exocytic compartment that is rapidly emptied of nascent class I molecules after exocytosis at the ER is blocked. A report that exogenous peptides induce assembly of class I heavy and light chains and transport from the ER in a mutant cell line deficient in these functions (*17*) favors the first possibility. Additional studies are needed to firmly establish the site of antigen association with class I molecules and to assess the contributions of other cellular gene products to the process.

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25. Cells fixed and permeabilized in 70% ethanol were washed and incubated with the following MAbs: H28-E23, HA-specific; H19-S24, NP-specific; NS1-1A7, NS1-specific; 170-1C12, PB2-specific. Cells were then washed and incubated with fluorescein-labeled rabbit immunoglobulin to mouse immunoglobulin, washed, and then analyzed via cytofluorography.

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T Cells Responsive to Myelin Basic Protein in Patients with Multiple Sclerosis

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Gene mutation in vivo in human T lymphocytes appears to occur preferentially in dividing cells. Individuals with multiple sclerosis (MS) are assumed to have one or more populations of dividing T cells that are being stimulated by autoantigens. Mutant T cell clones from MS patients were isolated and tested for reactivity to myelin basic protein, an antigen that is thought to participate in the induction of the disease. The hypoxanthine guanine phosphoribosyltransferase (*hprt*) clonal assay was used to determine mutant frequency values in MS patients with chronic progressive disease. Eleven of 258 thioguanine-resistant (*hprt*$^-$) T cell clones from five of the six MS patients who were tested proliferated in response to human myelin basic protein without prior in vitro exposure to this antigen. No wild-type clones from these patients, nor any *hprt*$^-$ or wild-type clones from three healthy individuals responded to myelin basic protein. Thus, T cell clones that react with myelin basic protein can be isolated from the peripheral blood of MS patients.

ALTHOUGH THE ETIOLOGY OF MS IS unknown, an autoimmune basis for this demyelinating disorder has been proposed (*1*). The similarity of MS to experimental allergic encephalomyelitis (EAE), a relapsing disease of mice, has suggested that MS, like EAE, might be a consequence of autosensitization to myelin basic protein (MBP) (*2*). However, attempts to isolate MBP-reactive T cells from the blood, cerebrospinal fluid, or plaque tissue obtained at autopsy from MS patients has been unsuccessful without stimulation

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with MBP in vitro (*3*). Although it is possible to clone MBP-reactive T cells from peripheral blood of MS patients through sequential antigenic stimulation, the significance of this observation is unclear, since these clones can also be derived from normal individuals. In EAE, the frequency of MBP-reactive clones in the target organ and peripheral blood is low (*4*). Hence any attempts to isolate directly the clones critical to the induction and maintenance of disease is difficult. Our strategy to enhance the likelihood of obtaining the critical clones in MS has been to use somatic mutation as a marker for in vivo cell division.

Mutations in the hypoxanthine guanine phosphoribosyltransferase (*hprt*) gene can be analyzed by the isolation of rare thioguanine-resistant (*hprt*$^-$) T lymphocytes in the peripheral blood. A clonal assay developed for this purpose is useful in monitoring humans for somatic cell mutations that occur spontaneously or are induced by exposure to environmental mutagens. The mean mutant frequency (MF) of *hprt*$^-$ T cells in normal adults is $(5.4 \pm 4.8) \times 10^{-6}$ (*5*).

We showed earlier that mutation occurs preferentially in dividing T cells (*6*), presumably as a result of errors in replication, temporally insufficient DNA repair, or fixation of mutation. Therefore, the mutant fraction of clones will be enriched in cells that have undergone recent division in vivo. On the basis of this assumption and the assumption that MS patients have an underlying population of T cells that are dividing in response to autoantigens, we tested the hypothesis that MS patients have a greater frequency of mutant T cells than do normal controls. Furthermore, the population of *hprt*$^-$ T cell clones will contain cells that show reactivity to the candidate autoantigen MBP, which induces demyelinating disease in animals. This approach implies that the clonal assay for *hprt*$^-$ T cells can be used to restrict the population of T cells isolated to favor those that have undergone recent division in vivo and are presumably relevant to the disease process.

Frequencies of mutant T cells were obtained by an *hprt* clonal assay of 24 (11 male, 13 female) MS patients (Table 1). Cloning efficiencies ranged from 4.7% to 100%. Four categories were used for disease severity: nine males and four females had chronic progressive–progressive (CP-P) disease; one male and one female had chronic progressive–stable (CP-S) disease; no males and six females had recurring remitting–acute (RR-A) disease; and two females and one male had recurring remitting–stable (RR-S) disease. The mean MFs for the four groups were CP-P, 24.7×10^{-6}; CP-S, 20.7×10^{-6}; RR-A, 10.9×10^{-6}; and RR-S, 16×10^{-6}. The mean MF for the males was 33.9×10^{-6} and for the females was 8.2×10^{-6}. This apparent sex effect may reflect the fact that most of the patients with chronic progressive disease were males (10 of 15). A scatter plot shows that normal MFs in healthy adults range from 1×10^{-6} to 13.9×10^{-6}, with a mean of 5.4×10^{-6} (Fig. 1). In patients with chronic progressive disease the MF was significantly higher than that of normal adults ($P = 0.014$, Welch t test; $P = 0.018$, Wilcoxon rank sum test). An increased MF in one patient with RR-S disease may be an indication of disease duration.

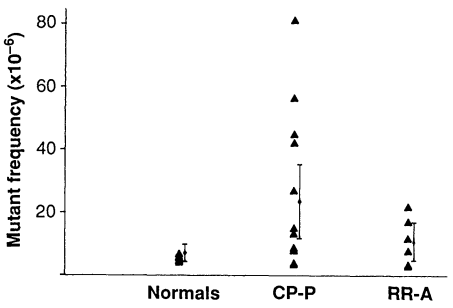

Fig. 1. Scatter plot of MF related to disease status. Cloning efficiencies (nonselection and thioguanine selection) were calculated by the Poisson relation $P_0 = e^{-x}$, which defines the average number of clonable cells per well (x), and then divided by the number of cells added to wells. Mutant frequency is defined as the cloning efficiency in selection divided by the cloning efficiency in nonselection. Cloning assays were performed as described (*5*). Error bars represent 95% confidence limits of the means.

Table 1. Clinical history and mutant frequency (MF) of 24 MS patients with varying disease severity. Patients were selected from the MS clinic at the Medical Center Hospital of Vermont. Informed consent was obtained from each patient. The mean age of the females was 44.2 and of the males was 47.6. Both groups had similar ages of disease onset (males, 31.3; females, 30.8). Values ranged from 2.0 to 8.5 on the Kurtzke disability status scale. Mutant frequency is expressed as number of mutants per 10^6 cells. Patients 1 and 2 had received cyclophosphamide 5 years ago and 7 years ago, respectively, before this study. Patients were categorized as RR-A if new neurological deficits developed and persisted for more than 24 hours and correlated with objective findings that were new on neurological exam. Evanescent symptoms, sensory or motor, were not considered. All patients who were in the acute exacerbation group had received steroids either orally or parenterally in the preceding 48 hours. Patients were categorized as CP-P if they had changed by at least one Kurtzke scale in the preceding year. Patients who were CP-S had remained unchanged in their Kurtzke scale value during the preceding year.

Patient no.	Age and sex	Age at onset	Disease duration	Clinical status	Disability by Kurtzke scale	MF
1	31/M	19	11	CP-P	7.5	45.0
2	41/M	19	22	CP-P	8.5	81.3
3	61/M	56	6	CP-P	6.0	15.1
4	44/F	24	20	CP-P	7.0	4.0
5	51/F	36	15	CP-P	6.5	3.6
6	46/F	21	26	RR-S	7.0	2.3
7	47/F	32	15	RR-S	3.0	4.0
8	43/M	38	5	CP-P	6.5	8.1
9	62/M	42	20	RR-S	2.0	41.8
10	51/M	26	25	CP-P	6.5	42.2
11	48/M	41	8	CP-P	6.5	56.5
12	27/M	21	7	CP-P	4.0	27.0
13	41/F	27	14	CP-P	7.0	8.0
14	51/F	46	5	RR-A	2.5	11.9
15	59/M	10	49	CP-S	6.5	29.8
16	44/F	37	7	RR-A	2.5	22.0
17	39/F	37	2	RR-A	2.5	17.2
18	46/M	31	15	CP-P	6.0	13.4
19	46/F	15	31	CP-P	5.0	8.0
20	40/F	36	4	RR-A	5.0	7.9
21	36/F	32	4	RR-A	3.0	3.1
22	55/M	41	14	CP-P	4.0	8.9
23	37/F	32	5	RR-A	3.5	3.4
24	53/F	26	27	CP-S	6.0	11.6

In order to determine the functional specificity of wild-type and *hprt*$^-$ clones, we performed proliferation assays to test reactivity to MBP. Clones were also tested for response to two irrelevant antigens as controls. A total of 497 clones from six MS patients and three normal individuals was tested. The three normal controls, males aged 30, 25, and 26, had MFs of 8.3×10^{-6}, 5.5×10^{-6}, and 3.9×10^{-6}, respectively. Eleven of the 258 *hprt*$^-$ T cell clones isolated from five of six patients showed a proliferative response to MBP (stimulation index >3.5) (Table 2). None of the 93 wild-type clones from MS patients and none of 114 *hprt*$^-$ or 32 wild-type clones isolated from three normal individuals responded to MBP. Each clone was tested with at least two irrelevant antigens, and no reactivity to streptokinase, varicella zoster, candida, or tetanus toxoid was observed. If it is assumed that all control clones represent the same

population of T cells (a total of 239 MS wild-type, normal *hprt*$^-$, and normal wild-type clones), the probability of finding this distribution (11 MBP-reactive clones in 258 versus 0 in 239) by chance alone is low ($P = 0.001$, binomial approximation for a conditional Poisson distribution).

We assume that the *hprt* mutation in each of these clones is unrelated to their reactivity. The observation that many other *hprt*$^-$ clones from these patients, as well as *hprt*$^-$ clones from normal individuals, did not respond to MBP supports this assumption. Selection for mutation at another locus would presumably allow the isolation of similar clones, simply by virtue of their clonal expansion and susceptibility to mutation in vivo. At present, this assumption is not proven. There are genes on the X chromosome that control functions related to immune responsiveness. In addition, adrenoleukodystrophy syndrome (an inherited demyelinating disorder) maps to the long arm of the X chromosome (Xq28) near *hprt* (*7*). This syndrome shares several symptoms with MS, and structural changes in this region of the X chromosome may influence similar immune functions in both diseases.

Two previous studies have demonstrated normal *hprt* mutant frequencies in patients with MS (*8*); however, the disease status of these patients was not reported, and MFs may be increased only in chronic disease. We have thus studied a larger number of patients of varying disease status and found high MFs in MS patients with chronic disease. Several explanations may account for the absence of high MFs among patients with recurring remitting disease. A high MF may be an indicator of the clonal expansion of a single *hprt*$^-$ T cell clone in vivo (*9*). We used DNA blot analysis of T cell receptor rearrangements on 145 *hprt*$^-$ T cell clones from three CP-P patients to show that this is not the case (*10*). A high MF may also indicate duration of disease, and the accumulation of *hprt*$^-$ T cells with time would be expected to result in a high value. Therefore, disease of long standing may be required for a sufficient number of *hprt*$^-$ T cells to accumulate. In addition, compartmentalization of MBP-reactive clones in the central nervous system and lymph nodes may occur in remitting disease and may result in the depletion of such clones from the peripheral blood.

Much effort has been focused on the relation of MBP to MS, and the success of immunotherapy in animal models such as EAE has heightened interest in finding MBP-reactive T cell clones in MS patients. The observation that T cell clones from two divergent species (rat and mouse) use strikingly homologous Vβ and Vα genes in the recognition of distinct encephalitogenic epitopes of MBP (*11*) has intensified the search for analogous associations in humans.

Extensive studies have shown that autoreactive T cell clones as such are absent or rare in MS patients (*3*), even when compartmentally restricted T cell clones are analyzed. However, it is clear that there are increases in certain populations of activated T cells in the peripheral blood of MS patients (*12*). It is possible that the *hprt*$^-$ MBP-reactive T cell clones we isolated may not be unique to MS. Low-level reactivity to MBP has been observed in clones obtained from the cerebrospinal fluid of patients with postinfectious encephalomyelitis (*3*). Since the frequency of MBP-reactive clones within the mutant fraction of MS patients is low, it does not entirely account for the increased MFs. Other mechanisms such as increased susceptibility to mutation, in vivo selection for the mutant phenotype, or in vivo expansion in response to other antigens may give rise to the increase in MF. Several of these clones express a helper T cell phenotype and may contribute to the oligoclonal immunoglobulin production often seen in these patients.

The availability of MBP-specific clones will allow the characterization of those aberrant T cell functions that persist in the peripheral blood of MS patients. Other autoimmune diseases with a suspected T cell involvement should lend themselves to this approach.

Table 2. Proliferation of 11 *hprt*$^-$ T cell clones from five MS patients. Numbers represent [^{3}H]thymidine incorporation as mean counts per minute ± standard error of the mean of triplicates in a 72-hour proliferation assay. Stimulation index of MBP is shown in parentheses. Wild-type and *hprt*$^-$ T cell clones were generated as described (*13*). Human MBP was isolated according to the method of Oshiro and Eylar (*14*), as modified by Brostoff and Mason (*15*). T cell clones were tested for reactivity to MBP (*16*). Briefly, between 1×10^4 and 5×10^4 cloned cells were placed with an equal number of irradiated (3000 rad) autologous peripheral blood mononuclear cells as antigen-presenting cells in flat-bottomed 96-well microtiter plates. Wells received either optimal concentrations of growth factor preparations containing recombinant interleukin-2 (IL-2) with phytohemagglutinin (PHA) (0.1 μg/ml), medium, or irrelevant antigen as controls. Candida (Hollister-Stier) was tested as a crude extract at a final dilution of 1:100 (w/v); tetanus toxoid was adsorbed on aluminum phosphate (Wyeth) and tested at 0.2 unit/ml; streptokinase (SK) was tested at 100 units/ml (Kabivitrum); and Varicella zoster was a dilution of supernatant from in vitro infection of fibroblast monolayers. All irrelevant antigen preparations had repeatedly tested positive in routine immunocompetency tests. MBP was tested at serial log dilutions of either 1, 10, and 100 or 3, 30, and 300 μg/ml; data shown are from the concentration that gave optimal stimulation. All clones showed maximal incorporation with MBP at 100 μg/ml except clone 17 from patient 1, which responded maximally to 30 μg/ml. Cultures were treated with [^{3}H]thymidine at 1 μCi per well for 16 hours before being harvested on a multichannel automated sample harvester and quantitated in a scintillation counter.

Clone	Media	IL-2/PHA	Candida	Tetanus	SK	V. zoster	MBP
				Patient 1			
17	810 ± 597	3,575 ± 877	707 ± 478	1,148 ± 532	1,100 ± 15	1,080 ± 140	3,727 ± 200 (4.6)
				Patient 2			
14	885 ± 240	56,779 ± 2,725	742 ± 74	1,049 ± 156			4,232 ± 771 (4.7)
37	203 ± 122	8,144 ± 549	145 ± 27	478 ± 241			1,773 ± 989 (8.7)
				Patient 3			
12	4,697 ± 673	107,289 ± 3,750	5,552 ± 654	4,809 ± 591			23,096 ± 2,618 (4.9)
13	4,625 ± 1,763	86,297 ± 2,877	6,481 ± 1,379		8,457 ± 1,523		28,767 ± 3,062 (6.2)
18	666 ± 273	67,824 ± 4,712	287 ± 49	761 ± 591			6,192 ± 1,100 (9.3)
26	652 ± 491	67,741 ± 742	502 ± 141	659 ± 207			3,221 ± 244 (4.9)
27	1,813 ± 83	75,710 ± 738	1,868 ± 158		1,689 ± 190		8,871 ± 1,493 (4.8)
31	1,463 ± 61	111,394 ± 4,655	2,317 ± 581	3,079 ± 716			11,022 ± 410 (7.5)
				Patient 19			
2	1,284 ± 457	43,916 ± 1,486	1,289 ± 276			1,455 ± 1,124	7,040 ± 3,459 (5.5)
				Patient 22			
14	1,532 ± 704	151,773 ± 4,375	1,632 ± 1,027		2,148 ± 303		7,370 ± 1,450 (4.8)

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13. Mononuclear cells were obtained from peripheral blood after separation with Sepracell, diluted to 10^6 cells per milliliter, and incubated with phytohemagglutinin (1 μg/ml). After 36 to 40 hours, the cells were suspended and counted. Cells were inoculated at 1, 2, 5, and 10 cells per well to determine cloning efficiency, and at 10^4 cells per well for selection conditions, in 96-well round-bottom microtiter plates. Growth medium consisted of RPMI 1640 with 10% fetal bovine serum (FBS), 20% HL-1 medium (Ventrex), recombinant interleukin-2, phytohemagglutinin (0.1 μg/ml), and an *hprt*$^-$ derivative of the lymphoblastoid line WIL-2 (TK_6) as a source of feeder cells. Supernatants from lymphokine-activated killer cells were used as a source of interleukin-2. Selection wells contained 10^{-5} *M* thioguanine. Cells were incubated for 10 to 14 days and scored for colony growth by use of an inverted phase microscope. Positive cultures were grown in medium described above.
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Influence of Scene-Based Properties on Visual Search

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The task of visual search is to determine as rapidly as possible whether a target item is present or absent in a display. Rapidly detected items are thought to contain features that correspond to primitive elements in the human visual system. In previous theories, it has been assumed that visual search is based on simple two-dimensional features in the image. However, visual search also has access to another level of representation, one that describes properties in the corresponding three-dimensional scene. Among these properties are three dimensionality and the direction of lighting, but not viewing direction. These findings imply that the parallel processes of early vision are much more sophisticated than previously assumed.

IT IS EASY TO DETECT A VERTICAL LINE placed among a group of horizontal lines. The vertical line "pops out," drawing attention to itself regardless of how many horizontal lines are present. In contrast, searching for a T-shaped target among L-shaped distractors requires conscious effort, and search time increases linearly with the number of L-shaped distractors in the display. These two classes of search exemplify the visual search paradigm, a useful tool for determining the primitive elements of early human vision.

In theories of visual search it is hypothesized that there are two subsystems (*1–3*). The first is a preattentive system capable of detecting simple features (for example, oriented lines) in parallel across the image. Processes at this stage do not detect spatial relations between features (for example, the relative locations of line segments). These spatial relations can only be determined by a second system that inspects each collection of features in a serial fashion.

When talking about features, however, one must distinguish between the world of objects in three-dimensional space (that is, the scene) and its projection onto a two-dimensional array (that is, the image). In a scene of objects illuminated by a distant point source, the array of image intensities is determined by: (i) direction of lighting, (ii) surface locations and orientations, (iii) surface reflectances, and (iv) viewing direction. These properties are only captured by the relations among image features.

The experiments in this report tested the sensitivity of preattentive vision to several of these relations. Target and distractor items were composed of polygons shaded with one of three intensities: white (all pixels lit), gray (alternate pixels lit), and black (no pixels lit) (Figs. 1 through 4). Some of the items corresponded to projections of simple blocks under various orientation, viewing, and lighting conditions (Figs. 1A, 2A, and 3). Others could not be interpreted as three-dimensional objects (Fig. 1, B to D, and Fig. 2, B and C). We asked whether visual search could distinguish among these items, and if so, which scene properties were relevant.

A Macintosh computer was used to generate the displays, control the experiments, and collect the data (*4*). Observers searched for a single target item among 1, 6, or 12 items (*5*). The target was present in half the trials and randomly distributed throughout the trial sequence (*6*). Observers were instructed to maintain fixation and to keep errors below 10% (*7*). Target presence or

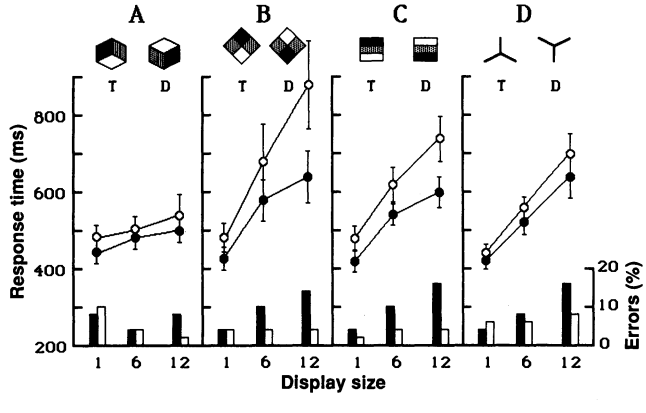

Fig. 1. Experiment 1. The target (T) and distractor (D) items in the four conditions (A to D). Filled circles and bars represent data from target-present trials; open circles and bars represent target-absent trials. (**A**) Search is rapid when the items correspond to three-dimensional blocks of different orientation and lighting. (**B** to **D**) Search is slow when the items are two dimensional. Values are mean ± SEM. Display size indicates number of items presented in a trial.

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