IMPAIRED MEASLES VIRUS–SPECIFIC CYTOTOXIC T CELL RESPONSES IN MULTIPLE SCLEROSIS

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Multiple sclerosis (MS)[1] is a chronic demyelinating disease of the central nervous system (CNS) which can have a highly variable clinical course. While the etiology of this disorder is unknown, the majority of current research in this field is focussed on the elucidation of an infectious agent, an autoimmune process, or a combination of the two (1, 2). Numerous studies (1–4) have demonstrated immune abnormalities in MS. However, the majority of these reports have employed assays that either measured an aspect of cellular recognition without consideration of function (eg. lymphoproliferative assays), or assessed the function of antigen-nonspecific cell activity, such as natural killer (NK) cell activity. Little is known of those populations of cells that are antigen-specific and have a demonstrable function, such as cytotoxic T cells (CTL) or T helper cells, which regulate T or B cell functions.

Measles virus has been considered as a possible etiological factor in the pathogenesis of MS, based primarily on serological findings (5–8). For the most part, assays designed to demonstrate a functional cellular immune response to this agent (eg. CTL) have been unsuccessful, and consequently, the assessment of such antigen-specific immune responses in MS patients has not been possible. Using T cell clones (9, 10) and bulk culture populations (Jacobson, S., M. L. Flerlage, and H. F. McFarland, manuscript in preparation)[2], we have shown that measles virus–specific CTL have a cell surface phenotype that was $OKT4^+$, and that they lysed infected cells by recognition of measles virus antigen in association with HLA class II molecules. To determine whether a measles virus–specific cellular immune abnormality is associated with MS, we analyzed the capacity of lymphocytes from MS patients to generate measles virus–specific CTL. In comparison, we have studied normal individuals and other disease controls (ODC). The vast majority of MS patients either failed to generate measles virus–specific CTL, or had significantly lower CTL responses than either normal individuals or ODC. Moreover, this decreased CTL response in MS patients was specific for

measles virus, since all three groups generated equivalent CTL responses to influenza virus. These results suggest that a measles virus–specific immune abnormality exists in MS.

Materials and Methods

Patient Profiles. Patients included in this study were evaluated in the MS clinic, Neuroimmunology Branch, NIH. Each patient had a diagnosis of clinically definite MS. 14 of the 16 patients had a chronic progressive form of disease. Degree of disease activity is indicated in Table I. Disability was established using the Kurtzke Disability Scale (DSS). All MS patients were ambulatory. 5 of the 16 MS patients and 4 of the 13 ODC patients required assistance for ambulation. None of the patients had received steroid or other immunosuppressive treatment for at least 2 mo before being studied. Control individuals were participants in the normal volunteer program. The procedures used in this study were reviewed and approved by the human research subpanel. Informed consent was obtained from each individual participating in the study.

Lymphocyte Preparation. Lymphocytes were obtained from MS patients and normal individuals by leukopheresis, and they were purified by density gradient centrifugation using Ficoll-diatrizoate (LSM solution, Litton Bionetics Inc., Charleston, SC). Venous blood was obtained from ODC, and was drawn in preservative-free heparin (Abbott Laboratories, Irving, TX). Lymphocytes were frozen in cryopreservative media (12-132A, M. A. Bioproducts, Walkersville, MD) in a programmable cell freezer (Cryomedics, Inc., Bridgeport CT), and were stored in liquid nitrogen vapor until used.

Cytotoxic Assay. Peripheral blood lymphocytes (PBL) were stimulated with either the Edmonston strain of measles virus at a multiplicity of infection (moi) of one, a 1:1,000 dilution of influenza virus A/Bangkok/1/79-RX73 (H3N2), with a titer of 256 hemagglutinating units per milliliter, or media alone for 90 min in a humidified 5% CO_2 incubator. Cells were cultured for 7 d in RPMI 1640 (Gibco Laboratories, Grand Island, NY) plus 5% human AB serum. Effectors were harvested and incubated with ^{51}Cr-labelled targets at a ratio of 40:1 for 5 h. Mean percent lysis was calculated as: (^{51}Cr-release with sensitized effectors − spontaneous release) × 100/(detergent release − spontaneous release). Mean percent specific lysis was calculated as the mean lysis of sensitized lymphocytes minus mean lysis of unsensitized lymphocytes on the appropriate virus-infected autologous target. The mean percent lysis of measles virus–infected targets by unsensitized lymphocytes from all three groups were comparable, and not statistically significant between groups: MS, 5.3 ± 5.9%; Normals, 3.5 ± 3.1%; ODC, 1.8 ± 2.6%. Epstein-Barr virus (EBV)-transformed B cell lines were generated for each individual. For targets, autologous EBV-transformed lymphoblastoid cell lines were infected with the Edmonston strain of measles virus at an moi of one for 90 min, and incubated for 4 d in RPMI 1640 media supplemented with glutamine and antibiotics plus 20% fetal calf serum. For influenza virus–infected targets, autologous B cell lines were infected as described (9). Effector/target ratios for all experiments are at 40:1. Spontaneous release from ^{51}-Cr-labelled infected and uninfected targets ranged from 10 to 15%. Mean percent lysis of uninfected targets by sensitized lymphocytes from all three groups were comparable, and not statistically significant between groups; MS, 6.6 ± 6.7%, Normals, 7.7 ± 3.3%, ODC, 5.7 ± 4.7%. NK activity was measured by the cytolytic activity of measles virus–primed lymphocytes on the NK-sensitive target, K562. Values are expressed as means of triplicate cultures.

Cold-target Blocking Experiments. CTL were generated from normal control 17 and MS patient 8 by stimulation with measles virus, as described above. After 7 d of incubation, cells were harvested, and 4 × 10^5 effectors were incubated with 10^4 ^{51}Cr-labelled measles virus–infected autologous B cell targets (group without cold blockers in Fig. 2) in a standard 5-h ^{51}Cr-release assay. Effectors were also incubated with a mixture of increasing amounts of unlabelled (cold) autologous infected and uninfected B cell targets to a constant number (10^4) ^{51}Cr-labelled (hot) measles virus–infected targets. The data are plotted as

TABLE I

Characteristics of Patients and Controls in Study Population

Patient	Age	Sex	DSS	Disease activity	Course
			MS patients		
1	30	F	3	Remission	Relapse-remit
2	23	F	4	Remission	Relapse-remit
3	28	F	6	Active	Rapid progress
4	38	M	6	Active	Rapid progress
5	24	M	6	Active	Rapid progress
6	28	F	4	Active	Chronic progress
7	46	F	4	Stable	Chronic progress
8	45	F	5	Stable	Chronic progress
9	33	M	5	Active	Chronic progress
10	33	F	3	Stable	Chronic progress
11	49	M	4	Stable	Chronic progress
12	38	M	4	Stable	Chronic progress
13	39	M	4	Active	Chronic progress
14	50	F	5	Stable	Chronic progress
15	44	F	3	Stable	Chronic progress
16	55	M	4	Stable	Chronic progress
			ODC patients		
				Disease	
1	22	F	Myasthenia Gravis		
2	42	M	Myasthenia Gravis		
3	19	F	Myasthenia Gravis		
4	50	M	Spinal cerebellar degeneration		
5	52	F	Spinal cerebellar degeneration		
6	50	F	Spinal cerebellar degeneration		
7	44	M	Multisystem degeneration		
8	37	F	Multisystem degeneration		
9	37	F	Rheumatoid Arthritis		
10	23	F	Charcot-Marie Tooth		
11	35	M	Peripheral neuropathy		
12	43	M	Guillain-Barré syndrome		
13	59	M	Amyotrophic Lateral Sclerosis		
	Healthy controls				
1	33	M			
2	41	M			
3	42	M			
4	32	M			
5	39	M			
6	26	M			
7	31	F			
8	38	F			
9	22	M			
10	28	F			
11	28	F			
12	36	M			
13	35	F			
14	44	M			
15	41	F			
16	25	F			
17	28	M			

percent specific lysis vs. increasing amounts of cold/hot targets. Values are expressed as means of triplicate cultures.

Statistical Analysis.   Statistics were performed by the student *t* test and the *p* values are reported with a two-tailed analysis. Results are expressed as means ± SEM.

Results

Generation of Measles and Influenza Virus–specific CTL.   The ability to generate measles virus and influenza virus-specific CTL was studied in MS patients, ODC, and normal individuals. The level of NK activity after measles virus stimulation was also examined. These results are shown in Fig. 1. Normal individuals whose PBL were stimulated with influenza virus lysed their influenza virus–infected autologous B cell line at a group mean level of 47.0 ± 2.36% specific lysis (at an effector/target ratio of 40:1). MS patients and ODC generated comparable levels of influenza virus–specific CTL responses, with a group mean specific lysis of 43.12 ± 3.3%, and 42.3 ± 3.64%, respectively (Fig. 1*B*). There was not a significant difference between the ability of MS patients to generate influenza virus–specific CTL and that of normals or ODC ($p > 0.8$).

In contrast, the capacity of MS patients to generate measles virus–specific CTL, compared to either normal individuals or ODC was markedly diminished. The measles virus–specific CTL response for the normal group, as assayed on measles virus–infected autologous B cell lines was 26.9 ± 2.86% specific lysis. ODC gave a comparable level of lysis, with a group mean level of 26.7 ± 2.8% specific lysis. PBL stimulated with measles virus from MS patients, however, lysed their measles virus–infected autologous B cell line at a group mean level of only 6.0 ± 1.4% specific lysis (Fig. 1*A*). This measles virus–specific lysis was significantly lower in the MS group than in normal individuals ($p < 0.00001$) or ODC ($p < 0.00001$). All MS patients were tested at least twice, and repeated samples from selected individuals gave similar low measles virus–specific CTL responses. These findings indicated that the depressed cytolytic responses from MS patients were measles virus–specific, and did not represent a generalized decrease in CTL.

Previous studies (9, 10) have shown that measles virus–specific CTL were virus- and HLA class II–restricted. To eliminate the possibility that the differences in measles virus–specific CTL from MS patients was due to changes in NK activity, we compared the capacity of measles virus–stimulated lymphocytes from MS patients, normal individuals, and ODC to lyse the K562 cell line (Fig. 1). Measles virus–stimulated NK activity from MS patients had a group mean level of lysis of 51.3 ± 5.0%. Measles virus–stimulated NK activity from normals or ODC had group mean levels of lysis of 54.1 ± 3.7% and 46.5 ± 3.4%, respectively. There was no statistically significant difference between the MS and the normal or ODC groups ($p > 0.8$). These results further substantiate the assertion that the lowered measles virus–specific CTL responses of MS patients do not reflect a generalized depressed cytolytic activity.

Cold-target Blocking Experiments.   Measles virus–stimulated PBL from MS patients 5, 8, and 9 (Fig. 1*A*, asterisks) had significantly higher levels of lysis of measles virus–infected autologous B cell lines (mean specific lysis of 16.8 ± 0.34%) than the other members of this group ($p < 0.0001$). However, these
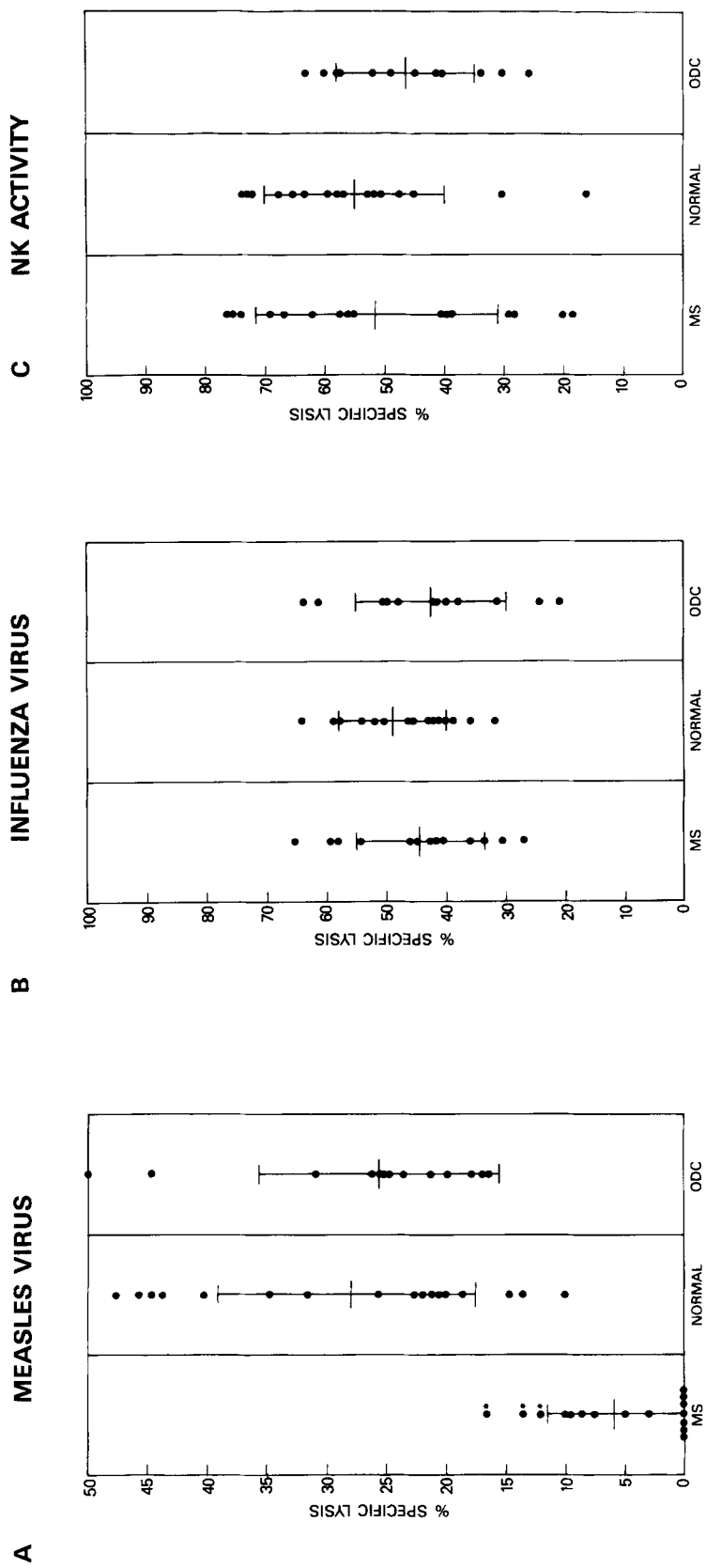
FIGURE 1. Percent specific lysis (as described in Materials and Methods) of the indicated target B cell line by either CTL generated against measles virus (A) or influenza virus (B) is plotted for MS patients, ODC, and normal individuals. NK activity (C) of PBL stimulated with measles virus is plotted as percent specific lysis for all three groups. Effector/target ratios are 40:1. Bars indicate means ± SD. Means are given in the text. Measles virus–stimulated PBL from MS patients denoted by asterisk had significantly higher levels of lysis of measles virus–infected and –uninfected autologous B cell lines than other members of this group (see text).
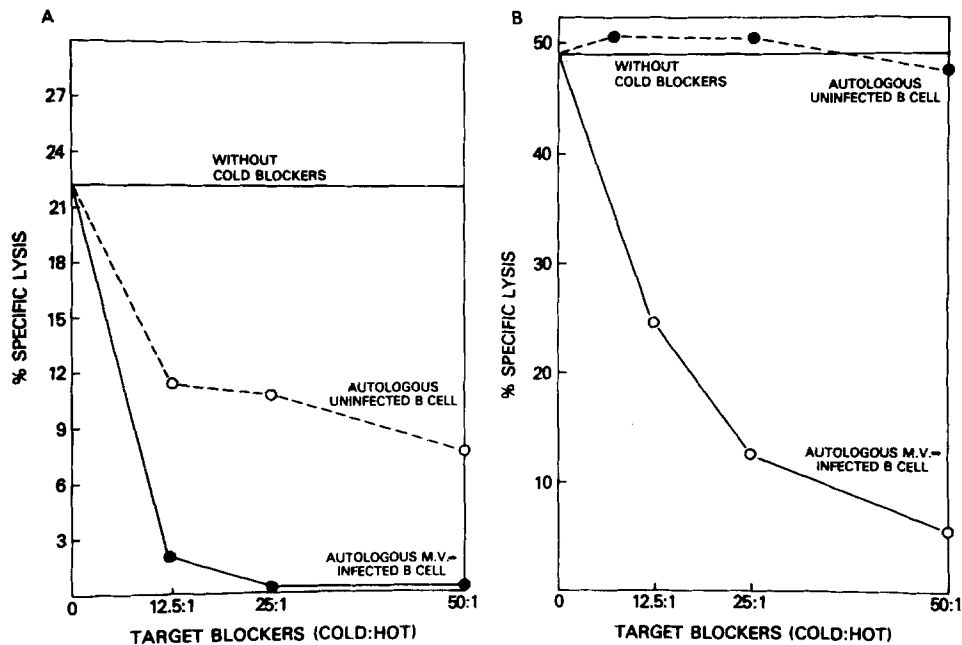
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FIGURE 2.   Cold-target blocking experiments of CTL generated against measles virus (MV) from MS patient 8 (A) and normal individual 17 (B) were performed as described in Material and Methods. Percent specific lysis is plotted vs. increasing cold/hot targets. Open circles represent uninfected autologous B cell lines, and filled circles represent measles virus–infected autologous targets. Effector/hot target ratio is 40:1.

sensitized lymphocytes lysed their uninfected autologous B cell line at a mean level of lysis of 17.7 ± 3.3%. This indicated a non–measles virus–specific response in PBL stimulated with measles virus from these three MS patients. None of the normal individuals or ODC who had high levels of lysis on measles virus–infected autologous B cell lines significantly lysed their uninfected autologous B cell targets (data not shown). To confirm this apparent lack of measles virus–specific killing in the MS patients who had high levels of lysis on uninfected autologous B cell targets, cold-target blocking experiments were performed. The results of a representative experiment are shown in Fig. 2. The ability of measles virus–stimulated PBL from normal individual 17 to lyse the ^{51}Cr-labelled measles virus–infected autologous B cell line could only be blocked by increasing numbers of unlabelled cold measles virus–infected B cell targets, and not by the addition of cold uninfected cells. In contrast, the capacity of measles virus–stimulated PBL from MS patient 8 to lyse its infected, ^{51}Cr-labelled B cell target could be blocked by both infected and uninfected autologous B cell lines (Fig. 2A). This result showed that there is a non–measles virus–specific lysis by measles virus–stimulated PBL from this MS patient.

Susceptibility of MS Patients Measles Virus–infected B Cell Lines to Be Lysed by Measles Virus–specific CTL.   The possibility that the lowered measles virus–specific CTL responses from MS patients was due to the inability of MS patients B cell lines to functionally express measles virus antigens was next examined.

TABLE II

Susceptibility of Measles Virus–infected B Cell Lines from MS Patients to Lysis by CTL Generated from Normal Individuals

Exp.	Measles virus–stimu-lated effectors*	Percent lysis of measles virus–infected B cell targets[‡]		
		MS patient 2	MS patient 7	Normal 15
I	MS patient 2	4.3	NT	−10.9
	MS patient 7	NT	−4.9	NT
	Normal 15	16.0	21.5	24.2
		MS patient 16	Normal 12	Normal 13
II	MS patient 16	1.3	NT	NT
	Normal 12	22.5	22.1	32.4
	Normal 13	15.4	16.8	30.1
		MS patient 1		Normal 12
III	MS patient 1	5.9		NT
	Normal 12	15.2		24.6

* Effectors were generated from the indicated individual by a 7-d in vitro stimulation of PBL with measles virus, as described in Materials and Methods. Effectors were used at an effector/target ratio of 40:1.
[‡] Data are presented as percent lysis of measles virus–infected B cell line targets, as described in Materials and Methods. NT, not tested.

Initially, the expression of measles virus from infected B cell lines from MS patients and normal individuals was examined by fluorescent antibody techniques, using a fluorescein-conjugated serum from a patient with subacute sclerosing panencephalitis (11). The intensity of staining and percent of positive cells was similar in the two populations (data not shown). To more clearly eliminate the possibility of differences in target cell susceptibility, the ability of measles virus–specific CTL from normal individuals to lyse measles virus–infected B cell lines from MS patients was examined (Table II). Those measles virus–infected B cell lines from MS patients that could not be lysed by measles virus–stimulated autologous PBL were shown to be susceptible to CTL from class II HLA–matched normal individuals. This indicated that the diminished measles virus CTL responses from MS patients was not due to a failure to express measles virus antigen on target cell membranes.

Discussion

Both immunological and infectious mechanisms have been suggested in the etiology of MS (1, 2). In support of an infectious agent in the pathogenesis of this disorder, evidence has been obtained from both epidemiological (12) data and serological studies. The latter experiments have demonstrated elevated serum and cerebrospinal fluid antibody levels to a number of viruses, most notably to measles virus in patients with MS (7, 8). Since the report in 1962 by Adams and Imagawa (7), this specific increased antibody production to measles virus from patients with MS has been confirmed by other laboratories (6, 8). The demonstration of an antigen-specific cellular immune abnormality in MS

has been less clear. While there is evidence to suggest that the cellular immune response to measles virus is altered in MS (13, 14), this has not been well confirmed (15–17).

Other types of immune abnormalities have been reported in MS, and have been extensively reviewed (1, 2, 4). These studies are based largely on experiments that have shown changes in circulating lymphocyte subsets during the course of the disease, the presence of specific lymphocyte subpopulations in CNS lesions, and alterations of antigen nonspecific in vitro lymphocyte functions. What clearly has been lacking is the demonstration of an antigen-specific cellular immune abnormality in MS. The importance of analyzing antigen-specific events is the possibility that the antigen under investigation may be related to the disease, and that such antigen-specific systems provide greater sensitivity in examining alterations in immunoregulatory mechanisms in MS. A previous study of twins with MS has shown an enhanced proliferative response to measles virus in the affected member of some discordant twin sets (18). This has not been shown in the vast majority of sporadic MS in nontwins (15–17), nor could this be demonstrated in our MS population sample (data not shown). An enhanced proliferative response to measles virus in a limited set of MS twins may reflect a different disease mechanism in this unique population, or could be related to twinning. Because of these and other findings (13), we have been interested in defining measles virus–specific lymphocyte functions. Recently, we have shown that measles virus–specific, OKT4$^+$ CTL clones recognize measles virus antigen in association with HLA class II molecules (9, 10). In subsequent experiments, we have shown that measles virus–specific CTL generated in bulk culture are also restricted predominantly to HLA class II determinants, while influenza virus–specific CTL generated in bulk culture could be both HLA class I– and II–restricted (Jacobson, S., M. L. Flerlage, and H. F. McFarland, manuscript in preparation)[2].

In this study, the capacity of MS patients (relative to normal individuals and ODC) to generate measles virus– and influenza virus–specific CTL in a standard 7-d in vitro–stimulated culture system assay has been examined. CTL targets were generated by infection of autologous lymphoblastoid B cell lines. These targets express both HLA class I and class II antigens, and are an essential feature of this assay system to detect measles virus–specific, HLA class II–restricted CTL. The K562 line was used to assess NK activity from these 7-d-stimulated bulk cultures.

The major finding in this study was that measles virus–specific CTL responses were markedly diminished in patients with MS. The mean specific lysis of autologous measles virus–infected B cells for this group was 6.0 ± 1.4%, while normal individuals and ODC could generate measles virus–specific CTL and lyse their respective infected B cell targets at a group mean level of lysis of 26.9 ± 2.86% and 26.7 ± 2.8%, respectively. Importantly, this diminished CTL response was specific for measles virus, since it could be shown that this group of MS patients had comparable levels of influenza virus CTL and NK responses, as did a panel of normal individuals and ODC. The lowered measles virus–specific CTL responses observed in MS patients was disease-specific, since patients with other

disorders had measles virus CTL responses comparable to normal individuals. No correlation could be made with the diminished measles virus CTL activity and the disability status of this group of MS patients (Table I). Further, patients with other diseases and moderately severe disability did not show a reduction in measles virus–specific CTL responses. Although three MS patients in this study did lyse their measles virus–infected autologous B cell targets at somewhat higher levels than the majority of MS patients tested (Fig. 1 A, asterisks), they also lysed their uninfected B cell targets at comparable levels. Lysis of uninfected cells by in vitro–primed lymphocytes was indicative of a non–measles virus–specific event, and cold-target blocking experiments confirmed this (Fig. 2). When this is taken into consideration in the interpretation of these data, the observation on these three MS patients also supports the concept that measles virus CTL responses are decreased in MS.

The evidence presented in this report couples two hypotheses in the pathogenesis of MS: (a) There is an infectious agent that may be an etiological factor in this disorder; and (b) There is an ongoing immune dysfunction that may be related to the infectious agent. The demonstration of diminished measles virus–specific CTL activity suggests a number of possible interpretations. If measles virus–specific CTL are absent or in lower numbers in the peripheral blood, one possibility is that these cells may be sequestered in other areas. As has been suggested previously, MS may be due to antigen-specific lymphocytes that migrate to the CNS and destroy white matter (19). Although an infiltration of T cells surrounding MS plaque lesions has been demonstrated in several laboratories (19–21), there is conflicting evidence with respect to the phenotype of these T cells. Observations from one group have indicated a predominance of OKT4+ cells, suggesting a role for this subpopulation of T cells in MS plaque lesion development (19, 21). Our results are also of interest with respect to the recent studies (22) in murine experimental allergic encephalomyelitis, a disease model with pathological and clinical similarities to MS, that show a redistribution of Lyt-1+ lymphocytes (the murine equivalent to OKT4+ cells in humans) from the periphery to the CNS. We have evidence that demonstrates that the majority of effector CTL generated in bulk culture from normal individuals are OKT4+ and recognize measles virus in association with HLA class II molecules (manuscript in preparation)[2]. There is no evidence for decreased OKT4+ cells in the peripheral blood from MS patients in this (data not shown) or other studies (23).

Other mechanisms that could account for the diminished measles virus CTL response in MS deserve consideration. First, several lines of evidence indicate that the parameters of immunization can determine the nature of the resulting immune response (24). Intravenous inoculation of haptenated cells will initiate a CTL response but suppress a delayed-type hypersensitivity response. In some cases, these differences may be due to selective induction of suppressor cells. As suggested (13), a measles virus infection could eliminate a clone of measles virus–reactive cells (OKT4+ CTL?), which may result in a suppression of cellular reactivity to this agent. Consequently, differences between MS patients and controls during the initial immunization to measles virus could account for our

findings. These differences could include age when exposed, strain of virus, or severity of infection.

A second consideration is that our findings could reflect a specific reduction in OKT4$^+$ cytotoxic cells. The major portion of influenza virus–specific CTL is HLA class I–restricted, and would not be affected by such a mechanism. Although there was no evidence for an overall reduction in OKT4$^+$ cells, a reduction in a subset of this population with CTL function may occur. This possibility cannot be excluded until other examples of HLA class II–restricted killing are examined. Alternatively, an increase in suppressor cells (presumably with an OKT8$^+$ phenotype) could also serve to selectively reduce measles virus–specific CTL. Although we saw no increase in OKT8$^+$ cells in the peripheral blood from the MS patients we studied, functional assays for measles virus–specific suppressor cells would be required to resolve this possibility.

As suggested previously (1), it may not be possible, in a single hypothesis, to account for all the observations that have been reported in studies of MS. Indeed, MS may be a heterogeneous disorder, with various etiologies, and more than one pathogenic mechanism. More MS patients in different stages of the disease with varying degrees of disability must be examined for their cellular immune responses to a number of infectious agents.

Summary

To assess whether an virus–specific immune defect may be associated with multiple sclerosis (MS), we have examined the ability to generate measles virus– and influenza virus–specific cytotoxic T cells (CTL) in patients with MS, normal individuals, and other disease controls (ODC). The mean ($\pm$ SEM) measles virus–specific CTL response for normal individuals and ODC was 26.9 $\pm$ 2.9% ($N = 17$) and 26.7 $\pm$ 2.8% ($N = 13$) specific lysis, respectively. In contrast, the capacity of MS patients to generate measles virus–specific CTL was markedly diminished. Peripheral blood lymphocytes from MS patients stimulated with measles virus lysed their measles virus–infected autologous B cell line at a group mean level of 6.0 $\pm$ 1.4% ($N = 16$) specific lysis. MS patients had significantly lower measles virus–specific CTL responses than normal individuals ($p < 0.00001$) or ODC ($p < 0.0001$). Importantly, this lowered response did not reflect a generalized depressed cytolytic activity of MS patients, since influenza virus–specific CTL and NK activity from these patients were comparable to normals and ODC. Thus, in MS there is a significant depression of measles virus–specific CTL which suggests that this virus–specific immune dysfunction may play a role in the pathogenesis of this disorder.

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