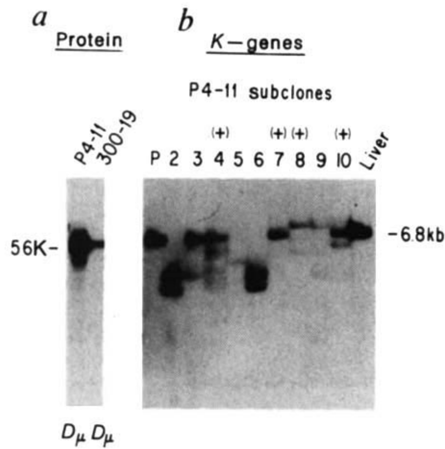

Fig. 2 High-level expression of $D\mu$-chains is associated with V_K assembly. *a*, $D\mu$ expression. Approximately 10^7 300-19 or subclone P4-11 cells were lysed, fractionated by polyacrylamide gel electrophoresis, electrotransferred to a nitrocellulose filter, and the bound μ-chains detected by a sandwich technique using goat anti-IgM and ^{125}I-labelled monoclonal IgM antibody B1-8 (see ref. 7 for details). *b*, Genomic DNA from the P4-11 line (lane P), various P4-11 secondary subclones (lanes 2-10) and mouse liver (liver) was assayed for J_κ rearrangement as described in Fig. 1 legend.

ordered rearrangement). A clue to some of the necessary molecular determinants of this putative signal may come from the observation that high-level expression of a truncated heavy-chain protein containing just D and C_μ portions (D_μ protein) may also signal the onset of V_L assembly and the cessation of V_H assembly.

Several 300-19 subclones which produce both μ- and κ-chains contain a large percentage of cells (30%) that express these chains on their surface in the form of IgM; in contrast, the parental line or μ-only intermediates do not express surface immunoglobulin (data not shown). The acquisition of surface IgM by the μ- and κ-producing subclones was often accompanied by the appearance of additional surface markers diagnostic of the B-cell stage of differentiation, including surface IgD (unpublished results). Thus, the 300-19 line represents, to our knowledge, the first permanent cell line of any type that clearly undergoes all of the sequential gene reorganization and expression events which lead to the surface IgM-positive stage of B-lymphocyte differentiation. Previously characterized A-MuLV transformants which were shown to undergo V_H to DJ_H rearrangement did not appear to go on to the V_L assembly stage. However, these lines, which were derived from the fetal liver of BALB/c mice, rearranged a highly restricted set of V_H gene segments[16] and usually generated V_HDJ_H-containing progeny in which these rearrangements were non-productive[17], suggesting a detrimental effect of the expression of the frequently used V_H gene segments. The 300-19 line, on the other hand, uses a very different set of V_H gene segments to form V_HDJ_H rearrangements[8] and generates, at high frequency, progeny which have formed productive rearrangements (see above). Taken together, our observations suggest that the relative ability of these lines to differentiate to the V_L assembly stage may be a function of their inherent capacity to generate progeny which have assembled and expressed productive V_HDJ_H rearrangements, rather than some consequence of the A-MuLV transformation event *per se*. The *in vitro* differentiation of the 300-19 line appears to occur spontaneously and rapidly with no additional stimulaton needed beyond that potentially provided by components of the growth medium. This portion of the normal B-cell differentiation pathway probably also occurs in the spontaneous and autoregulatory manner observed in 300-19 cells, with the product of one differentiation stage signalling the onset of the next stage.

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T-cell clones specific for myelin basic protein induce chronic relapsing paralysis and demyelination

Scott Zamvil*, Patricia Nelson†, Jacqueline Trotter*, Dennis Mitchell*, Robert Knobler‡, Robert Fritz§ & Lawrence Steinman*

* Departments of Pediatrics and Neurology, Stanford University School of Medicine, Stanford, California 94305, USA
† Carcinex, Inc., Burlingame, California 94010, USA
‡ Department of Neurology, Jefferson Medical College, Philadelphia, Pennsylvania 19107, USA
§ Department of Microbiology, Emory University Medical School, Atlanta, Georgia 36322, USA

Experimental allergic encephalomyelitis (EAE) serves as a model for autoimmune diseases mediated by T lymphocytes[1,2]. Following sensitization to rat, mouse or guinea pig myelin basic protein (MBP) in complete Freund's adjuvant, inbred mouse strains PL/J (*H–2[u]*), SJL/J (*H–2[s]*) and (PL/J × SJL/J)F$_1$((PLSJ)F$_1$) develop EAE[3,4]. Whereas sensitization to the N-terminal 37 amino-acid peptide of rat or guinea pig MBP [MBP(1–37)] induces EAE in PL/J mice, immunization to the C-terminal peptide (89–169) leads to EAE in SJL/J mice[4,5]. The immune response to MBP in (PLSJ)F$_1$ mice is not co-dominant; sensitization to the N-terminal peptide induces EAE, while sensitization to the C-terminal peptide does not[3,4]. We have generated MBP-specific T-cell clones restricted to class II (Ia) antigens of the major histocompatibility complex (MHC) from PL/J and (PLSJ)F$_1$ mice following sensitization to rat MBP. Two such I–A[u]-restricted T-cell clones that proliferate in response to the encephalitogenic N-terminal MBP peptide and recognize a shared determinant with mouse (self) MBP cause paralysis in 100% of (PLSJ)F$_1$ mice tested. Paralysis is induced even when recipients are injected with as few as 1×10^5 cloned T cells. Relapsing paralysis followed in two-thirds of the recipients after recovery from acute paralysis, whereas one-third developed chronic persistent paralysis, a form of EAE not usually seen. Histopathology revealed intense perivascular inflammation, demyelination and remyelination within the central nervous system of paralysed mice. The experimental disease induced with these clones shares important features with human demyelinating diseases such as multiple sclerosis. This is the first demonstration that T-cell clones that respond to a defined self-antigen can induce clinical and histological autoimmune disease.

T lymphocytes sensitized to MBP can cause clinical and histological EAE when adoptively transferred to naive

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Table 1 Response of T-cell clones to MBP

Encephalitogenic clones	Antigen-presenting cells	Response to MBP (mean c.p.m. ^{3}H-thymidine incorporation) No antigen	Rat MBP	Bovine MBP	Mouse MBP
Clone PJR-25	PL/J	276	88,341	27,859	73,421
	SJL/J	319	266	145	292
	(PLSJ)F_1	99	76,726	4,170	22,574
Clone F1-12	PL/J	530	54,743	6,013	16,038
	SJL/J	1,659	1,663	2,150	1,964
	(PLSJ)F_1	530	42,666	2,712	5,358
Non-encephalitogenic clones					
Clone F1-13	PL/J	281	666	314	355
	SJL/J	766	37,094	447	262
	(PLSJ)F_1	174	15,544	166	230
Clone F1-15	PL/J	176	288	ND	ND
	SJL/J	2,110	2,404	ND	ND
	(PLSJ)F_1	1,789	239,244	2,169	2,924
Clone F1-16	PL/J	73	279	ND	ND
	SJL/J	152	216	ND	ND
	(PLSJ)F_1	126	35,173	138	206

Proliferative T-cell lines specific for MBP were generated from PL/J and (PLSJ)F_1 mice following protocols previously described[23]. Five mice per group of each strain were immunized subcutaneously at the base of the tail with 200 μg rat MBP emulsified in complete Freund's adjuvant (CFA). Seven days later, draining inguinal and para-aortic lymph nodes were removed, cells were suspended in media and cultured in 24-well dishes (Costar, 3524) at 6×10^6 cells per well with 100 μg ml^{-1} rat MBP. Eight days later, cells were collected, washed twice and 5×10^5 viable cells were transferred to 25-cm^2 tissue culture flasks (Corning 25100). These cells were stimulated with 3×10^7 γ-irradiated (3,300 rad) APC and 100 μg ml^{-1} MBP. Twelve days after the previous stimulation, viable resting T cells were separated by centrifugation over a Ficoll gradient. At this time T cells were cloned by limiting dilution in 96-well flat-bottom microtitre plates (Linbro, 76-003-05) at 0.3 cells per well with 5×10^5 syngeneic APC, 100 μg ml^{-1} rat MBP and 10% supernatant from concanavalin A-stimulated rat spleen cells (CAS)[23]. After 12 days, cell growth was observed in only 8–10% of the wells. Cells from individual wells were expanded in 24-well Costar plates in the same manner with 5% CAS and eventually transferred to 25-cm^2 tissue culture flasks. Then 5×10^5 cloned T cells were routinely stimulated every 12–14 days without CAS. Clone PJR-25 was subcloned by sorting at 1 cell per well using FACS. Complete culture media consisted of RPMI 1640 (Gibco) supplemented with 10% fetal calf serum (M.A. Bioproducts), 5×10^{-5} M 2-mercaptoethanol, 2 mM glutamine, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin. To assay for proliferative response to rat, bovine or mouse MBP, 10^4 T cells were cultured with 5×10^5 irradiated APC from PL/J, SJL/J or (PLSJ)F_1 mice in 0.2 ml culture media in 96-well flat-bottomed microtitre plates. MBP from each species was isolated as described previously[24]. Each clone was tested with and without rat MBP. Cultures were pulsed using 1 μCi ^{3}H-thymidine at 48 h and collected 16 hours later. The mean c.p.m. of thymidine incorporation was calculated for triplicate cultures. The standard deviations from replicate cultures were within 10% of the mean value. ND, not done.

recipients[6-10]. The T cells that are transferred in these experimental systems are heterogeneous polyclonal populations capable of responding to various antigenic determinants on MBP[9]. It is important to define precisely which T cells are involved in induction of EAE, because many cells contained in bulk T-cell cultures[6,7], or even long-term T-cell lines[8-10], may not be involved in the induction of autoimmunity. In an investigation of the properties of the cells participating in the pathogenesis of EAE, examining cloned populations of T cells that react to MBP is advantageous. We established proliferative T-cell lines specific for MBP in PL/J and (PLSJ)F_1 mice following immunization with rat MBP. Clones were then derived from these T-cell lines. The response of the clones to mouse (self) MBP and peptides derived from MBP was analysed. Since T cells recognize antigen in association with class II MHC antigens, these clones were further characterized on the basis of their pattern of restriction to I-A or I-E molecules. The clones were then tested for their ability to induce EAE in (PLSJ)F_1 mice. The properties of two encephalitogenic clones, PJR-25 derived from PL/J mice and F1-12 derived from (PLSJ)F_1 mice, are described here. The characteristics of three MBP-specific non-encephalitogenic 'control' clones, F1-13, F1-15 and F1-16, are also presented.

Clones PJR-25 and F1-12 proliferate in response to rat, bovine or mouse MBP, but only in association with H-2^u MHC molecules expressed on PL/J and (PLSJ)F_1 antigen-presenting cells (APC) (Table 1). Neither clone is alloreactive for H-2^s or

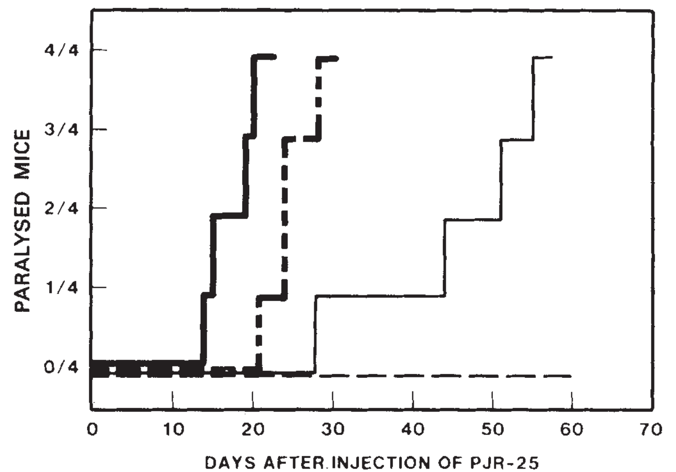

Fig. 1 Onset of paralysis in (PLSJ)F_1 mice after injection of MBP-specific T-cell clone PJR-25.

Methods. (PLSJ)F_1 mice were injected intravenously (i.v) with 1×10^6 (thick solid line), 5×10^5 (thick broken line) or 1×10^5 (thin solid line) PJR-25 cells, or 5×10^6 control T-cell clones specific for MBP, which do not proliferate to mouse MBP (thin broken line). Fourteen days after stimulation with rat MBP and irradiated APCs, viable T cells were separated by centrifugation through a Ficoll gradient. These cells were then stimulated for 24 h in complete media containing 10% CAS (see Table 1 legend) in the absence of APC. Recipient mice were given low-dose whole-body irradiation (350 rad) and 10^{10} heat-inactivated *B. pertussis* i.v. prior to T-cell injection, and a second *B. pertussis* injection was given ~48 h after injection of PJR-25 cells. Onset of paralysis is defined as the first signs of hind-limb paraparesis or paraplegia and loss of tail tone.

H-2^{uxs} F_1 antigens expressed on (PLSJ)F_1 APC. Both PJR-25 and F1-12 respond to peptides composed of guinea pig and bovine MBP(1–37) (Table 2). Rat and guinea pig MBP have identical amino-acid sequences for residues 1–37, and differ from mouse MBP at only two residues. Mouse MBP is missing His 10 and Gly 11 (ref. 11). Bovine MBP(1–37) also has this deletion, but differs from rat, guinea pig and mouse MBP at residues 2 and 17 (see Table 2 legend).

In PL/J and (PLSJ)F_1 mice, two similar but separately encoded Ia antigens, I-A and I-E, can be expressed. Whether antigen recognition occurs in association with a particular Ia complex can be determined by using monoclonal antibodies that recognize only specific I-A or I-E gene products[12,13]. These monoclonal antibodies bind to I-A or I-E molecules on APC and block T-cell recognition, thus inhibiting T-cell proliferation. Using this approach, we found that PJR-25 and F1-12 are both I-A^u-restricted (Table 3).

Clones PJR-25 and F1-12 were considered good candidates to test *in vivo* for induction of EAE because each clone recognizes an epitope within the encephalitogenic N-terminal peptide that is shared with mouse (self) MBP. We predicted that MBP-specific T-cell clones that do not respond to mouse MBP *in vitro* would be unlikely to cause EAE in mice. Clone PJR-25, which has the phenotype of a T-helper cell (Lyl^+, Lyt 2^-, $L3T4^+$) as determined by fluorescent-activated cell sorter (FACS) analysis (data not shown), was the first clone tested *in vivo*.

In five consecutive experiments, 100% of (PLSJ)F_1 mice developed paralysis after a single injection of PJR-25 (Fig. 1). Even when injected with as few as 10^5 cells, the smallest number yet tested, all of four mice developed paralysis. The onset of paralysis is, however, dependent on the number of PJR-25 cells injected. With greater numbers of cells, clinical signs appear earlier (Fig. 1) and death is more likely. Of 12 mice injected with 5×10^6 PJR-25 cells, all developed acute EAE within 10–14 days (data not shown). Eight mice died 1–5 days after onset of paralysis, and the other four mice were used for histological studies when found moribund.

After an injection of 10^6 or 5×10^5 PJR-25 cells, some mice

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Table 2 Response of T-cell clones to peptide fragments of MBP (mean c.p.m. ^{3}H-thymidine incorporation)

Encephalitogenic clones	No antigen	Rat MBP	Guinea pig MBP	Guinea pig MBP(1-37)	Bovine MBP(1-37)	Bovine MBP(89-169)
PJR-25	143	117,592	83,489	108,853	39,715	392
F1-12	125	48,326	19,223	53,839	11,125	126
Non-encephalitogenic control clones						
F1-13	55	32,779	53	37	42	52
F1-15	50	36,580	39,011	43,108	32	26
F1-16	68	74,286	83,687	95,650	133	247

All MBP peptides are products of pepsin cleavage from either intact guinea pig MBP or bovine MBP. These peptides have been isolated and purified as previously described[3-5]. Guinea pig MBP(1-37) has the identical sequence to rat MBP in this N-terminal region and bovine MBP (89-169) shares the same amino-acid sequence as large rat MBP (89-169)[11]. Bovine MBP(1-37) differs from the rat and guinea pig MBP(1-37) sequence at residue 2, where Ala replaces Ser, and residue 17, where Ser replaces Thr. Bovine MBP has a deletion of the His 10 and Gly 11 residues. Although these clones do not proliferate to bovine MBP(89-169), this peptide does not suppress the proliferation to either MBP(1-37) (not shown). MBP(89-169) has been found to stimulate certain MBP-specific proliferative T-cell clones derived from SJL/J mice[9]. All peptides were added at initiation of the cultures to give a final concentration of 50 μg ml^{-1}. Intact proteins were also added but at a concentration of 100 μg ml^{-1}. Syngeneic irradiated APC were used in all cultures (see Table 1). Proliferative responses were determined as described in Table 1 legend.

Table 3 Pattern of I-region restriction of T-cell clones (mean c.p.m. ^{3}H-thymidine incorporation)

Encephalitogenic clones	No antigen	Rat MBP + anti-I-$A_\beta^{u,s}$: alone	Rat MBP + anti-I-$A_\beta^{u,s}$: (10-2.16)	Anti-I-A_β^u (40 M)	Anti-I-E_α^u (14-4-4)	Anti-I-$E^{s/u}$ (Y-17)
PJR-25	213	125,562	7,018	5,181	127,836	108,853
F1-12	125	48,326	8,828	2,474	47,324	63,078
Non-encephalitogenic control clones						
F1-13	361	33,002	28,025	27,303	34,635	38,253
F1-15	394	99,266	345	364	75,923	102,890
F1-16	68	69,552	133	185	52,599	65,603

All monoclonal antibodies have been described previously[25-28]. Antibody 10-2.16 binds determinants Ia.17 on certain I-A_β chains including A_β^s and A_β^u (ref. 25). Antibody 40 M binds Ia.1, a determinant on I-A_β^u, but not on I-A_β^s (ref. 26). Antibody 14-4-4 binds determinant Ia.7 expressed on certain E_α chains including E_α^u (ref. 27). Antibody Y-17 binds a determinant, Ia.m44, on certain hybrid I-E molecules including $E_\alpha^u E_\beta^s$ (ref. 28). In these blocking studies 1 μg of each antibody was added at the beginning of culture to those wells containing cloned T cells, syngeneic APC and rat MBP. 10-2.16, 14-4-4 and Y-17 were provided by Dr P. P. Jones (Stanford University). Proliferative responses were determined in the same manner as described in Table 1 legend.

have developed acute paralysis followed by death, as was seen when 5×10^6 cells were injected. Many, however, develop chronic relapsing paralysis, and a small number develop chronic unremitting paralysis, a form not usually observed. In three experiments in which a total of 28 mice were injected with either 1×10^6 or 5×10^5 PJR-25 cells, 13 mice died 3-9 days following the onset of paralysis. Eleven of the 15 mice that survived the initial onset of paralysis developed hind-limb paraplegia and complete loss of tail tone, which lasted 6-10 days and then improved with no other observable signs of paralysis. These 11 mice relapsed 10-25 days later, developing at least hind-limb paraparesis and usually complete paraplegia of 6-20 days duration. Of these 11 mice, 8 underwent a second remission, with 3 of those relapsing for a second time (20 days later). The other 3 mice from the 11 remained chronically paralysed (>40 days). The remaining 4 of the 15 mice that survived the intial onset of paralysis were chronically paralysed (either severe paraparesis or complete paraplegia) for more than 80 days without signs of deterioration or remission. This chronic persistent form of paralysis is unusual in mice with EAE.

Classic histological features of EAE, including perivascular infiltrates in the central nervous system and meningeal inflammation, were observed (Fig. 2). Demyelination and remyelination accompanied the cellular inflammation in mice with both chronic relapsing and chronic unremitting paralysis (Fig. 2).

A subclone of PJR-25 was also observed to cause paralysis in mice. This subclone, called PJr-25, was derived by sorting the parent clone at one cell per well using FACS analysis. When injected with 1×10^6 PJr-25 cells, all of four (PLSJ)F_1 recipient mice developed clinical EAE with chronic paralysis. Two of these four mice relapsed. The encephalitogenic potential of this clone was not diminished by subcloning or by continuous culture *in vitro* for more than 10 months.

T-cell clone F1-12 was also tested *in vivo*. This clone, derived from (PLSJ)F_1 mice, has a similar MBP recognition pattern as PJR-25. It responds to the encephalitogenic N-terminal peptide (Table 2), recognizes a conserved epitope with mouse MBP (Table 1), and is also I-A^u-restricted (Table 3). When tested *in vivo*, paralysis was observed in four of four (PLSJ)F_1 recipient mice. These mice developed paraparesis and, in two cases, the paraparesis evolved to monoparesis. In one case a mouse developed paraparesis followed 14 days later by right leg monoparesis. Ten days later paraparesis recurred and 40 days later right leg monoparesis developed a second time.

MBP-specific T-cell clones that respond to rat MBP but not to mouse MBP were administered to (PLSJ)F_1 mice in the same manner as the encephalitogenic clones PJR-25, PJr-25 and F1-12. Three different control clones, F1-13, F1-15 and F1-16, were injected (5×10^6 cells) into (PLSJ)F_1 recipients. None out of 21 of these recipient mice developed clinical paralysis (see Fig. 1 legend). Demyelination was not observed in tissue sections from mice given control clones. These T-cell clones were derived from (PLSJ)F_1 mice immunized with rat MBP. One of these clones, F1-13, responds to rat MBP only (Table 1), and solely in association with SJL/J APC. The other two clones, F1-15 and F1-16, recognize a determinant within the encephalitogenic N-terminal peptide of rat and guinea pig MBP that is not shared by mouse MBP (Table 2). This determinant probably includes His 10 and Gly 11, present in guinea pig MBP and rat MBP, but deleted in mouse MBP as this is the only difference in amino acids 1-37 between mouse MBP and rat or guinea pig MBP. Both F1-15 and F1-16 respond to rat MBP only in association with (PLSJ)F_1 hybrid APC, not parental APC (Table 1). Monoclonal antibodies to I-A_β^u block proliferation of these clones to rat MBP. Clones F1-15 and F1-16 are thus restricted to I-$A_\alpha^s A_\beta^u$ (Table 3).

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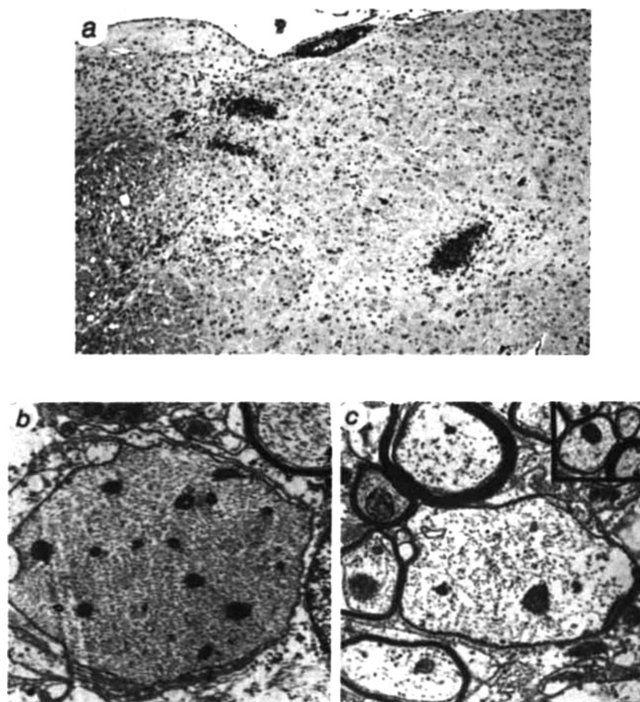

Fig. 2 *a*, Histological evidence of EAE. Representative perivascular cuffs from cortex of mouse with paraparesis 25 days after receiving PJR-25, 5×10^5 cells. ×150, haematoxylin and eosin stain. *b*, A completely demyelinated axon, demonstrating primary demyelination in a mouse injected with MBP-specific T-cell clone PJR-25. This mouse was killed during a relapse, 45 days after initial signs of paralysis. A portion of a remyelinated axon is present in the upper right corner. ×17,500. *c*, A partially remyelinated axon is evident in the centre of the field, which is surrounded by several remyelinated axons. The inset in the upper right corner shows several additional remyelinated axons, characterized by a thin myelin sheath around the axonal profile. A more normal axonal sheath profile is present in the lower right corner of the inset. ×17,500.

The failure of any of the three control clones to induce EAE mitigates against passive transfer of MBP as a mechanism for EAE induction. These clones all proliferate in response to rat MBP as well as to PJR-25 or F1-12. Thus, if any trace amount of rat MBP is passively transferred with PJR-25, PJr-25 or F1-12, similar amounts are probably transferred with the control clones handled in the same manner.

As in other systems in which murine T-cell lines have been used to induce EAE[8,9,14], we have found that low-dose irradiation of the recipients (see Fig. 1 legend) facilitates induction of severe clinical EAE. Thus, all of eight $(PLSJ)F_1$ mice receiving 1×10^6 PJR-25 T cells with radiation (350 R) but without *Bordetella pertussis* developed relapsing paralysis, while none of eight mice without irradiation that received *B. pertussis* intravenously developed EAE. One of eight mice developed EAE without irradiation or *B. pertussis*. Preliminary results revealed that *in vivo* administration of recombinant human interleukin-2 (provided by Cetus) permitted induction of EAE with clone PJR-25 without the need for either radiation or *B. pertussis* (S.Z. *et al.*, in preparation).

These experiments demonstrate for the first time that EAE can be induced with individual T-cell clones. Class II (I-A)-restricted T cells bearing the L3T4 phenotype characteristic of helper-inducer cells, which recognize the N-terminal peptide of MBP, are highly encephalitogenic. We have shown previously that EAE can be prevented and that progressive paralysis can be reversed with *in vivo* administration of either anti-I-A or anti-L3T4 antibody[15-17]. These antibodies may be effective through blockade of activation of T cells restricted to class II MHC molecules[15-18]. It is intriguing that astrocytes isolated from the central nervous system express class II MHC antigens, when cultured *in vitro* with T-cell lines specific for MBP, and are capable of presenting MBP to these T-cell lines[19,20]. Ia antigens can be demonstrated on endothelial cells in the central nervous system early in the development of EAE[21]. Thus, it is likely that these T-cell clones recognize MBP in association with Ia antigens present in the brain and spinal cord.

These T-cell clones induce a variety of clinical states, including acute fulminant paralysis, relapsing-remitting paralysis and chronic persistent paralysis. It is clear that a single cloned T-cell population can induce relapsing paralysis. The number of cloned T cells that are administered is a critical factor in determining the clinical course. Whether recurrent paralysis and demyelination in part results from sensitization to MBP plus lipids—a question raised by others[7]—can now be excluded by the finding that cloned T cells reactive to MBP peptides induce relapses and demyelination.

The availability of T-cell clones specific for defined regions of the MBP molecule that mediate EAE, the archetypal model for autoimmunity induced with T cells, provides several opportunities to study the pathophysiology of this disease. Because clinical relapses and demyelination are induced with these clones, this model disease shares important features with human demyelinating diseases such as multiple sclerosis. These T-cell clones provide decisive advantages for studying such questions as the migration of T cells to the central nervous system in disease[22], and the molecular biology of the T-cell receptors for the encephalitogenic epitope of MBP. Using smaller peptides, we are currently defining the region within MBP (1-37) that is recognized by disease-inducing clones. If encephalitogenic T cells responding to the N-terminus of MBP recognize the same epitope, they possibly share structural similarities in their MBP-specific T-cell receptors. If so, the development of clonotypic antibodies against MBP-specific T-cell clones can be tested in this system to determine whether they could be beneficial in the therapy of T cell-mediated autoimmune diseases. Finally, because these clones induce relapsing disease, the regulatory mechanisms leading to relapse and remission can be studied following a single injection of a clonally derived population.

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