Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 7219–7223, August 1991
Immunology

T-cell receptor peptide immunization leads to enhanced and chronic experimental allergic encephalomyelitis

(disease enhancement/multiple sclerosis)

LISE DESQUENNE-CLARK, THOMAS R. ESCH, LASZLO OTVOS, JR., AND ELLEN HEBER-KATZ*

The Wistar Institute of Anatomy and Biology, 3601 Spruce Street, Philadelphia, PA 19104

Communicated by Hilary Koprowski, May 23, 1991

ABSTRACT **It has previously been reported that synthetic peptides corresponding to sequences derived from T-cell receptor variable regions identified as dominant in the T-cell-mediated autoimmune disease experimental allergic encephalomyelitis in both the mouse and the rat can down-regulate disease in Lewis rats. In contrast to these results, we have found that immunization of Lewis rats with such peptides in complete Freund's adjuvant prior to induction of experimental allergic encephalomyelitis with myelin basic protein leads to responses ranging from profound disease enhancement to lack of disease. In some cases, enhanced disease was followed by a prolonged neurologic deficit that resembles multiple sclerosis more closely than does acute experimental allergic encephalomyelitis. These findings, on the one hand, support previous results showing T-cell receptor peptide-induced modulation of the disease experimental allergic encephalomyelitis and, on the other, indicate that such immunization is not a reliable method for inducing suppression of encephalitogenic effector cells.**

The finding that a limited set of T cells, in the mouse (1–3) and the rat (4, 5), mediates the autoimmune disease of the central nervous system experimental allergic encephalomyelitis (EAE) suggests the possibility of immune intervention by clonal-specific regulation. In both species, T cells bearing the T-cell receptor (TCR) variable (V) regions Vβ8.2 and Vα2/4 are the dominant disease-causing population (6). Monoclonal antibodies directed against these TCR V regions have been shown to block the expression of EAE in response to myelin basic protein (MBP) in Lewis rats (7), and similar antibodies have both blocked disease and suppressed ongoing disease in mice (1–3). Recently, evidence of disease suppression in rats after immunization with synthetic peptides corresponding to portions of the V α and V β chains of identified autoreactive T-cell clones has been seen (8, 9). We now report that immunization of Lewis rats with peptides representing the V regions of these dominant TCRs can hasten disease onset and increase the severity and duration of disease.

We constructed three peptides derived from both TCR chains of the T-cell hybridoma 5.10, which is specific for the encephalitogenic determinant of MBP (4): the Jα39 peptide with the sequence RFGAGTRLTVK (8), the CDR2-Vβ8 peptide with the sequence DMGHGLRLIHYSYDVN-STEKG (9), and TK20 representing the N-terminal residues 5–24 of rat Vβ8.2 (4) with the sequence TQSPRNKVALTG-GKVTLSCK. Rats were immunized with these peptides in complete Freund's adjuvant (CFA) prior to induction of EAE with MBP, and responses ranging from profound disease enhancement to the lack of disease were obtained. In some cases, enhancement of disease was followed by a prolonged neurologic deficit, something we have not previously observed in MBP-induced EAE, which is generally an acute self-limiting disease. This chronic condition is of considerable interest in that it more closely resembles multiple sclerosis than does the acute form of EAE, presently the most generally used animal model for that disorder. This report, then, describes a model for multiple sclerosis using MBP as the immunogen. Lack of a measurable T-cell or antibody response to the peptide has led us to consider the possibility that a regulatory cell normally functioning in EAE that is able to limit the disease *in vivo* has been tolerized.

MATERIALS AND METHODS

Animals. Eight-week-old female Lewis rats were purchased from the Charles River Breeding Laboratories and maintained in the animal facility of The Wistar Institute.

EAE Induction. MBP was dissolved in saline and emulsified with an equal volume of CFA containing *Mycobacterium tuberculosis* (H37Ra) at 2 mg/ml (Difco). Rats were injected subcutaneously in the thigh with a total volume of 100 μl containing 50 μg of MBP. Animals were observed for clinical signs of EAE. In experiments 1–3 (see Table 1), the observations were made by an observer without knowledge of the immunization protocol. Disease severity was recorded daily using the following paralysis scale: 1, tail paralysis; 2, hind limb weakness and/or partial paralysis; 3, hind limb paralysis; 4, hind and fore limb paralysis.

Flow Cytometry Analysis. Approximately 1×10^5 cells were incubated with antibody in phosphate-buffered saline (PBS)/0.1% bovine serum albumin/0.02% azide for 1 hr at 4°C. After washing three times, the cells were incubated with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin (Pel-Freez Biologicals) for 30 min. The cells were washed three times and analyzed with an Ortho model 50HH cytofluorograph connected to an Ortho model 2150 data handling system (Ortho Diagnostics).

MBP. MBP was extracted from guinea pig spinal cords using chloroform/methanol [1:1 (vol/vol)] followed by acetone lipid extraction as described by Deibler *et al.* (14) and modified by Hickey *et al.* (10).

Peptide Synthesis. Peptide synthesis was done on a Biosearch model SAM2 peptide synthesizer using a combination of fluoren-9-ylmethoxycarbonyl-amino acid symmetrical anhydrides and pentafluorophenyl esters for the acylation reactions. Cleavage from the solid support was achieved with trifluoroacetic acid. The CDR2-Vβ8 peptide was dialyzed in tubing with a M_r 1000 cutoff and purified on an Altex Ultrasphere ODS reversed-phase HPLC column. The isolated peak was subjected to positive-ion fast atom bombardment/mass spectrum analysis, which revealed a single ion at 2392 mass units as predicted. This analysis and amino acid analysis showed purity and the appropriate amino acid com-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EAE, experimental allergic encephalomyelitis; CFA, complete Freund's adjuvant; MBP, myelin basic protein; TCR, T-cell receptor; V region, variable region.

*To whom reprint requests should be addressed.

position according to the reported sequence (4) and the peptide used previously (9).

Peptide Immunization. TCR peptides were dissolved in PBS and emulsified with an equal volume of CFA containing *M. tuberculosis* (H37Ra) at 1 mg/ml. Unless otherwise indicated, each animal was injected subcutaneously in both hind foot pads with a total of 100 μg of peptide in 200 μl of emulsion.

RESULTS

Lewis rats were injected with CFA or CFA plus the CDR2-Vβ8 peptide (9) in the rear footpads (see Table 1). One month later each rat was injected with an encephalitogenic dose of MBP in CFA. Those control animals initially injected with CFA alone displayed classic symptoms of EAE starting on day 10 and peaking on day 15 (Table 1, experiment 1, and Fig. 1*B*). Two of the three CDR2-Vβ8-peptide-primed animals did not show suppression of disease, as reported (9), but in fact showed a higher average disease level and, possibly more important, earlier onset of disease. The third animal showed no effect of the peptide treatment, the time of onset and level of disease being indistinguishable from that in the controls (Table 1, experiment 1, and Fig. 1*A*). The two animals that showed enhanced disease also developed a chronic disease state, unusual in rats receiving MBP: one animal displayed a flaccid tail that never improved and the other, which initially displayed typical fore and hind limb paralysis, had improved by day 16 but then went on to develop severe asymmetric clonus. Enhanced disease severity and development of chronic disease were also seen in one rat given the CDR2-Vβ8 peptide 2.5 months prior to the challenge with MBP in CFA (Table 1, experiment 2).

To determine whether these effects were due to the particular peptide used for immunization, we repeated these experiments with each of the three TCR peptides. Immunization with the CDR2-Vβ8 peptide followed by MBP again resulted in severe disease with early onset (Table 1 and Fig. 2). Similar results were obtained with the Jα39 peptide. Although disease was slightly more severe in rats immunized with the TK20 peptide than in the controls, the time of onset did not differ. It should be pointed out that in some cases the controls did exhibit a less-severe EAE than is generally reported, due to the fact that the MBP in CFA had been given in the thigh where it is generally less encephalitogenic. Nevertheless, the CR2-Vβ8 and Jα39 peptides cause early onset of disease with dramatic enhancement over control levels and with low levels of disease in the controls.

Table 1. Summary of all animals injected with TCR peptides

Exp.	Rat	Day of onset	Peak time of disease, day	Peak level of disease	Chronic disease
1	CDR2-Vβ8 + CFA primed (1 mo)*				
	1	10	13	4	Yes
	2	11	13	4	Yes
	3	13	14	2	No
	CFA primed				
	1	10	15	3	No
	2	12	15	2	No
	3	13	13	1	No
2	CDR2-Vβ8 + CFA primed (2.5 mo)				
	1	10	12	3	Yes
	Unprimed				
	1	13	13	1	No
3	CDR2-Vβ8 + CFA primed (1 mo)				
	1	11	14	3	No*
	2	Before day 10	12	3	Yes*
	3	11	13	3	No
	4	Before day 10	10	3	Yes
	5	10	12	3	No
	Jα39 + CFA primed				
	1	10	13	3	No
	2	13	14	1	No
	TK20 + CFA primed				
	1	15	16	1	No
	2	15	16	2	No
	3	14	15	2	No
	CFA primed				
	1	15	15	1	No
	2	15	15	1	No
	3	14	15	1	No
4	CDR2-Vβ8 + CFA primed (1 mo)				
	1	8	10	3	Yes
	2	—	—	—	†
	3	13	15	2	No
	CFA primed				
	1	12	13	3	No
	2	11	13	3	No
	3	12	14	2	No

Lewis rats were injected with 100 μg of TCR peptides in CFA. One to 2.5 months (mo) later, MBP in CFA was injected. Animals were then observed for appearance of clinical signs.

*Rats were injected with 200 μg, instead of 100 μg, of TCR peptides in CFA.

†No clinical disease was observed.

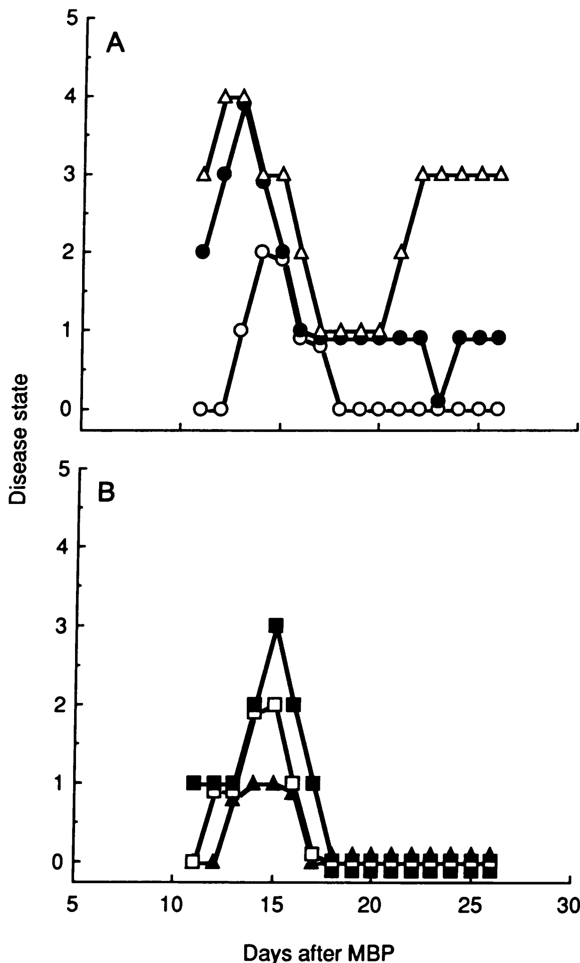

FIG. 1. Enhancing effect of TCR peptides on MBP-induced EAE in Lewis rats. Rats were injected with either CDR2-Vβ8 peptide emulsified in CFA (*A*) or CFA alone (*B*). One month later, rats were injected with MBP in CFA. Results are presented for individual animals (shown by various symbols).

To exclude the possibility that our results were due to an impurity in the peptide preparations, an HPLC-purified CDR2-Vβ8 peptide was used to immunize the rats (Table 1, experiment 4, and Fig. 3). In this experiment, one animal developed chronic disease, one developed self-limiting EAE, and the third remained healthy.

Besides following clinical symptoms and pathology, we also examined sera from the peptide-immunized rats for their ability to bind to 5.10, the MBP-(68–88)-specific T-cell hybridoma from whose TCR sequences the peptides were derived. As can be seen in the flow cytometry profiles in Fig. 4, there was no binding activity in any of the sera derived from rats immunized with the TCR peptide followed by MBP (from animals in experiment 3) or TCR peptide in CFA alone (data not shown) as compared to rats immunized first with CFA followed by MBP (Fig. 4*B*), though the level of TCR expression on the cell surface of the hybridoma was high as determined by the binding of R73, an anti-TCR monoclonal antibody (11) (Fig. 4*A*).

DISCUSSION

The limited clonality, as determined by shared TCR V gene usage, which characterizes EAE (1–5), naturally invokes the possibility of immune network control of self reactivity. Indeed, the original observations by Cohen and colleagues (12) support this idea. We find that priming animals to

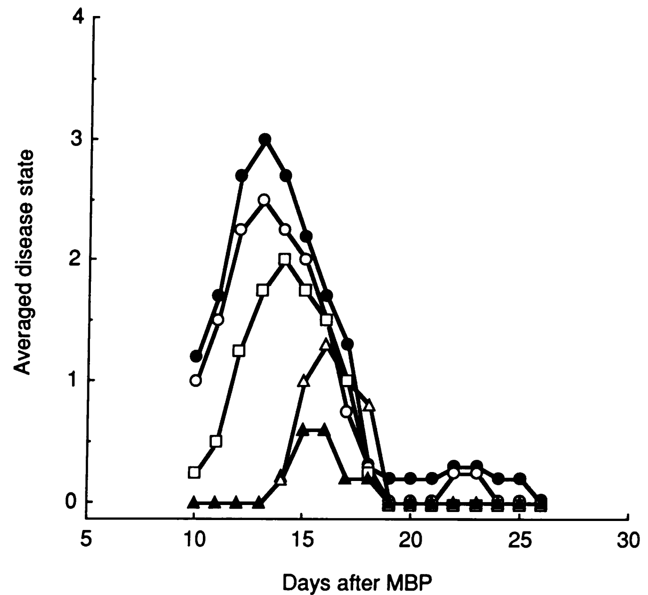

FIG. 2. Various TCR peptides induce enhancement of EAE in Lewis rats. Animals were injected with the CDR2-Vβ8 peptide at 200 μg (●, $n = 2$) or 100 μg (○, $n = 3$), the Jα39 peptide at 100 μg (□, $n = 2$), the TK20 peptide at 100 μg (△, $n = 3$), or CFA alone (▲, $n = 3$). One month later, animals were injected with MBP in CFA and observed for signs of EAE. Results are presented as an average for all animals in each peptide treatment group.

TCR-derived self antigens frequently leads to enhancement of EAE. In 9 of 12 animals injected with the previously tested peptide CDR2-Vβ8 (Table 2), profound enhancement of disease was observed, whereas in 2 of 12 animals no effect was seen and only 1 of 12 animals showed no signs of disease, which could be the same as the reported suppression (9).

Enhancement of disease in TCR peptide-primed animals in these experiments was manifested not only as a greater average state of disease as measured by a subjective judgment on a standard paralysis scale but also as a significant shift (2–5 days) of the kinetics curves to the left, to an earlier onset of severe disease. Since early onset has historically been taken as the *sine qua non* of second-set responses in graft rejection as well as in antibody titers, we believe that this shift is clear evidence of a second-set response. Further evidence of a qualitatively different disease state lies in the fact that 50% of the animals developed chronic disease, a phenomenon not often seen in animals receiving MBP alone.

EAE has historically been used as an animal model of multiple sclerosis due to the similarities between the two conditions. The most commonly studied form of EAE, however, is an acute self-limiting condition that confers resistance to reinduction of active disease. These characteristics contrast sharply with the clinical course of multiple sclerosis, which is typically seen as a chronic illness with exacerbations and remissions over many years. The data presented above, showing that prior immunization with TCR peptides can result in chronic disease, may thus provide a convenient means to mimic more closely the course of multiple sclerosis, and elucidation of the mechanism underlying this phenomenon may provide clues about the state of the immune system during the development and progression of that disease.

Besides evaluating the effect of the TCR peptides on the outcome of immunization with MBP and subsequent disease, we also examined T-cell and B-cell responses of animals injected with these peptides. First, as shown above, we examined the animals that received TCR peptides followed by MBP and were unable to detect antibody that could bind to an MBP-reactive Lewis T-cell population or the MBP-

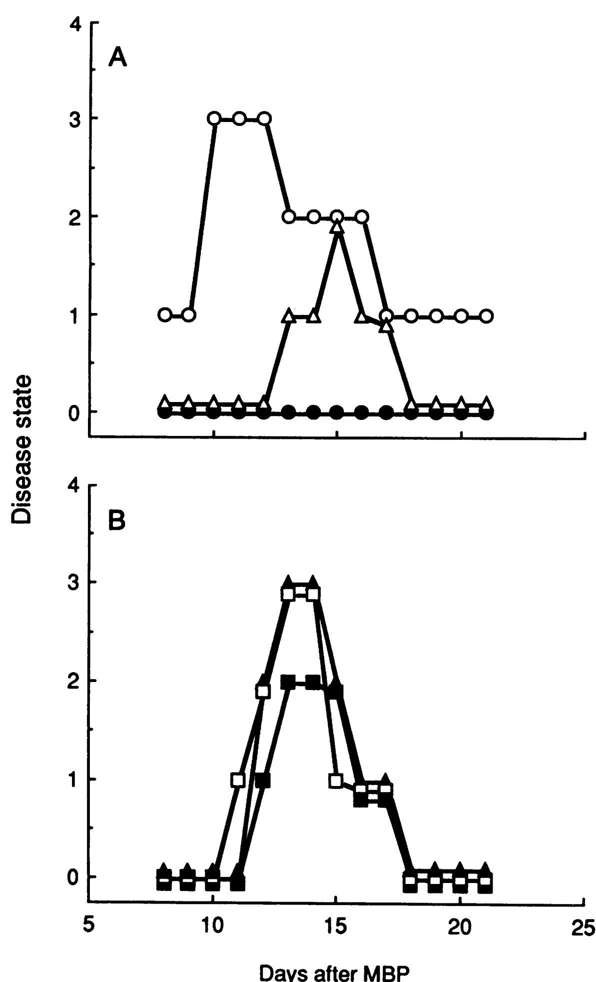

FIG. 3. Effect of HPLC-purified peptide on disease course. Rats were injected with HPLC-purified peptide in CFA (*A*) or CFA alone (*B*). One month later, animals were injected with MBP. Data from individual animals are shown with various symbols.

reactive Vβ8-positive T-cell hybridoma 5.10 from which the TCR peptide sequences were derived. We also examined rats that received TCR peptides in CFA for T-cell responsiveness. Lymph node cells from these primed animals, on primary *in vitro* stimulation with TCR peptide, demonstrated a small degree of blastogenesis, but these cells could neither be propagated *in vitro* nor incorporate thymidine as compared to control cells responsive to other antigens such as MBP or ovalbumin.

The data presented above demonstrate that immunization of Lewis rats with the TCR peptides previously reported to suppress subsequent induction of EAE with MBP (8, 9) can also result in more severe EAE, followed in some instances by chronic neurologic abnormalities. Thus, it appears that immune responses to these peptides do not necessarily favor suppression over enhancement.

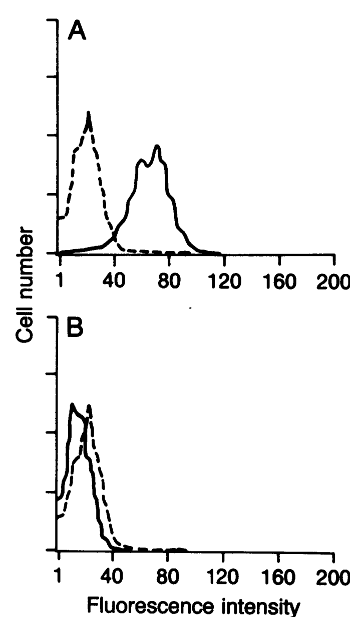

FIG. 4. Binding specificity of sera from rats immunized with TCR peptide and then with MBP. Flow cytometry analysis of the MBP-(68–88)-specific Lewis × BW5147 T-cell hybridoma 5.10 treated with $F(ab')_2$ goat anti-mouse immunoglobulin (---) or R73 plus anti-immunoglobulin (—) (*A*) and with sera pooled from rats immunized with CFA followed by MBP (---) or sera from rats immunized with the CDR2-Vβ8 peptide followed by MBP (—) (*B*).

Why do some animals immunized with TCR peptides develop enhanced disease whereas others either remain unaffected by this treatment or become resistant to the induction of EAE as reported (8, 9)? One possible explanation is that different colonies of rats are being used, each with a different immune status and a different set of normal pathogens leading to different outcomes upon immunization. In terms of mechanisms responsible for these different responses to the same TCR peptides, it has been proposed that regulatory T cells function in the control of EAE (12) and that TCR peptides activate or enhance the down-regulating function of these T cells (9). Support for this proposal comes from the ability of TCR peptide-specific T cells to suppress disease when adoptively transferred into rats that have received MBP in CFA (9). In this context, our finding of up-regulation, early onset, and chronic neurologic deficits of EAE along with the lack of a B-cell or a T-cell response is consistent with the tolerization of such a regulatory cell. One might imagine that the rats used in our studies provide an environment leading not to stimulation of regulatory cells but rather to a state of unresponsiveness or anergy (13). The lack of cells that down-regulate the encephalitogenic effector cells would then result in less regulation and more severe disease.

On the other hand, a T cell that down-regulates may not be involved at all. Rather, in our case, a helper cell that exists at low frequency and thus is not seen responding *in vitro* may be activated to positively stimulate the EAE effector cell.

Table 2. Evaluation of TCR peptide immunization in disease

	No. positive/no. immunized				Total no. positive/ total no. immunized
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	
Early onset and enhancement					
CDR2-Vβ8 peptide + CFA	2/3	1/1	5/5	1/3	9/12
CFA	0/3	0/1	0/5	0/3	0/12
Chronic disease					
CDR2-Vβ8 peptide + CFA	2/3	1/1	2/5	1/3	6/12
CFA	0/3	0/1	0/5	0/3	0/12

Finally, it is also possible that some unknown stochastic process predisposes individuals to enhancement, suppression, or lack of effect from immunization with these peptides.

We thank Drs. C. Hackett, B. B. Knowles, H. Ertl, and I. Cohen for their careful review of this manuscript. This work was supported by Grant NMS RG 1593 from the National Multiple Sclerosis Society and National Institutes of Health Grant NS 11036 from the U.S. Public Health Service. L.D.-C. is supported by the Cancer Research Institute. T.R.E. is supported by a U.S. Public Health Service training grant.

1. Acha-Orbea, H., Mitchell, D. J., Timmermann, L., Wraith, D. C., Tausch, G. S., Waldor, M. K., Zamvil, S. S., McDevitt, H. O. & Steinman, L. (1988) *Cell* **54**, 263–273.
2. Urban, J. L., Kumar, V., Kono, D. H., Gomez, C., Horvath, S. J., Clayton, J., Ando, D. J., Sercarz, E. E. & Hood, L. (1988) *Cell* **54**, 577–592.
3. Zamvil, S. S., Mitchell, D. J., Lee, N. E., Moore, A. C., Waldor, M. K., Sakai, K., Rothbard, J. B., McDevitt, H. O., Steinman, L. & Acha-Orbea, H. (1988) *J. Exp. Med.* **167**, 1586–1596.
4. Burns, F. R., Li, X., Shen, N., Offner, H., Chou, Y. K., Vandenbark, A. A. & Heber-Katz, E. (1989) *J. Exp. Med.* **169**, 27–39.
5. Chluba, J., Steeg, C., Becker, A., Wekerle, H. & Epplen, J. T. (1989) *Eur. J. Immunol.* **19**, 279–284.
6. Heber-Katz, E. & Acha-Orbea, H. (1989) *Immunol. Today* **10**, 164–169.
7. Owhashi, M. & Heber-Katz, E. (1988) *J. Exp. Med.* **168**, 2153–2164.
8. Howell, M. D., Winters, S. T., Olee, T., Powell, H. C., Carlo, D. J. & Brostoff, S. W. (1989) *Science* **246**, 668–670.
9. Vandenbark, A. A., Hashim, G. & Offner, H. (1989) *Nature (London)* **341**, 541–544.
10. Hickey, W. F., Gonatas, N. K., Kimura, H. & Wilson, D. B. (1983) *J. Immunol.* **131**, 2805–2809.
11. Hunig, T., Wallny, H.-J., Hartley, J. K., Lawetzky, A. & Tiefenthaler, G. (1989) *J. Exp. Med.* **169**, 73–86.
12. Ben-Nun, A., Wekerle, H. & Cohen, I. (1981) *Nature (London)* **292**, 60–61.
13. Schwartz, R. H. (1989) *Cell* **57**, 1073–1076.
14. Deibler, G. E., Martenson, R. E. & Kies, M. W. (1972) *Prep. Biochem.* **2**, 139–165.