A single amino acid change in a myelin basic protein peptide confers the capacity to prevent rather than induce experimental autoimmune encephalomyelitis

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ABSTRACT Experimental autoimmune encephalomyelitis (EAE) is an experimental demyelinating disease of rodents. In (PL/J × SJL)F₁ mice, it is induced by immunization with the myelin basic protein peptide Ac1–11. Ac1–11[4A], a myelin basic protein peptide analog with a single amino acid substitution, (i) binds to class II major histocompatibility complex molecules and stimulates encephalitogenic T cells *in vitro* better than Ac1–11, (ii) is nonimmunogenic and nonencephalitogenic *in vivo* in (PL/J × SJL)F₁ mice, (iii) prevents EAE when administered before or at the time of immunization with Ac1–11, and (iv) prevents EAE when administered later, near the time of disease onset. Initial studies suggest that Ac1–11[4A] does not prevent EAE by competitive inhibition or by activation of regulatory cells. Thus, substitution of a single amino acid in a myelin basic protein peptide confers the capacity to prevent rather than induce EAE, even after peptide-specific encephalitogenic T cells have been activated.

Immunological encounter with antigen results in a number of vastly different consequences, depending on such diverse factors as the stage of development of the immune system and the conditions under which antigen is displayed. In the thymus, encounter with antigen can result in either positive or negative selection, processes that are poorly understood (1, 2). The immune system is especially sensitive to tolerance induction during early stages of development, but it is clear that mature lymphocytes are also susceptible to signals that prevent a subsequent response (3–10). Autoimmunity represents a situation in which the immune signals that usually lead to unresponsiveness fail.

Experimental autoimmune encephalomyelitis (EAE) is an autoimmune demyelinating disease induced by immunizing susceptible mouse, rat, or guinea pig strains with myelin basic protein (MBP) or with encephalitogenic peptide fragments. CD4⁺ T cells are involved in this immune response (11–13), and genetic susceptibility in mice is based partly on the capacity of encephalitogenic peptides to bind to particular class II major histocompatibility complex molecules (14, 15). (PL/J × SJL)F₁ mice are susceptible to EAE induced with the acetylated N-terminal 1–11 peptide of MBP (Ac1–11), which binds to A_uA_β expressed in these mice (13, 15). A second MBP peptide 35–47 is also encephalitogenic in (PL/J × SJL)F₁ mice and is restricted to E_uE_β (16).

Previous studies of the role of individual residues of Ac1–11 in binding of the peptide to A_uA_β and in stimulating encephalitogenic T cells *in vitro* showed that residue 4 influences binding of the peptide to A_uA_β. In contrast, residues 3 and 6 are important T-cell interaction residues (15,

17). Substitution of alanine for the lysine normally found at residue 4 creates a peptide analog (Ac1–11[4A]), which binds at least 10- to 100-fold better to A_uA_β than the normal peptide. Ac1–11[4A] also stimulates encephalitogenic T cells *in vitro* in an enhanced fashion. It is not known whether this augmented response is due exclusively to increased A_uA_β binding or to an additional effect on T-cell stimulation as well. Although Ac1–11[4A] was expected to be highly encephalitogenic *in vivo*, it proved to be poorly immunogenic. Moreover, coimmunization of (PL/J × SJL)F₁ mice with Ac1–11 and Ac1–11[4A] significantly reduced the incidence of EAE, but not the *in vitro* T-cell proliferative response to Ac1–11 (15).

This report describes a more extensive examination of the *in vivo* activity of the Ac1–11[4A] peptide. Pretreatment with the peptide prior to disease induction effectively prevents EAE, and administration of the peptide at or near the time of disease onset prevents EAE as well. Disease inhibition studies with a structurally similar peptide suggest that Ac1–11[4A] prevents EAE by a mechanism other than competitive binding to A_uA_β. Moreover, transfer studies do not support a role for inhibitory regulatory cells. Thus, two peptides (Ac1–11 and Ac1–11[4A]), which are recognized by the same T-cell clone *in vitro* and which differ by only one amino acid, can effect markedly different immunological consequences when administered *in vivo*.

MATERIALS AND METHODS

EAE Induction and Scoring. EAE was induced by immunizing 10- to 15-week-old (PL/J × SJL)F₁ mice (The Jackson Laboratory) subcutaneously at the base of the tail with 50–150 nmol of Ac1–11. The peptide was emulsified in complete Freund's adjuvant containing an additional 400 μg of heat-killed *Mycobacterium tuberculosis* H37RA (Difco). For coimmunization, inhibitor peptides were mixed in the same emulsion. Pertussis toxin (200 ng) (List Biological Laboratories, Campbell, CA, or JRH Biosciences, Woodland, CA) was injected intravenously at the time of immunization and again 2 days later. Mice were observed daily beginning at day 9 for signs of EAE and were graded according to the following clinical scale: 1, limp tail; 2, partial hind limb paralysis; 3, complete hind limb paralysis; 4, hind and front limb paralysis; 5, moribund. Animals were cared for in accordance with Stanford University guidelines. Mean maximum severity is the mean of the maximum severity of EAE for each mouse in the group calculated over the total number of mice in the entire group. Cumulative incidence of disease is expressed as a percentage.

Abbreviations: EAE, experimental autoimmune encephalomyelitis; MBP, myelin basic protein; IFA, incomplete Freund's adjuvant.

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T-Cell Proliferation *in Vitro*. (PL/J $\times$ SJL) F_1 mice (The Jackson Laboratory) were immunized with 150 nmol of peptide as described for disease induction, with the omission of pertussis toxin from the immunization regimen. Draining lymph node lymphocytes were harvested 10 days after immunization and cultured in triplicate at 5×10^5 cells per well in microtiter dishes. Synthetic peptides, purified protein derivative, or Con A were added as stimulators. After 72 hr of incubation, cultures were pulsed with 0.5 μ Ci of [3 H]thymidine (1 Ci = 37 GBq) for an additional 16 hr before harvesting. Culture medium was RPMI 1640 medium supplemented with 1% fresh normal mouse serum, glutamine, 2-mercaptoethanol, and penicillin/streptomycin.

Peptides. Peptides were synthesized with an ABI 431A or a MilliGen 9050 peptide synthesizer using 9-fluorenylmethoxycarbonyl chemistry. N-terminal MBP peptides and peptide analogs were acetylated at the N terminus during the synthesis procedure. Peptides were cleaved by using standard protocols and were HPLC purified if necessary. Peptides were 95–99% pure as determined by HPLC, had correct amino acid compositions, and had correct molecular weights as determined by mass spectroscopy. Peptide sequences were as follows: Ala-Ser-Gln-Lys-Arg-Pro-Ser-Gln-Arg-His-Gly for MBP Ac1-11, Ala-Ser-Gln-Ala-Arg-Pro-Ser-Gln-Arg-His-Gly for Ac1-11[4A], Ala-Ser-Ala-Ala-Arg-Pro-Ser-Gln-Arg-His-Gly for Ac1-11[3A,4A], and Thr-Gly-Ile-Leu-Asp-Ser-Ile-Gly-Arg-Phe-Phe-Ser-Gly for MBP 35–47. MBP 89–101 was synthesized by using *t*-butoxycarbonyl chemistry rather than 9-fluorenylmethoxycarbonyl chemistry, and its

sequence was Val-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro.

RESULTS

Previous studies showed that coimmunization of (PL/J $\times$ SJL) F_1 mice with Ac1-11 and Ac1-11[4A] significantly reduced the incidence of EAE (15). In five separate experiments, including two that were published previously (15), 47/57 mice (82%) immunized with 50–150 nmol of Ac1-11 developed EAE. In contrast, only 15/54 mice (28%) developed EAE upon coimmunization with Ac1-11 and a 3- to 10-fold molar excess of Ac1-11[4A] ($P < 0.003$). Moreover, Ac1-11[4A] is not encephalitogenic in (PL/J $\times$ SJL) F_1 mice when administered by itself. In two separate experiments, 0/22 mice (0%) immunized with 100–400 nmol of Ac1-11[4A] developed EAE, while 14/20 control mice (70%) immunized with 50–150 nmol of Ac1-11 developed disease.

One possible mechanism by which nonencephalitogenic Ac1-11[4A] could inhibit EAE is by binding to $A_\alpha A_\beta$ with higher avidity than Ac1-11, interfering with the immune response to encephalitogenic Ac1-11. Ac1-11[4A] clearly binds to purified $A_\alpha A_\beta$ molecules *in vitro* 10- to 100-fold better than Ac1-11 (15) and potentially could compete for binding *in vivo*. Inhibition of the immune response by competitive binding of inhibitor peptides to class II molecules has been extensively studied for conventional antigens (18, 19) and also has been proposed as the mechanism for inhibition of both EAE and autoimmune myocarditis by peptides (20–23). If competitive binding to $A_\alpha A_\beta$ is the mechanism by which Ac1-11[4A] inhibits EAE, then another structurally similar peptide that binds as well as Ac1-11[4A] to $A_\alpha A_\beta$ might be expected to inhibit EAE also.

Ac1-11[3A,4A] is a doubly substituted MBP peptide analog that binds as well as Ac1-11[4A] to $A_\alpha A_\beta$, a property that is probably based on its alanine substitution at the $A_\alpha A_\beta$ binding residue 4 (15). Ac1-11[3A,4A] is itself immunogenic,

FIG. 1. *In vitro* lymph node proliferation in response to Ac1-11 ($\blacktriangle$) or Ac1-11[3A,4A] ($\square$). (PL/J $\times$ SJL) F_1 mice were immunized with 150 nmol of Ac1-11 (A) or Ac1-11[3A,4A] (B). (A) The following controls were used: no peptide, 260 cpm; purified protein derivative, 36,280 cpm; Con A, 19,234 cpm. (B) The following controls were used: no peptide, 676 cpm; purified protein derivative, 61,061 cpm; Con A, 13,569 cpm.

Disease induction	Incidence	Mean onset	Mean maximum severity
Ac1-11	7/10 (70%)	20	2.0
Ac1-11 + Ac1-11[3A,4A]	9/10 (90%)	14	3.6

FIG. 2. Coimmunization with Ac1-11[3A,4A] does not inhibit EAE. EAE was induced by immunizing (PL/J $\times$ SJL) F_1 mice with 50 nmol of Ac1-11 ($\blacktriangle$). For coimmunization, 500 nmol of the peptide Ac1-11[3A,4A] was mixed in the same emulsion ($\square$). Incidence of EAE for the coimmunized group was not significantly different from the control group ($P = 0.6$). In a second experiment, a 5-fold molar excess of Ac1-11[3A,4A] also did not inhibit EAE (data not shown).

and the bulk of the polyclonal T-cell immune response generated by immunization with Ac1-11[3A,4A] is not cross-reactive on the Ac1-11 peptide due to the alanine substitution at T-cell interaction residue 3 (Fig. 1). Based on these properties, Ac1-11[3A,4A] was considered to be a candidate for inhibiting EAE by competing for the binding of Ac1-11 to $A_{\alpha}A_{\beta}$. However, in contrast to expectations, this peptide does not inhibit EAE in (PL/J $\times$ SJL) F_1 mice when administered in up to a 10-fold molar excess in a coimmunization regimen (Fig. 2). This failure of Ac1-11[3A,4A] to inhibit EAE suggests that Ac1-11[4A] interferes with the disease by a mechanism other than competitive binding to $A_{\alpha}A_{\beta}$.

Further experiments examined the capacity of Ac1-11[4A] to prevent EAE after the initiation of the immune response, which leads to disease. EAE was induced with the encephalitogenic peptide Ac1-11. When signs of EAE began to appear at day 11, Ac1-11[4A] was administered subcutaneously in incomplete Freund's adjuvant (IFA) (Fig. 3). Such treatment with Ac1-11[4A] during the development of disease prevents EAE in the treated mice. EAE in mice is a phasic disease with remissions and exacerbations, and in this experiment 33% of the mice in the two control groups suffered relapses between day 21 and day 42. Mice treated with Ac1-11[4A] were followed for 56 days with no late onset signs observed. In a second experiment, mice were treated with Ac1-11[4A] at day 9 with an equally dramatic reduction in disease incidence (Fig. 4). Administration of the encephalitogenic peptide Ac1-11 or the control peptide MBP 89-101 [not encephalitogenic in (PL/J $\times$ SJL) F_1 mice (24)] at the onset of disease does not reduce the incidence of EAE.

Administration of some antigens under special tolerogenic conditions has been shown to prevent a subsequent immune response, even in adults. Pretreatment with MBP administered in IFA prevents the subsequent induction of EAE (25,

Day 11 treatment	Incidence	Mean onset	Mean maximum severity
None	6/10 (60%)	13	1.9
MBP 89-101	8/8 (100%)	16	3.4
Ac1-11[4A]	0/10 (0%)		

FIG. 3. Treatment with Ac1-11[4A] near the time of disease onset at day 11 prevents EAE. Two of 27 mice (7%) developed signs of EAE by day 11 after disease induction with 50 nmol of Ac1-11. Groups were treated on this day with a single subcutaneous injection between the scapulae of 250 nmol of Ac1-11[4A] (Δ) or MBP 89-101 emulsified in IFA ($\square$). A control group received no injection ($\blacktriangle$). The difference between the incidence of EAE in mice treated with Ac1-11[4A] versus the control group was statistically significant (6/10 vs. 0/10; $P = 0.015$).

26). Moreover, induction of adult tolerance to conventional antigenic peptides by pretreatment in IFA has been reported (27). The mechanism of such adult tolerance is poorly understood and may involve complex cellular interactions. Table 1 shows that treatment with the normally encephalitogenic peptide Ac1-11 in IFA before disease induction with Ac1-11 dramatically reduces the incidence of EAE in an epitope-specific fashion. Fig. 5 shows that treatment with Ac1-11[4A] in IFA 10 days before disease induction also prevents the subsequent induction of EAE. Although Ac1-11[4A] is not encephalitogenic, it is as effective as Ac1-11 in generating unresponsiveness to EAE induction when administered under conditions expected to lead to adult tolerance. This protection from EAE induction is not readily transferred into irradiated recipients (Fig. 6). This result suggests that active suppression does not explain protection from EAE by Ac1-11[4A] and that an alternative mechanism may be operating.

DISCUSSION

Table 2 summarizes the *in vitro* and *in vivo* biological properties of the encephalitogenic MBP peptide Ac1-11 and the disease-inhibiting MBP peptide analog Ac1-11[4A]. Structurally, these two peptides differ only at residue 4 at which the neutral amino acid alanine has been exchanged for the basic amino acid lysine. Alanine at residue 4 in Ac1-11[4A] (i) causes increased binding to $A_{\alpha}A_{\beta}$ and increased stimulation of encephalitogenic T-cell proliferation *in vitro*, (ii) interferes with the peptide's immunogenicity and its encephalitogenic activity in (PL/J $\times$ SJL) F_1 mice, (iii) leads to inhibition of EAE when administered prior to or at the time

Day 9 treatment	Incidence	Mean onset	Mean maximum severity
None	8/8 (100%)	12	3.8
MBP 89-101	8/8 (100%)	15	4.0
Ac1-11	8/8 (100%)	14	4.4
Ac1-11[4A]	3/8 (37.5%)	16	1.0

FIG. 4. Treatment with Ac1-11[4A] near the time of disease onset at day 9 prevents EAE. Four of 32 mice (12.5%) developed signs of EAE by day 9 after disease induction with 50 nmol of Ac1-11. Groups were treated on this day with a single subcutaneous injection between the scapulae of 250 nmol of Ac1-11[4A] (Δ), Ac1-11 ($\circ$), or MBP 89-101 ($\square$) emulsified in IFA. A control group received no injection ($\blacktriangle$). The difference between the incidence of EAE in mice treated with Ac1-11[4A] versus the control group was statistically significant (8/8 vs. 3/8; $P = 0.031$).

Table 1. Treatment with Ac1-11 prior to disease induction prevents EAE in an epitope-specific fashion

Pretreatment* (day -21)	Induction of EAE† (day 0)	Incidence of EAE, diseased/total (%)
None	Ac1-11	7/9 (78)
Ac1-11/IFA	Ac1-11	0/5 (0)
None	MBP 35-47	4/7 (57)
Ac1-11/IFA	MBP 35-47	7/7 (100)

* (PL/J × SJL)F₁ mice were treated 21 days before disease induction with a single subcutaneous injection of 100 nmol of Ac1-11 emulsified in IFA.

† EAE was induced with 100 nmol of Ac1-11 or 100 nmol of MBP 35-47.

of disease induction, and (iv) inhibits EAE when administered at the time of disease onset, after the activation of encephalitogenic cells.

In contrast, Ac1-11[3A,4A], a doubly substituted peptide analog that binds to A_αA_β as well as Ac1-11[4A] but does not stimulate encephalitogenic T cells, fails to inhibit EAE. Ac1-11[3A,4A] was expected to inhibit EAE by competitive binding to A_αA_β, as has been described for several other peptides (20-22). Insufficient binding avidity to A_αA_β, or, alternatively, susceptibility to serum proteases (19), may explain the failure of Ac1-11[3A,4A] to inhibit EAE. Ac1-11[4A] and Ac1-11[3A,4A] differ substantially from one another in terms of their immunological properties. However, since the two peptides are structurally similar and bind equally well to A_αA_β, the failure of Ac1-11[3A,4A] to inhibit EAE suggests that Ac1-11[4A] interferes with the disease by a mechanism other than competitive binding to A_αA_β.

Pretreatment in IFA	Incidence	Mean onset	Mean maximum severity
None	16/19 (84%)	11	3.3
Ac1-11	3/13 (23%)	14	1.1
Ac1-11[4A]	2/16 (12%)	11	0.2
IFA	5/7 (71%)	14	2.8

FIG. 5. Treatment with Ac1-11 or Ac1-11[4A] before disease onset prevents EAE. Mice were treated 10 days before induction with a single intraperitoneal injection of 250 nmol of either Ac1-11 (○) or Ac1-11[4A] (△) emulsified in IFA. EAE was induced with 150 nmol of Ac1-11. Results are pooled from two similar experiments. The reduction in incidence of disease was statistically significant for both Ac1-11 ($P < 0.003$) and Ac1-11[4A] ($P < 0.003$). ●, Pretreatment with IFA alone; ▲, untreated control.

Transferred cells	Incidence	Mean onset	Mean maximum severity
None	7/7 (100%)	16	4.3
IFA pretreated	7/10 (70%)	16	3.5
Ac1-11[4A] pretreated	9/10 (90%)	16	4.0

FIG. 6. Protection by Ac1-11[4A] is not transferred. Mice were pretreated with either 250 nmol of Ac1-11[4A] (△) emulsified in IFA or with IFA alone (●). After 10 days, 3×10^7 splenocytes from the two groups of mice were transferred into recipient mice that had received 150 rads (1 rad = 0.01 Gy) from an x-irradiation source. An additional group of control mice received irradiation but no transferred cells (▲). EAE was induced in these mice with 50 nmol of Ac1-11.

It is quite remarkable that Ac1-11[4A] prevents the encephalitogenic consequences of an ongoing immune response to Ac1-11 even when it is administered near disease onset. This result would not have been predicted from the *in vitro* stimulation data, in which Ac1-11[4A] stimulates rather than inhibits primed T cells. *In vivo*, Ac1-11[4A] is a potent inhibitor of activated encephalitogenic cells. One explanation for the prevention of disease by Ac1-11[4A] under these circumstances is that the peptide is recruiting encephalitogenic cells away from the site of injury in the central nervous system. However, the recruited encephalitogenic cells might be expected eventually to escape and cause late disease, perhaps at a time when control mice are experiencing relapses. If encephalitogenic cells are recruited away from the central nervous system by Ac1-11[4A], they appear to be rendered incapable of causing subsequent disease for at least 6 weeks. Moreover, Ac1-11 does not have a similar effect of preventing disease when administered at the time of onset.

Table 2. Ac1-11 vs. Ac1-11[4A]: Comparison of *in vivo* and *in vitro* activities

	Ac1-11	Ac1-11[4A]
<i>In vitro</i>		
Binds to A _α A _β	+	+++
Activates T-cell clones to proliferate	+	+++
Activates primed LN to proliferate	+	+++
<i>In vivo</i>		
Immunizes for T-cell proliferation	+	-
Induces EAE	+	-
Inhibits EAE upon coimmunization	-	+
Inhibits EAE by pretreatment in IFA	+	+
Inhibits EAE by treatment at onset	-	+

LN, lymph node.

These findings argue that simple recruitment of encephalitogenic cells is an insufficient explanation for the inhibition of EAE and that another regulatory mechanism must be operating.

In light of the markedly different immunological properties of Acl-11 and Acl-11[4A] *in vivo* and *in vitro*, it is perhaps surprising that pretreatment with both peptides induces protection from subsequent induction of EAE. The results illustrate that the antigenic requirements for induction of immunity may differ from those for induction of unresponsiveness. It is clear that Acl-11 and Acl-11[4A] are both capable of interacting with encephalitogenic T cells. However, *in vivo* Acl-11 usually activates these autoimmune cells and is capable of inducing unresponsiveness only under special conditions. In contrast, Acl-11[4A] always tends to induce unresponsiveness *in vivo*, even in previously activated encephalitogenic cells. Structurally, Acl-11 and Acl-11[4A] differ only at residue 4 in which the neutral amino acid alanine replaces the basic amino acid lysine. This single amino acid change results in dramatically different immunological consequences, conferring on Acl-11[4A] the capacity to prevent EAE even after the initiation of the active immune process leading to disease. This unusual capacity of Acl-11[4A] to disarm activated autoreactive cells demonstrates a unique approach for ameliorating an autoimmune disease.

The mechanism by which Acl-11[4A] prevents EAE remains elusive. The experiments described here do not support the idea that Acl-11[4A] is inhibiting EAE solely by competitive binding to A₂A₂ (20–23) or by activating suppressive regulatory cells (25, 26, 28–30). Moreover, coimmunization with Acl-11 and Acl-11[4A] does not induce typical anergy (3–10) in the entire population of cells capable of responding to Acl-11. Coimmunized mice still mount a proliferative response to Acl-11 *in vitro*, although they are protected from EAE. It may be necessary to invoke a more complex explanation for the unusual activity of Acl-11[4A]. One model is that Acl-11[4A] leads to the delivery of a dominant negative signal to encephalitogenic T lymphocytes, possibly involving differential signaling by the T-cell receptor (31–34). Encephalitogenic T lymphocytes comprise a subpopulation of all those lymphocytes that proliferate in response to Acl-11 (13, 17) and are not readily identified in polyclonal mixtures of lymphocytes. Perhaps only the encephalitogenic subpopulation is susceptible to the negative signal induced by Acl-11[4A], so that no inhibition of the overall *in vitro* proliferative response to Acl-11 is observed. This model is purely speculative but provides a framework for further investigation of the prevention of EAE by the MBP peptide analog Acl-11[4A].

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- Blackman, M., Kappler, J. & Marrack, P. (1990) *Science* **248**, 1335–1341.
- von Boehmer, H. & Kieselow, P. (1990) *Science* **248**, 1369–1373.
- Ramsdell, F. & Fowlkes, B. J. (1990) *Science* **248**, 1342–1348.
- Schwartz, R. H. (1990) *Science* **248**, 1349–1356.
- Sprent, J., Gao, E.-K. & Webb, S. R. (1990) *Science* **248**, 1357–1363.
- Webb, S. R. & Sprent, J. (1990) *Cell* **63**, 1249–1256.
- Kawabe, Y. & Ochi, A. (1990) *J. Exp. Med.* **172**, 1065–1070.
- Rellahan, B. L., Jones, L. A., Kruisbeek, A. M., Fry, A. M. & Matis, L. A. (1990) *J. Exp. Med.* **172**, 1091–1100.
- Rocha, B. & von Boehmer, H. (1991) *Science* **251**, 1225–1228.
- Schonrich, G., Kalinke, U., Momburg, F., Malissen, M., Schmitt-Verhulst, A.-M., Malissen, B., Hammerling, G. J. & Arnold, B. (1991) *Cell* **65**, 293–304.
- Waldor, M. K., Sriram, S., Hardy, R., Herzenberg, L. A., Herzenberg, L. A., Lanier, L., Lim, M. & Steinman, L. (1985) *Science* **227**, 415–417.
- Brostoff, S. W. & Mason, D. W. (1984) *J. Immunol.* **133**, 1938–1942.
- Zamvil, S. S., Nelson, P., Trotter, J., Mitchell, D., Knobler, R., Fritz, R. & Steinman, L. (1985) *Nature (London)* **317**, 355–358.
- Fritz, R. B., Skeen, M. J., Chou, C.-H. J., Garcia, M. & Egorov, I. K. (1985) *J. Immunol.* **134**, 2328–2332.
- Wraith, D. C., Smilek, D. E., Mitchell, D. J., Steinman, L. & McDevitt, H. O. (1989) *Cell* **59**, 247–255.
- Zamvil, S. S., Mitchell, D. J., Powell, M. B., Sakai, K., Rothbard, J. B. & Steinman, L. (1988) *J. Exp. Med.* **168**, 1181–1186.
- 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.
- Adorini, L. & Nagy, Z. A. (1990) *Immunol. Today* **11**, 21–24.
- Lamont, A. G., Powell, M. F., Colon, S., Miles, C., Grey, H. M. & Sette, A. (1990) *J. Immunol.* **144**, 2493–2498.
- Sakai, K., Zamvil, S. S., Mitchell, D. J., Hodgkinson, S., Rothbard, J. B. & Steinman, L. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 9470–9474.
- Gautam, A. M. & Glynn, P. (1990) *J. Immunol.* **144**, 1177–1180.
- Lamont, A. G., Sette, A., Fujinami, R., Colon, S. M., Miles, C. & Grey, H. (1990) *J. Immunol.* **145**, 1687–1693.
- Smith, S. C. & Allen, P. M. (1991) *J. Immunol.* **147**, 2141–2147.
- Sakai, K., Zamvil, S. S., Mitchell, D. J., Lim, M., Rothbard, J. B. & Steinman, L. (1988) *J. Neuroimmunol.* **19**, 21–32.
- Bernard, C. C. A. (1977) *Clin. Exp. Immunol.* **29**, 100–109.
- Lando, Z., Teitelbaum, D. & Arnon, R. (1979) *J. Immunol.* **123**, 2156–2160.
- Ria, F., Chan, B. M. C., Scherer, M. T., Smith, J. A. & Geffer, M. L. (1990) *Nature (London)* **343**, 381–383.
- Ben-Nun, A., Wekerle, H. & Cohen, I. R. (1981) *Nature (London)* **292**, 60–61.
- Ellerman, K. E., Powers, J. M. & Brostoff, S. W. (1988) *Nature (London)* **331**, 265–267.
- Lider, O., Reshef, T., Beraud, E., Ben-Nun, A. & Cohen, I. R. (1988) *Science* **239**, 181–183.
- Evavold, B. D. & Allen, P. M. (1991) *Science* **252**, 1308–1310.
- Sussman, J. J., Mercep, M., Saito, T., Germain, R. N., Bonvini, E. & Ashwell, J. D. (1988) *Nature (London)* **334**, 625–628.
- Mercep, M., Weissman, A. M., Frank, S. J., Klausner, R. D. & Ashwell, J. D. (1989) *Science* **246**, 1162–1165.
- O'Rourke, A. M., Mescher, M. F. & Webb, S. R. (1990) *Science* **249**, 171–174.