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Synthetic copolymer 1 inhibits human T-cell lines specific for myelin basic protein

(multiple sclerosis/experimental allergic encephalomyelitis/major histocompatibility complex blocking/T-cell activation/immunotherapy)

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ABSTRACT **Copolymer 1 (Cop 1) is a synthetic basic random copolymer of amino acids that has been shown to be effective in suppression of experimental allergic encephalomyelitis and has been proposed as a candidate drug for multiple sclerosis. Cop 1 is immunologically cross reactive with myelin basic protein (BP) and was shown to inhibit murine BP-specific T-cell lines of various H-2 restrictions. In the present study these findings were extended to include human T-cell lines. Cop 1 competitively inhibited the proliferative responses and interleukin 2 secretion of six BP-specific T-cell lines and 13 clones with several DR restrictions and epitope specificities. Conversely, BP inhibited—albeit to a lesser extent—the response of all the Cop 1-specific T-cell lines and clones, irrespective of their DR restrictions. Another random copolymer of tyrosine, glutamic acid, and alanine, denoted TGA, had no effect on these lines. Neither Cop 1 nor BP inhibited the response of lines and clones specific for purified protein derivative. Cop 1 and BP exerted their cross-inhibitory effects only in the presence of antigen-presenting cells. These results suggest that Cop 1 can compete with BP for the binding to human major histocompatibility complex molecules. In view of recent studies implicating BP reactivity in multiple sclerosis, these findings suggest a possible mechanism for the beneficial effect of Cop 1 in this disease.**

Myelin basic protein (BP) is the major autoantigen involved in the induction of experimental allergic encephalomyelitis (EAE), a cell-mediated autoimmune disease of the central nervous system (1). EAE serves as an animal model for human demyelinating diseases including multiple sclerosis (MS) (2). Although the myelin components that act as the autoantigen in MS have not been identified yet, recent studies suggest that T-cell reactivity to BP may be of significance in this disease (3).

We have reported that suppression of EAE in various animal species may be affected by copolymer 1 (Cop 1), a synthetic basic random copolymer of amino acids (4–8). The immunological cross reactivity of Cop 1 with BP was conclusively established at the humoral level, using monoclonal antibodies to either BP or Cop 1 (9), and at the cellular level (10, 11). Studies in mice suggest two possible mechanisms for Cop 1 activity in EAE. (*i*) Induction of antigen-specific suppressor cells; Cop 1 was found to induce suppressor T cells specific to BP that mediate prevention of clinical EAE (7). (*ii*) Competition with BP for binding to major histocompatibility complex (MHC) molecules. It was demonstrated that Cop 1 can competitively inhibit the response to BP of murine T-cell lines and clones specific to different epitopes of BP and involving different H-2 restrictions (12).

In a double-blinded clinical trial, Cop 1 was demonstrated to reduce the number of relapses in early exacerbating-remitting MS (13). To find out whether the MHC-blocking mechanism may be relevant also in MS, we have exploited human T-cell line technology. Others demonstrated (14–18) the isolation of BP-specific T-cell lines reactive to multiple T-cell epitopes on BP, from MS patients and normal individuals. We now report that Cop 1 can inhibit the response of various BP-specific human T-cell lines and clones, irrespective of their DR restriction. Similarly, BP inhibited T-cell clones specific to Cop 1 of various HLA origins. The results also indicate that the site of competition between BP and Cop 1 is most probably the MHC. This activity may form the basis for the effect of Cop 1 in MS.

MATERIALS AND METHODS

Antigens. BP was isolated from spinal cords of rat, bovine, and human white matter as described (19). The synthetic peptides p1-11, p5-18, p89-101, p114-122, p147-162, and p160-177 of the human BP [according to the 177-residue numbering system (16)] were synthesized by the Merrifield solid-phase method (38) and purified by HPLC. Cop 1, a random copolymer composed of L-alanine, L-glutamic acid, L-lysine, and L-tyrosine in a residue molar ratio of 6.0:1.9:4.7:1.0 (4) was obtained from Teva (Petach Tikva, Israel). TGA, a random polymer of tyrosine, glutamic acid, and alanine in a residue molar ratio of 2.0:1.0:1.0, was synthesized and characterized as described (20). Purified protein derivative (PPD) of tuberculin was obtained from Statens Serum Institute (Copenhagen).

Antibodies. Monoclonal anti-DR and anti-DQ antibodies were obtained from Serotec.

T-Cell Lines. Human T-cell lines were derived from peripheral blood mononuclear cells (MNCs) according to Burns *et al.* (14), by incubating 5×10^6 cells in a 24-well culture plate with BP (100 μg/ml), Cop 1 (50 μg/ml), and PPD (25 μg/ml) in an enriched RPMI 1640 medium supplemented with 10% (vol/vol) heat-inactivated autologous serum. After 7 days of culture, the cells were transferred to culture medium containing 10% fetal calf serum (BioLab, Jerusalem) and recombinant human interleukin 2 (IL-2; 20 units/ml; Boehringer Mannheim).

The cells were grown continuously in this medium with periodic exposure to antigen presented on irradiated (3000 rad; 1 rad = 0.01 Gy) autologous MNCs as antigen-presenting cells (APCs), every 14–18 days.

T-Cell Clones. T-cell clones were generated according to Ota *et al.* (21) by plating limited number of cells in 96-well plates. For each antigen, 120 wells were plated; each well contained 2×10^5 cells and rat BP (RBP; 50 μg/ml), 5×10^4

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Abbreviations: APC, antigen presenting cell; BP, basic protein; BBP, bovine BP; RBP, rat BP; Cop 1, copolymer 1; EAE, experimental allergic encephalomyelitis; MHC, major histocompatibility complex; IL-2, interleukin 2; MNC, mononuclear cell; MS, multiple sclerosis; PPD, purified protein derivative.

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cells and Cop 1 (25 μg/ml), or 3×10^4 cells and PPD at 12.5 μg/ml. Under these conditions less than 20% of the wells were positive for the selecting antigen.

Epstein–Barr Virus-Transformed B-Cell Lines. These lines were initiated according to Brenner *et al.* (22) by culturing 20×10^6 MNCs with B95.8 cell line supernatant (gift from Talma Brenner, Hadassah Hospital, Jerusalem), for 1 h at 37°C. The cells were then washed and cultured in RPMI medium with 10% fetal calf serum and cyclosporin A (10 μg/ml) to deplete T cells.

T-Cell Proliferation Assay. T cells (1×10^4 cells per well) were cultured with 0.5×10^5 to 1×10^5 irradiated (3000 rad) MNCs or 0.25×10^5 to 0.5×10^5 Epstein–Barr virus-transformed B cells (10,000 rad) in the presence of various concentrations of antigen. Cultures were plated in 200 μl of enriched medium containing 10% heat-inactivated autologous serum in microtiter plates. At the end of a 48 h incubation, the cultures were pulse-labeled with 1 μCi of [^{3}H]thymidine (1 Ci = 37 GBq) and harvested 16 h later. The mean thymidine incorporation (cpm) was calculated for triplicate cultures. Standard deviations from triplicate cultures were within 10% of the mean value.

Assay of IL-2 Secretion. Cultures were plated as described for the proliferation assay, and 24–48 h later, 50 μl of culture supernatant was collected. The supernatants were tested for IL-2 activity, either immediately or stored at −20°C until assayed. IL-2 activity was measured by the ability of the supernatants to support the growth of IL-2-dependent CTLD cell line. CTLD cells (10^4 cells per well) were cultured with the tested supernatants diluted 1:1 with culture medium in a final volume of 0.1 ml. After a 24-h incubation, 1 μCi of [^{3}H]thymidine was added for 5 h. The cultures were further processed as described above.

Inhibition Studies. Inhibition of the T-cell proliferative activity and IL-2 secretion was studied by adding various concentrations of the inhibitors tested plus the stimulating antigen to the assay system. Inhibition was calculated as percent inhibition = [1 − (Δcpm in the presence of inhibitor/Δcpm in the absence of inhibitor)] × 100.

Inhibition of T-Cell Response in the Absence of APCs. We used a modification of the protocol described by Burns *et al.* (23) for induction of antigen-specific tolerance *in vitro*. The lines tested were taken 9 days after the last stimulation, when the number of MNCs is minimal, washed twice, and split into three portions. These samples were further incubated with culture medium containing IL-2, IL-2 medium plus bovine BP (BBP; 50 μg/ml), or IL-2 medium plus Cop 1 (50 μg/ml). After a 72-h incubation under these conditions, the cells were washed three times and tested in the T-cell proliferation assay described above.

RESULTS

Characterization of Human T-Cell Lines and Clones. Several human T-cell lines specific to BP of various origins, human BP, BBP, and RBP, were isolated from peripheral blood lymphocytes of one normal individual and three MS patients. In addition, lines specific to Cop 1 or PPD were selected from these four donors. The reactivity of these T-cell lines could be measured by both T-cell proliferation and IL-2 secretion assays. The results summarized in Table 1 demonstrate that all the lines are highly reactive and specific for the selecting antigen.

From one healthy individual, SI, 13 T-cell clones reactive with BP, 17 clones specific to Cop 1, and 17 clones specific to PPD were generated. Representative clones of each specificity are listed in Table 2. The HLA restriction of the lines and clones was determined by testing the responses of the clones in the presence of anti-DR and anti-DQ monoclonal antibodies. All the tested lines and clones, regardless of their specificity, were shown to be DR restricted. Furthermore, by using APCs from the four donors to assess the responses of the clones, it was possible to assign precisely the DR-restricting allele of each clone. As can be seen (Table 2), the BP-specific clones exhibited two DR restriction patterns—nine clones that reacted only with autologous or matched APCs of the donor RG, were identified as DRw11-restricted, represented in Table 2 by clones I-11 and I-39. Four clones were DR7-restricted (reacted also with APCs from the donor SS), e.g., clones II-9 and II-18. Cop 1-specific clones showing the same types of restrictions could also be demonstrated. On the other hand, all the PPD clones were DR7-restricted.

The epitope specificity of the BP clones was determined using the synthetic peptides corresponding to these epitopes. Two epitope specificities were defined: the sequence 1-11 in five DRw-11 clones and the sequence 160-177 in two DR-7 clones. In some clones the epitope specificity could not be determined, as the clones were not reactive with any of the peptides tested.

Table 1. Specificity of T-cell lines

			Response (stimulation index)					
			BP		Cop 1		PPD	
Donor	HLA-DR haplotype	Line	Proliferation	IL-2 secretion	Proliferation	IL-2 secretion	Proliferation	IL-2 secretion
SI	7,w11	S-BBP	8.5	35.0	1.0	0.9	1.1	1.2
		S-Cop 1	0.9	0.8	25.2	13.9	0.9	0.8
		S-PPD	1.0	1.1	0.9	1.1	53.9	27.5
RG	7,w11	R-BBP	21.0	10.5	1.2	1.1	0.9	0.8
		R-HBP	5.4	6.3	0.9	0.8	0.8	0.7
		R-Cop 1	0.9	1.2	11.2	11.1	0.9	0.8
		R-PPD	0.8	1.2	0.9	0.8	53.5	27.3
SS	2,7	SS-HBP	5.8	10.2	1.1	NT	0.9	NT
		SS-Cop 1	1.1	0.9	11.2	23.4	0.8	NT
		SS-PPD	1.0	NT	0.9	NT	41.1	29.3
LL	2,w13	L-BBP	8.4	12.3	1.0	NT	0.8	NT
		L-RBP	19.2	10.2	1.1	NT	1.2	NT
		L-Cop 1	1.1	0.9	20.8	32.2	1.3	NT
		L-PPD	0.9	1.1	1.2	NT	45.9	20.7

Donor SI was a normal control; donors RG, SS, and LL had MS. BP lines were tested with the corresponding BP at 2.5 μg per well. The Cop 1 and PPD lines were tested with BBP. Cop 1 and PPD were used at 1 μg per well. NT, not tested. Stimulation index = cpm with antigen/cpm without antigen.

Table 2. Characterization of T-cell clones

Clone	Antigenic specificity	DR restriction	BP epitope	Stimulation index	
				Proliferation	IL-2 secretion
I-11	RBP	w11	1-11	21.2	33.2
I-39	RBP	w11	ND	55.3	26.7
II-9	RBP	7	ND	56.2	7.6
II-18	RBP	7	160-177	37.7	40.1
C-54	Cop 1	w11		23.3	35.1
C-58	Cop 1	7		25.2	15.6
C-82	Cop 1	7		60.5	26.3
C-87	Cop 1	w11		20.7	11.3
P-35	PPD	7		58.5	28.6
P-96	PPD	7		143.5	81.5
P-97	PPD	7		73.1	144.6

Stimulation index is as defined in Table 1 in response to the selecting antigen. The antigen concentrations were as indicated in Table 1. Epitope specificity was defined when the stimulation index was at least half of that obtained with intact BP. ND, not defined.

Inhibition of BP-Specific T-Cell Lines and Clones by Cop 1. None of the BP-specific lines and clones could be directly stimulated by Cop 1. However, the effect of Cop 1 on the specific response of these lines and clones could be demonstrated by competition reactions, when incubation was performed with various concentrations of BP and Cop 1. The results with a representative line S-BBP, are shown in Fig. 1. Cop 1 inhibited BP activation of the S-BBP line in a competitive dose-dependent manner. As can be seen (Fig. 1*A*), 50% inhibition of the proliferative response to BP could be obtained when a 10-fold excess of Cop 1 was added to the culture, in the linear phase of the dose–response curve to BP, and up to 100% inhibition was obtained by using 20-fold excess of Cop 1. The response of this line to BP, as followed by IL-2 secretion, could also be completely inhibited by Cop 1 (Fig. 1*B*). The inhibition of the response to BP was specific to Cop 1, as another random polymer of amino acids, TGA, did not inhibit the response to BP assayed by cell proliferation (Fig. 1*A*) and IL-2 secretion (Fig. 1*B*).

The effect of Cop 1 was tested on all the BP clones isolated from donor SI and the various BP lines originating from the three MS patients. All the BP lines and clones, irrespective of their specificity, were inhibited by Cop 1 to various extents. The results obtained with various lines and clones are illustrated in Fig. 2; 70–100% inhibition of the proliferative response was achieved with the various clones when a 20-fold excess of Cop 1 was used.

Inhibition of Cop 1-Specific T-Cell Lines and Clones by BP. The Cop 1 lines and clones that were generated from the four donors were highly reactive and specific to Cop 1. No cross-stimulation with BP was observed by any of the lines or clones. To further establish the nature of competition between BP and Cop 1, the effect of BP on the Cop 1 lines and clones was tested. As can be seen in Fig. 1*D*, S-Cop 1 line

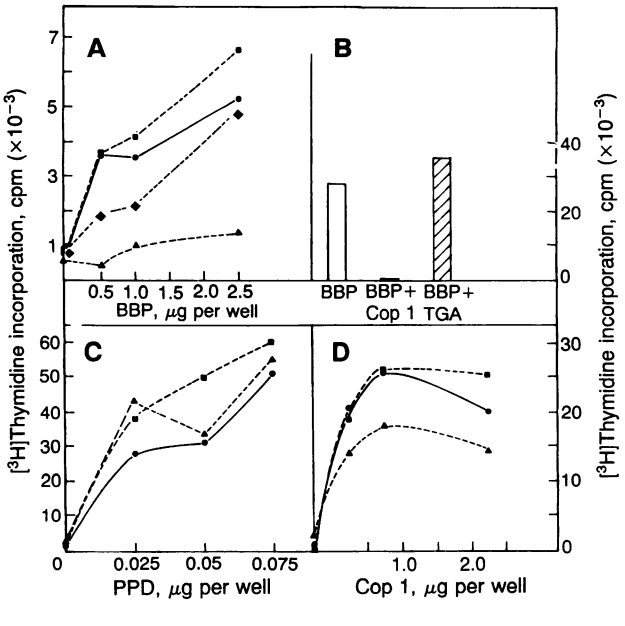

FIG. 1. Inhibition of lines derived from donor SI. (*A*) Inhibition of the proliferative response of S-BBP cells to BBP. ●, Response to BBP without inhibitors; ▲ and ◆, response to BBP plus Cop 1 (5 μg and 10 μg, respectively); ■, response to BBP plus TGA (10 μg). (*B*) Inhibition of BBP-induced IL-2 secretion by S-BBP line. Bars: open, response to BBP (1 μg) without inhibitors; solid, response to BBP plus Cop 1 (10 μg); hatched, response to BBP plus TGA (10 μg). (*C*) The effect of Cop 1 and BBP on the proliferative response of S-PPD line. ●, Response to PPD without inhibitors; ▲, response to PPD plus Cop 1 (10 μg); ■, response to PPD plus BBP (10 μg). (*D*) Inhibition of S-Cop 1 line proliferative response by BBP. ●, Response to Cop 1 without inhibitors; ▲, response to Cop 1 plus BBP (5 μg); ■, response to Cop 1 plus TGA (5 μg).

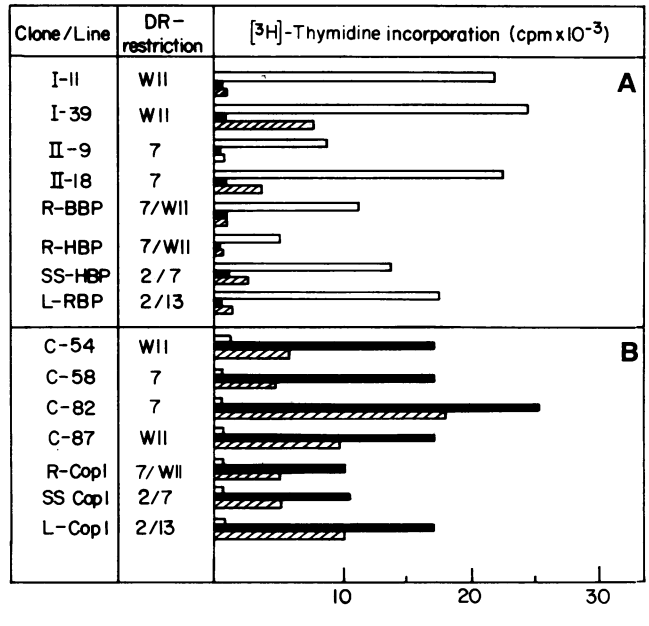

FIG. 2. Effect of Cop 1 and BP on the proliferative response of BP- and Cop 1-specific T-cell lines and clones. (*A*) The proliferative responses of BP-specific T-cell lines and clones. (*B*) The proliferative responses of Cop 1-specific T-cell lines and clones. The responses to BP alone (open bars), to Cop 1 alone (solid bars), and to BP plus Cop 1 (hatched bars) are shown.

could be competitively inhibited by BBP, but to a lesser degree than the inhibition of the response of the BP line by Cop 1 (Fig. 1*A*). A nonrelevant polymer such as TGA had no effect on the response to Cop 1. A similar extent of inhibition, ranging from 25 to 60%, was obtained with all the other lines and clones tested in the presence of a 20-fold excess of BP (Fig. 2*B*). The cross inhibitory effects of BP and Cop 1 seem to be specific as BP and Cop 1 did not inhibit any of the PPD lines and clones tested, as shown for S-PPD line (Fig. 1*C*).

Blocking of T-Cell Lines in the Absence of APC. To define more precisely the site of competition between BP and Cop 1, inhibition studies without APCs were performed. BP- and Cop 1-specific lines were preincubated with the tested antigens, added to the culture medium for 3 days. Subsequently, the cell lines were tested for their proliferative response to antigen presented on APCs. The response to IL-2 was followed in all cultures as a measure of the capacity of the cells to respond to proliferating signal. Results of such experiments are illustrated in Fig. 3. Preincubation of the S-BBP line with BP for 3 days resulted in complete anergy of the line to antigen-induced proliferation, while it still proliferated in response to IL-2. Preincubation with Cop 1 did not affect at all the response of the line to BP (Fig. 3*A*). Conversely, when S-Cop 1 line was incubated with Cop 1, 92% inhibition of the proliferative response to Cop 1 was obtained, whereas BP had no effect on this activity (Fig. 3*B*). Similar results of antigen-specific induced anergy were demonstrated with R-BBP and R-Cop 1 lines (data not shown). These results indicate that BP and Cop 1 cannot exert cross-inhibition in the absence of APCs.

DISCUSSION

The present report extends our previous studies demonstrating inhibition by Cop 1 of the response of BP-specific murine T-cell lines (12) to humans. The results reported here conclusively establish that human T-cell lines of several HLA restrictions and BP epitope specificities are inhibited by Cop 1 (Table 2 and Fig. 2).

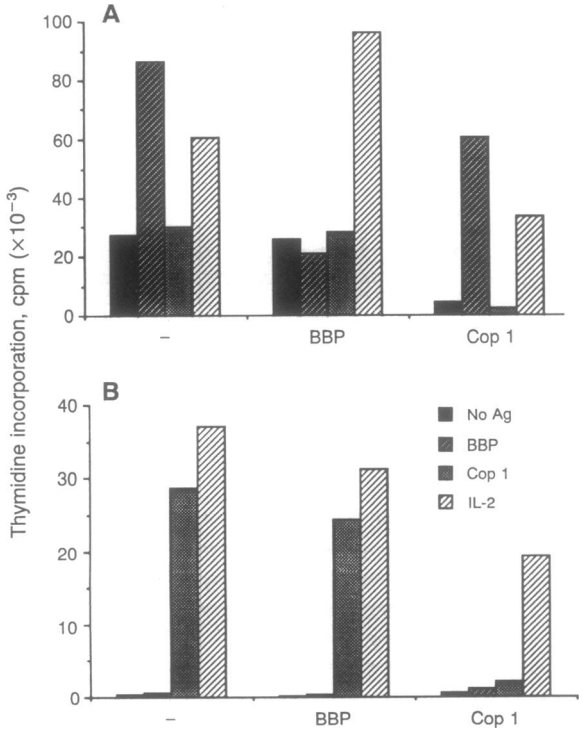

FIG. 3. Inhibition of lines derived from donor SI in the absence of APCs. (*A*) Inhibition of the proliferative response of S-BBP line. (*B*) Inhibition of the proliferative response of S-Cop 1 line. Groups of bars: –, responses of the line without preincubation with antigen; BBP, responses after a 3-day preincubation with BBP; Cop 1, response after a 3-day preincubation with Cop 1. The proliferative responses to BBP (5 μg), Cop 1 (5 μg), and IL-2 (20 units/ml) were tested. Ag, antigen.

In the EAE system, the role of BP in disease induction is well established and T-cell lines reactive with immunodominant regions of BP are indeed capable of mediating the disease (1). In MS, however, the role of BP has been debated for many years. Though we (24) and others (25, 26) have demonstrated that it is possible to detect cellular immune response to BP in MS patients, the responses have been generally quite low and inconsistent. The introduction of the T-cell line technology (27) enabled the isolation of BP-specific lines and clones from MS patients and normal individuals, as demonstrated by several groups (14–18). The uniform feature of these T cells is their expression of the CD4 molecule and the recognition of BP in the context of MHC class II products. In EAE there is usually dominance of one major encephalitogenic epitope that is recognized by BP-sensitized cells (1). A similar phenomenon was reported for T cells from MS patients, which seem to interact preferentially with a distinct BP epitope, 84-102 (21). In contrast, several other reports demonstrated responses of human T-cell lines from MS and healthy controls to multiple epitopes on BP (15–18). Our results, showing that different BP-specific T-cell clones isolated from one individual responded to at least three epitopes corresponding to the sequences 1-11 and 160-177 or undefined epitopes (Table 2), therefore, confirm the finding of multiple epitopes of BP.

In addition to BP-specific lines and clones, we could select T-cell lines specific to PPD from all donors; this is expected since most people respond vigorously to this antigen, due to vaccination against tuberculosis. We were also able to select lines specific to Cop 1, which is less expected since this is a synthetic amino acid copolymer. Yet peripheral blood lymphocytes from most individuals proliferate in response to Cop 1 (28) and the isolation of Cop 1-specific T-cell lines was also demonstrated by another group (23). All the lines and clones prepared in the present study, irrespective of their antigenic specificities, were restricted by DR alleles. The BP- and Cop 1-specific clones were restricted to the two DR alleles of the donor, whereas PPD-specific clones showed dominant restriction to only one allele (Table 2). This may indicate some heterogeneity in the response to BP and Cop 1, both of which are multideterminant antigens.

BP and Cop 1 are immunologically cross reactive, on the humoral level (9) and on the cellular level, by using *in vivo*-sensitized primary lymphocytes (10, 11). However, on the level of T-cell lines, most murine BP-specific T-cell lines do not cross react with Cop 1 (12). The present study and the results of Burns *et al.* (23) demonstrate the same phenomenon for human T-cell lines and clones. This may be due to the fact that the isolation of T-cell lines favors a specific selected population of CD4 T cells that respond to effector and not suppressor determinants. The present study demonstrates that, nevertheless, BP and Cop 1 seem to competitively inhibit T-cell lines specific to the counterpart antigen and do not inhibit the irrelevant PPD lines. Cop 1 was usually a more effective inhibitor of BP responses than BP was of Cop 1 responses. This inhibition is not due to toxic effects, as it can be reversed by increasing the concentration of the homologous antigen in culture.

It has been demonstrated that specific T-cell activation can be inhibited by unrelated peptides as well, due to competition for antigen presentation (29–32). To further establish the role of the MHC in the presently investigated system, experiments in which the competing antigens were added to the T-cell lines in the absence of APCs were performed. The results indicate that under these conditions T-cell inhibition was obtained only with the homologous antigen, probably by

blocking the specific T-cell receptor, whereas BP and Cop 1 showed no cross-inhibitory effects. These results indicate that indeed the site of competition between BP and Cop 1 is not the T-cell receptor and suggest it is likely to be the MHC molecules on the APCs. It should be noted that similar results were previously reported by Burns *et al.* (23) and were interpreted as showing a lack of cross reactivity. In the presence of APCs, we demonstrated the higher efficacy of inhibition by Cop 1 in the BP T-cell system as compared with that of BP in the Cop 1 T-cell system, which may be indicative of its higher affinity to MHC molecules. It was recently claimed that Cop 1 did not inhibit human T-cell lines reactive to BP (33). These latter results, which seem to contradict our data, may be due to the use of outdated Cop 1.

Of special importance is the finding that Cop 1 was found to block T-cell responses linked to several H-2 haplotypes in the murine system (12) and responses linked to a variety of DR alleles involving various epitopes of the BP in the human system (the present work). The ability of Cop 1 to interact with a large array of MHC molecules may be due to the fact that Cop 1, which is a random polymer, contains different sequences that are able to bind to different MHC haplotypes.

In addition to the capacity to inhibit antigen-specific proliferative responses, Cop 1 was also found to inhibit IL-2 secretion. Such activity may be of special relevance in MS *in vivo*, since the number of activated T cells was reported to be elevated in MS patients (34, 35). Such activated cells produce IL-2 as well as other cytokines that may contribute to the aggravation of the pathological symptoms and progression of the disease (36).

It has been reported that *in vivo* competition between pathogenic and nonpathogenic self peptides can be applied to prevention of EAE (37). Cop 1, when simultaneously injected with BP, also blocks EAE induction, probably by interfering with T-cell activation. If indeed BP is a pathogenic factor in MS, as suggested recently by several lines of evidence (3), then the ability of Cop 1 to compete with BP for binding to various DR molecules may be a significant element in the beneficial effect of Cop 1 in MS patients.

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1. Zamvil, S. S. & Steinman, L. (1990) *Annu. Rev. Immunol.* **8,** 579–621.
2. Paterson, P. Y. & Day, E. D. (1981–1982) *Clin. Immunol. Rev.* **1,** 581–697.
3. Wucherpfenning, K. W., Weiner, H. L. & Hafler, D. A. (1991) *Immunol. Today* **12,** 277–282.
4. Teitelbaum, D., Meshorer, A., Hirshfeld, T., Arnon, R. & Sela, M. (1971) *Eur. J. Immunol.* **1,** 242–248.
5. Teitelbaum, D., Webb, C., Meshorer, A., Arnon, R. & Sela, M. (1973) *Eur. J. Immunol.* **3,** 273–279.
6. Teitelbaum, D., Webb, C., Bree, M., Meshorer, A., Arnon, R. & Sela, M. (1974) *Clin. Immunol. Immunopathol.* **3,** 256–262.
7. Lando, Z., Teitelbaum, D. & Arnon, R. (1979) *J. Immunol.* **123,** 2156–2160.
8. Sela, M., Arnon, R. & Teitelbaum, D. (1990) *Bull. Inst. Pasteur (Paris)* **88,** 303–314.
9. Teitelbaum, D., Aharoni, R., Sela, M. & Arnon, R. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9528–9532.
10. Webb, C., Teitelbaum, D., Arnon, R. & Sela, M. (1973) *Eur. J. Immunol.* **3,** 279–286.
11. Webb, C., Teitelbaum, D., Arnon, R. & Sela, M. (1976) *Immunochemistry* **13,** 333–337.
12. Teitelbaum, D., Aharoni, R., Arnon, R. & Sela, M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9724–9728.
13. Bornstein, M. B., Miller, A., Slagle, S., Weitzman, M., Crystal, H., Drexler, E., Keilson, M., Merriam, A., Wassertheil-Smoller, S., Spada, V., Weiss, W., Arnon, R., Jacobsohn, I., Teitelbaum, D. & Sela, M. (1987) *N. Engl. J. Med.* **317,** 408–414.
14. Burns, J., Rosenzweig, A., Zweiman, B. & Lisak, R. P. (1983) *Cell. Immunol.* **81,** 435–440.
15. Richert, J., Robinson, E. D., Gladys, G. E., Martenson, R. E., Draqovic, L. J. & Kies, M. W. (1989) *J. Neuroimmunol.* **23,** 55–56.
16. Chou, Y. K., Vainiene, R., Whitham, D., Bourdette, D., Chou, C. H-J., Hashim, G., Offner, H. & Vandenbark, A. A. (1989) *J. Neurosci. Res.* **23,** 207–216.
17. Pette, M., Fujita, K., Kitze, B., Whitaker, J. N., Albert, E., Kappas, L. & Wekerle, H. (1990) *Neurology* **40,** 1770–1776.
18. Liblau, R., Tournier-Lasserve, E., Maciazek, J., Dumas, G., Siffert, O., Hashim, G. & Bach, M. A. (1991) *Eur. J. Immunol.* **21,** 1391–1395.
19. Hirshfeld, T., Teitelbaum, D., Arnon, R. & Sela, M. (1970) *FEBS Lett.* **7,** 317–320.
20. Sela, M., Fuchs, S. & Arnon, R. (1962) *Biochem. J.* **85,** 223–235.
21. Ota, K., Matsui, M., Milford, E. L., Mackin, G. A., Weiner, H. L. & Hafler, D. (1990) *Nature (London)* **346,** 183–187.
22. Brenner, T., Timore, Y., Wirguin, I., Abramsky, O. & Steinitz, M. (1989) *Neuroimmunology* **24,** 217–229.
23. Burns, J., Krasner, L. J. & Guerreo, F. (1986) *Neurology* **36,** 92–94.
24. Webb, C., Teitelbaum, D., Abramsky, O., Arnon, R. & Sela, M. (1974) *Lancet* **ii,** 66–68.
25. Lisak, R. P. & Zweinman, B. (1977) *N. Engl. J. Med.* **297,** 850–853.
26. Tournier-Lasserve, E., Jacque, C., Fradelizi, D. & Bach, M. A. (1986) *Ann. N.Y. Acad. Sci.* **475,** 404–406.
27. Ben-Nun, A., Wekerle, H. & Cohen, I. R. (1981) *Eur. J. Immunol.* **11,** 195–199.
28. Brosnan, C. F., Litwak, M., Neighbour, P. A., Lyman, W. D., Carter, T. H., Bornstein, M. B. & Bloom, B. R. (1985) *Neurology* **35,** 1750–1754.
29. Guillet, J., Lai, M., Briner, T., Smith, J. & Gefter, M. (1986) *Nature (London)* **324,** 260–263.
30. Brocke, S., Molly, D., Steinman, L., Rothbard, J. & Mozes, E. (1990) *Int. Immunol.* **2,** 735–742.
31. Adorini, L. & Nagy, Z. A. (1990) *Immunol. Today* **11,** 21–24.
32. Zisman, E., Sela, M. & Mozes, E. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 9738–9742.
33. Burns, J. & Littlefield, K. (1991) *Neurology* **41,** 1314–1319.
34. Allegretta, M., Nicklas, J. A., Sriram, S. & Albertini, R. J. (1990) *Science* **247,** 718–721.
35. Gambi, D., Porrini, A. M., Giampietro, A. & Macor, S. (1991) *J. Neuroimmunol.* **33,** 97–102.
36. Balkwill, F. R. & Burke, F. (1989) *Immunol. Today* **10,** 299–304.
37. Wraith, D. C., Smilek, D. E., Mitchell, D. J., Steinman, L. & McDevitt, H. O. (1989) *Cell* **59,** 247–255.
38. Merrifield, R. B. (1965) *Science* **150,** 178–185.