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Mini-Review

Copolymer 1: from basic research to clinical application

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Abstract. Copolymer 1 (Cop-1) is a synthetic amino acid copolymer effective in suppression of experimental allergic encephalomyelitis (EAE). The suppressive effect of Cop-1 in EAE is a general phenomenon and is not restricted to a particular species, disease type or encephalitogen used for EAE induction. In phase III clinical trials Cop-1 was found to slow progression of disability and reduce the relapse rate in exacerbating-remitting multiple sclerosis (MS). In vivo and in vitro studies suggest that the mechanism for Cop-1 activity in EAE and MS involves the binding of Cop-1 to major histocompatibility complex class II molecules as an initial step. This binding results both in competition with myelin antigens for T-cell activation and in induction of specific suppressor cells of the Th2 type. As an antigen-specific intervention, Cop-1 has the advantage of reduced probability of long-term damage to the immune system.
Key words. Experimental allergic encephalomyelitis; multiple sclerosis; Cop-1; Copaxone; autoimmunity.

Introduction

The topic of this article is the 28-year odyssey of developing a drug against multiple sclerosis (MS), starting from basic research without the prior intention of reaching a practical goal. It all began as basic research into the mechanisms involved in the induction and suppression of experimental allergic encephalomyelitis (EAE), which is the primary animal model for MS.

EAE is an acute neurological autoimmune disease mediated by CD4+ autoreactive T cells which recognize the encephalitogenic antigen(s) in association with major histocompatibility complex (MHC) class II molecules. These autoreactive cells migrate into the central nervous system (CNS) and mediate the pathogenic process. Three myelin proteins were demonstrated to be encephalitogenic: myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG), and all three were also implicated as putative autoantigens in MS [1]. However, when we started our research in 1967, the only encephalitogenic material identified in the CNS was the MBP, and the only information available about it was its general amino acid composition.

Our approach to the study of EAE and its suppression was the synthetic one, using copolymers of amino acids whose composition resembled to a certain extent that of natural MBP, in order to simulate its ability to induce or suppress EAE. None of the copolymers proved to be encephalitogenic even after conjugation with brain lipids, but some, particularly Cop-1, showed high efficacy in suppressing EAE [2].

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Suppression of EAE by Cop-1

Cop-1 is a synthetic amino acid copolymer composed of L-alanine, L-lysine, L-glutamic and L-tyrosine in a residue molar ratio of 4.2:3.4:1.4:1.0 [2]. It was shown to suppress EAE induced by MBP in a variety of animals, including guinea pigs, rabbits, mice and two species of monkeys – rhesus monkeys and baboons [3]. The results summarized in table 1 clearly indicate that there is a remarkable degree of suppression of EAE by Cop-1 in all species studied, although different encephalitogenic determinants of MBP are involved in disease induction in the different species.

The results described so far were obtained in the acute model of EAE. Chronic relapsing EAE (CR-EAE), which is characterized by two or more discrete periods with clinical or neurological signs, resembles the appearance of clinical signs in MS more closely than does the acute disease. CR-EAE can be induced in different species by the injection of either whole spinal cord homogenate, the purified PLP and MOG proteins, or synthetic peptides based on their sequences. The effect of Cop-1 on CR-EAE was tested in two species – guinea pigs and mice. Cop-1 was effective both in preventing and treating CR-EAE induced in juvenile strain 13 guinea pigs by whole spinal cord homogenate [4] (as demonstrated in table 1). Cop-1 also blocked CR-EAE induced in (SJL/J × BALB/c) F_1 mice by

Table 1. Suppression of EAE induced by different encephalitogens and in various species by Cop-1.

Encephalitogen	Disease type	Species	Inhibition by Cop-1 (%)
MBP	acute EAE	guinea pigs	70
		rabbits	73
		mice (SJL/J × Balb/c)	67
		rhesus monkeys	80
		baboons	78
Spinal cord homogenate	chronic-relapsing EAE	guinea pigs	58
		mice	55
PLP 139-151	chronic-relapsing EAE	mice (SJL/J × Balb/c)	100
PLP 178-191	chronic EAE		86
MOG 35-55	chronic EAE	mice (C3H.SW)	50

mouse spinal cord homogenate, or by the encephalitogenic peptides PLP 139-151 and PLP 178-191 [5]. Similarly, the disease induced in H-2^b mice by an MOG encephalitogenic peptide, MOG 35-55, could be considerably inhibited by Cop-1 [6]. Thus, the suppressive effect of Cop-1 in EAE is a general phenomenon and is not restricted to a particular species, disease type or the encephalitogen used for EAE induction.

Immunological mechanisms involved in the EAE-suppressive effect of Cop-1

Immunological cross-reactivity between MBP and Cop-1

Since EAE is autoimmune in nature, and its pathogenicity involves T cells sensitized to MBP, the specific inhibition by Cop-1 may be explicable in terms of an immunological cross-reaction between Cop-1 and MBP. Studies have been performed to test this hypothesis at both the cellular and humoral levels of the immune response [7–9].

Using monoclonal antibodies raised aganst MBP, we could demonstrate clearly that several monoclonal anti-MBP antibodies reacted with Cop-1 and vice versa [7]. At the cellular level, a marked cross-reaction was observed both in vivo in the delayed hypersensitivity skin test and in vitro by measuring lymphocyte transformation [8]. Of particular interest is the very good correlation between the extent of immunological cross-reactivity and suppressive effect on EAE of various materials. Thus, D-Cop-1, a polymer resembling Cop-1 in all parameters except that it is composed of D-amino acids rather than L-amino acids, does not cross-react with MBP and has no suppressing activity whatsoever [9].

Induction of antigen-specific suppressor cells

It was demonstrated that mice pretreated with Cop-1 in incomplete adjuvant became resistant to further EAE induction. This state of unresponsiveness could be adoptively transferred to normal recipients by spleen cells from Cop-1 treated donors, and the cells responsible for the suppressive activity were identified as T lymphocytes [10]. Furthermore, we have demonstrated the generation of suppressor T-cell hybridomas and lines from spleen cells of mice rendered unresponsive to EAE by Cop-1. Both cell types produce in vitro inhibition of MBP-specific effector lines and in vivo inhibition of clinical EAE [11]. Recent results revealed that these T-suppressor cells secrete Th2 cytokines after exposure to either Cop-1 or MBP [12]. These cytokines may mediate the therapeutic effect of Cop-1 in disease induced not only with MBP but also with PLP and MOG by the mechanism of 'bystander suppression'.

Inhibition of in vitro T-cell responses by Cop-1

It was demonstrated that Cop-1 can competitively inhibit the response to MBP of diverse MBP-specific murine T-cell lines and clones, which had different H-2 restrictions and responded to different epitopes of MBP [13]. Cop-1 also inhibited the specific proliferative response of T-cell lines reactive with two different encephalitogenic determinants of PLP in SJL/J mice (PLP 139-151 and PLP 178-191) [5]. Cop-1 could inhibit only the antigen-induced responses of the MBP and PLP lines, but it did not inhibit their response to the mitogen Con A, or to superantigen. Cop-1 also had no effect on other T-cell lines specific to PPD, lysozyme or ovalbumin. These findings were extended to the human T-cell response, demonstrating that Cop-1 competitively inhibited the proliferative response of various human MBP-specific T-cell clones, while having no effect on PPD-specific T-cell clones [14].

Direct binding of Cop-1 to MHC class II molecules

The studies described above on the inhibition of MBP and PLP T-cell lines and clones by Cop-1, irrespective of their MHC restriction, suggest that the site of competition between MBP or PLP and Cop-1 is most likely to be the MHC-binding site. In order to demonstrate the direct binding of Cop-1 to MHC molecules on antigen-presenting cells [APC] and to study the specificity, affinity and time course of these interactions, we used a

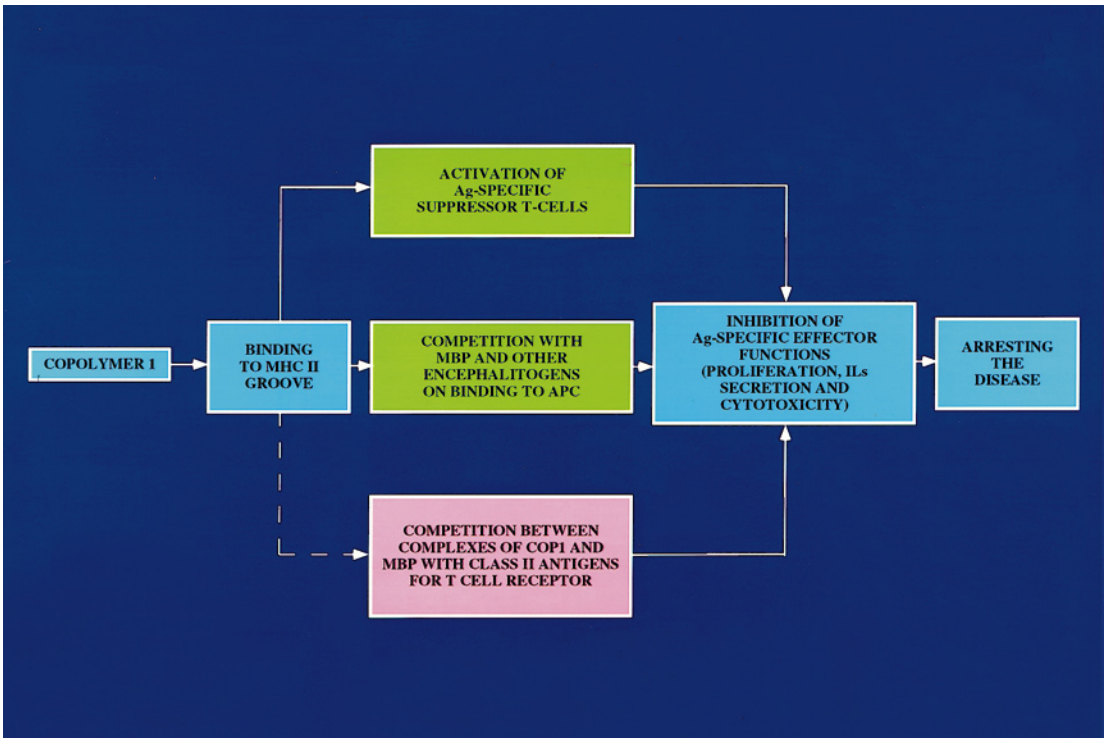

Figure 1. Proposed mechanism of action of Cop-1. Encephalitogens: MBP, PLP, MOG, other myelin proteins and peptides thereof.

biotinylated derivative of Cop-1 and a fluorimetric method to follow the binding [15]. Cop-1 exhibited a very high and indiscriminate binding to different types of APC of various H-2 and HLA haplotypes. The specificity of the binding was confirmed by its inhibition with either the relevant anti-MHC class II antibodies or unlabelled analogues. The binding of Cop-1 to MHC class II molecules was more rapid and efficient than that of either MBP or its peptide fragment p84-102, and each of these substances showed competition with the other for this binding site. Cop-1 was also capable of competing with other myelin associated proteins, such as PLP and MOG, for binding to the MHC class II molecule [16]. Moreover, Cop-1 efficiently displaced MBP, PLP and MOG-derived peptides from the MHC class II-binding groove, whereas it could not be displaced by these antigens once bound to the MHC. These results indicate the potential of Cop-1 as a broad spectrum drug for MS.

Proposed mode of action of Cop-1 in EAE and MS

The results described hitherto reveal that Cop-1 affects EAE, and therefore by extrapolation MS, at various levels of the immune response involved, which differ in their degree of specificity. The proposed mechanism for Cop-1 activity is depicted in figure 1. Binding of Cop-1 to the MHC class II molecules, which is the least specific step, is a prerequisite for its effect by any mechanism. Following this interaction two mechanisms were clearly shown to be effective: 1) Cop-1 binding to the relevant MHC leads to the activation of T suppressor cells, which are activated by suppressive determinants shared between MBP and Cop-1. This mechanism is a specific one and results from the cross-reactivity between Cop-1 and MBP. 2) Cop-1 can compete for binding to MHC class II molecules with several myelin-associated antigens, e.g. MBP, PLP and MOG, resulting in inhibition of antigen-specific T-cell effector functions (i.e. proliferation, interleukin secretion and cytotoxicity).

This mechanism may be less specific, as MHC blockade may lead to interference with other immune responses. However, this does not seem to be the case, as Cop-1 did not inhibit responses to OVA, lysozyme or PPD. Furthermore, D-Cop-1, which bound to MHC class II molecules as efficiently as Cop-1 and competed with MBP for binding, did not inhibit MBP-specific T-cell lines, and did not inhibit EAE when coinjected with the encephalitogenic emulsion. These findings may suggest that the nonspecific MHC blocking is a necessary but not sufficient step, which requires an additional step involving antigen-specific mechanisms such as induction of crossreactive T-cell tolerance, or T-cell receptor antagonism. Thus it is possible that competition also occurs at the level of the T-cell receptor between the complex of MBP-derived peptides with class II MHC antigen, and the complex of Cop-1 with class II antigen.

The inhibition of PLP and MOG-induced EAE by Cop-1 can easily be explained by the MHC blocking, as Cop-1 efficiently competes with PLP and MOG peptides for MHC binding. The effect of Cop-1 on PLP-induced

disease can also be explained by the specific mechanism of Ts cells. Thus, Cop-1 induces MBP-specific suppressor cells which upon stimulation in the target organ by MBP secrete suppressive cytokines and cause bystander suppression of PLP encephalitogenic responses, due to its in vivo colocalization with MBP. Such bystander suppression of PLP-induced EAE was demonstrated by Ts cells induced by feeding with MBP [17]. Another possible explanation may be that the immune reactivity towards PLP or MOG causes, through 'antigen spreading' [18], an immune response to the vicinal MBP which is suppressed by Cop-1. Regardless of the mechanism involved, the ability of Cop-1 to suppress disease which is induced not only by MBP but by other myelin-associated proteins as well is very important, since these antigens might be potential autoantigens in MS.

Clinical studies with Cop-1 in MS

In view of the putative resemblance between EAE and MS and the assumption that MBP may be involved in the pathogenesis of MS, preliminary clinical trials using Cop-1 were conducted in MS patients. These were begun after toxicity studies in experimental animals showed that Cop-1 was nontoxic after both acute and subchronic administration to mice, rats, rabbits and beagle dogs [11], and that there was no significant uptake by any of the animal organs.

Our clinical trials have included two preliminary open trials and two double blind phase II trials, one involving exacerbating-remitting ER patients [19] and another one in chronic progressive (CP) patients [20]. The results of the phase II trial in ER patients demonstrated a remarkable decrease in the number of relapses and rate of progression in Cop-1-treated patients compared with the placebo control. After a successful pivotal multicentre phase III clinical trial [21, 22], which was conducted in 11 medical centres in the United States and involved 251 patients, the US Food and Drug Administration decided to approve Cop-1 ('Copaxone') as a drug for MS.

Conclusions

Cop-1, a synthetic polypeptide, has a specific effect on the autoimmune process involved in EAE and probably also in MS. The results of clinical trials with Cop-1 indicate that it is a promising low-risk MS-specific drug for the treatment of relapsing MS, capable of slowing progression of disability and reducing the relapse rate. As an antigen-specific intervention, Cop-1 has the advantage of reduced probability of long-term damage to the immune system.

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