

INHIBITION OF ACUTE EXPERIMENTAL ALLERGIC  
ENCEPHALOMYELITIS IN MICE BY COLCHICINE

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Experimental allergic encephalomyelitis (EAE) is widely regarded as a laboratory analog of multiple sclerosis (MS). Recent evidence both in MS and EAE point to the fact that activated macrophages appear to be proximal agents of myelin destruction (1). In view of these findings and encouraged by the preliminary results of a trial of colchicine in MS patients, we asked the question whether or not colchicine might be effective in the prevention of EAE.

Colchicine is a unique antiinflammatory agent whose traditional use has been in the treatment and prophylaxis of acute gout (2). In addition, colchicine is also effective against a variety of inflammatory disorders such as familial Mediterranean fever and Behcet's disease which resemble MS in their cardinal features of recurrent inflammation and enhanced chemotaxis and phagocytosis.

Murine EAE was induced both by active sensitization of animals with neural antigens and by adoptive transfer of encephalitogenic T cells. The results obtained clearly show that colchicine blocks the appearance of EAE and appears to exert its inhibitory effects mainly on the efferent limb of the immune response.

#### Materials and Methods

*Animals.* Female SJL/J or (SJL/J × BALB/c)<sub>F1</sub> hybrid mice, 6–10 wk of age were obtained from The Jackson Laboratory, Bar Harbor, ME.

*Induction of Acute EAE.* The method of Linthicum and Frelinger (3) was used for the active induction of EAE. Mice were examined daily for signs of EAE and scored on a scale of 0–3, as described in this paper.

*Adoptive Transfer of EAE.* The method of Pettinelli and McFarlin (4) was followed, using SJL/J female mice.

*Colchicine Treatment.* Intravenous colchicine (Eli Lilly and Co., Indianapolis, IN), was injected in test mice intraperitoneally on alternate days or as otherwise indicated in the text at a dosage level of 15 µg in 0.1 ml. Peripheral blood counts of mice showed no change after 14 d of colchicine treatment. Control mice were injected intraperitoneally with water.

*Measurement of Delayed-Type Hypersensitivity (DTH).* The footpad swelling test was used to measure the effect of colchicine on DTH responses to bovine myelin basic protein (MBP) and the unrelated antigen PPD. Mice were injected subcutaneously at three sites in the flanks with the appropriate antigen in a total of 0.1 ml CFA with added *Mycobacterium tuberculosis* (H37RA; Difco Laboratories Inc., Detroit, MI). For challenge, antigen in saline or saline alone was injected into interdigital pads in a volume of 10 µl using a

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TABLE I  
Effect of Colchicine on the Development of Acute EAE

Group	Number of animals immunized*	Colchicine treatment (15 µg)	Number with clinical EAE per total number at risk	Disability score	Histopathology grade <sup>§</sup>	Ia expression
I	30	None	25/30	2-3	++++	+
II	30	Intraperitoneally from day -1 after infection and every other day	2/30 <sup>‡</sup>	0	±	-
III	15	Intraperitoneally from day +7 and every other day	11/15	2	+	ND

\* 6 µg dry of mouse spinal cord in 0.1 ml CFA with 500 µg *M. tuberculosis* (H37RA; Difco) added was injected into the four footpads. Immediately thereafter and 48 h later *B. pertussis*,  $25 \times 10^9$  cell in 0.2 ml, was injected via the tail vein. The *B. pertussis* was kindly provided by Dr. D. S. Linthicum, University of Southern California, Los Angeles.

<sup>‡</sup> Two animals had ruffled fur with weight loss and tail atonia for <2 d.

<sup>§</sup> In order of increasing severity, ± through ++++.

10µl syringe (Hamilton Co., Reno, NV) fitted with a 30-gauge needle. Footpad swelling was measured before and after challenge using a spring-loaded dial gauge caliper (Peacock Laboratories, Inc., Philadelphia, PA).

*Immunocytochemistry: Staining for Ia Antigen.* The avidin-biotin complex (ABC) technique as described by Traugott et al. was used on frozen sections of brain and spinal cord from colchicine-treated and control EAE hybrid mice. Ia-bearing cells were localized by use of mouse mAb against the H-2<sup>d</sup> phenotype (Becton Dickinson & Co., Mountain View, CA).

*Histopathology.* Glutaraldehyde perfused tissues were embedded in EPON and 1-µm sections were stained with toluidine blue for light microscopy. Perfusion with 20% formalin in PBS was used for the preparation of paraffin-embedded sections to be stained with H and E, luxol fast blue and Bodian stain.

## Results

*Effect of Colchicine on Development of Acute EAE.* EAE was induced in female SJL/J × BALB/c mice after immunization with spinal cord antigens in the presence of CFA and *Bordetella pertussis*. 25 of 30 immunized animals developed classical clinical signs of EAE between 12 and 18 d after immunization. Colchicine treatment begun on day -1 resulted in complete prevention of clinical EAE in an experimental group of 30 mice. When colchicine treatment was begun on day 7 after immunization, EAE was found to develop in 11 of 15 mice indicating that colchicine must be given early after sensitization to prevent disease. The results of these experiments are summarized in Table I.

Five animals from each group were killed for histology. All five EAE animals from the untreated group showed inflammatory cell infiltrates in the brain and spinal cord consisting of mononuclear cells and small numbers of polymorphonuclear leukocytes. Luxol fast blue and Bodian stains showed areas of demyelination with relative axonal sparing. Toluidine blue-stained Epon sections and electron microscopy (not shown) confirmed the presence of focal demyelination associated with inflammatory mononuclear cells.

Five colchicine-protected animals showed a mild mononuclear cell infiltrate in the leptomeninges at the base of the brain, and no demyelination was seen in the brain and spinal cord of these animals. EAE animals from the group with delayed colchicine treatment had pathological lesions similar to the untreated EAE animals, but of definite reduced severity.

TABLE II  
*Effect of Colchicine on Adoptive Transfer of EAE by Lymph Node Cells (LNC) Cultured for 96 h with MBP*

Group	Treatment of donor mice*	Treatment of recipient mice	Outcome: incidence of EAE
IA	Sensitized with MBP in CFA plus <i>M. tuberculosis</i>	$4 \times 10^7$ stimulated LNC injected intravenously.	5/5
IB	As in IA	$4 \times 10^7$ stimulated LNC injected intravenously; animals pretreated with colchicine day -3 and -1	5/5
IC	As in IA	$4 \times 10^7$ stimulated LNC injected intravenously; animals treated with colchicine from day of injection (day 0) and every other day	0/5
II	Sensitized as in group I; treated with colchicine from day -1 and every other day until LNC harvest on day 12	$4 \times 10^7$ stimulated LNC injected intravenously	0/5

\* Five SJL/J mice for each group were sensitized by subcutaneous injection into the flanks of 400  $\mu$ g bovine MBP in 0.1 ml of a CFA emulsion with 50  $\mu$ g *M. tuberculosis* (H37RA) added.

Immunostaining for endothelial cell Ia expression was performed on spinal cord tissue from three EAE animals and three colchicine-protected animals. The spinal cords of the mice with acute EAE exhibited significant Ia staining of blood vessel endothelium, while colchicine-treated animals were essentially negative.

*Effect of Colchicine on Adoptive Transfer of EAE.* Two groups of female SJL/J mice were immunized as described (4). One group was treated with colchicine beginning on the day before immunization and continued every other day until LNC harvest on day 12 after immunization. LNC were cultured with MBP for 96 hours, washed and counted and injected into four groups of recipient SJL/J mice according to the protocol outlined in Table II.

LNC from donor animals treated with colchicine from the day before immunization failed to transfer EAE (group II), while LNC from control animals produced EAE in all recipients between days 9–11 after transfer (group IA). Pretreatment of recipients with colchicine (on days -3 and -1 before cell transfer) had no effect on subsequent development of EAE (group IB). However, colchicine treatment of recipients concurrent with cell transfer resulted in complete prevention of EAE development (group IC). Histopathological evaluation of spinal cord from these animals showed the presence of sparse mononuclear infiltrates around blood vessels in 3 of 15 sections examined and no evidence of demyelination. In contrast, animals with EAE showed dense inflammatory cell infiltrations with associated areas of demyelination in 15 of 15 sections of spinal cord examined (see Fig. 1).

*Effect of Colchicine on DTH Responses.* Three groups of SJL/J mice were sensitized with MBP in CFA and added *M. tuberculosis* and were challenged 4 d later by intrafootpad injection of MBP in saline. One group was treated with colchicine on days 1 and 3 after sensitization, and a second group was treated on day 4. The third group served as a non-colchicine-treated control. The results are presented in Fig. 2. Colchicine treatment on days 1 and 3 after sensitization had a potentiating effect on DTH. Colchicine on day 4, however, suppressed DTH expression. Identical results were obtained when an unrelated antigen PPD was used in place of MBP for sensitization and challenge (results not shown).

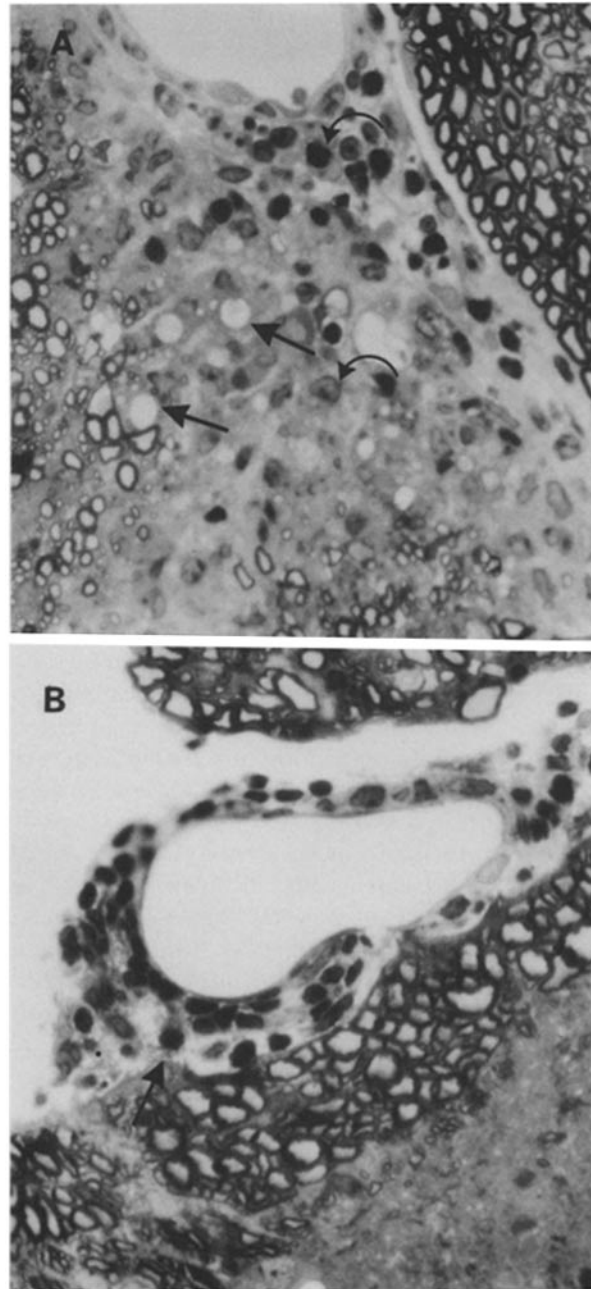


FIGURE 1. Photomicrographs of toluidine blue-stained 1- $\mu$ m EPON sections from lumbar spinal cords of mice with EAE (A) and without EAE after colchicine treatment during adoptive transfer (B). (A) Several demyelinated axons are seen in the central area (arrows) in proximity to numerous mononuclear inflammatory cells (curved arrows). Intact axons of the anterior root (peripheral nerve) are seen in upper right corner. (B) Mild perivascular infiltration is seen (arrow). Spinal cord axons appear normal (below vessel and lower left). Normal appearing posterior root axons are on upper right.  $\times 200$

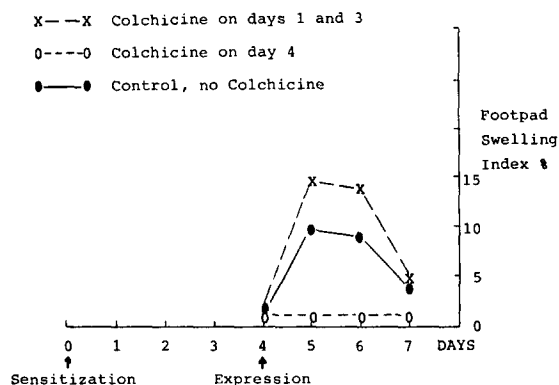


FIGURE 2. Effect of colchicine on DTH reaction to MBP in SJL/J mice. Three groups of five mice were sensitized as described in Materials and Methods using 200  $\mu$ g bovine MBP per mouse (day 0). Intrafootpad challenge used 50  $\mu$ g of MBP in saline (day 4). Footpad swelling was measured on days 5, 6, and 7 and group means were calculated. Footpad swelling index (FSI) was calculated from the following:  $FSI = \{[(L-L_0)/L_0] - [(R-R_0)/R_0] \times 100\}$  where  $L$  and  $L_0$  represent left footpad thickness after ( $L$ ) and before ( $L_0$ ) challenge with antigen, and  $R$  and  $R_0$  represent right footpad thickness after ( $R$ ) and before ( $R_0$ ) saline control inoculation.

### Discussion

The present studies show that colchicine effectively prevents the development of the clinical signs and histological lesions of acute, monophasic EAE in mice. However, if administration was delayed until day 7 after immunization, treatment was relatively ineffective (Table I).

As noted by Traugott et al. (5), Ia molecules can be shown on endothelial cells, as well as on small numbers of mononuclear inflammatory cells within the CNS, within 7 d after immunization, suggesting an important role for these molecules in the induction of EAE. This may explain the relative ineffectiveness of colchicine when administered from day 7 after immunization. Experiments now in progress will determine at what point after sensitization colchicine is effective in preventing EAE.

The adoptive cell transfer experiments indicate that colchicine may act at two different levels (Table II). First, cultured LNC from colchicine treated animals failed to transfer the disease. Secondly, colchicine completely prevented EAE from developing in animals when treatment was begun at the time of cell transfer. Thus, it is possible that colchicine interrupts the signaling mechanism provided by MBP-primed T cells by suppression of lymphokine secretion or by mitotic inhibition of a critical effector population. North (6) showed that a mitosis-inhibiting dose of vinblastin, an alkaloid similar to colchicine, could prevent the adoptive cell transfer of immunity to listeria organisms in normal recipient mice when immune donors were pretreated with the drug before spleen cell harvest.

Regarding the DTH experiments (Fig. 2), treatment with colchicine in the induction phase resulted in an enhanced DTH response. Others have noted a similar DTH enhancement after pretreatment with cytotoxic agents, notably cyclophosphamide, and they attribute such modulation to interruption of feedback control of effector cells by diminished suppressor T cell activity (7) or by suppressed specific antibody formation (8).

Additional experiments (our unpublished observations) showed that when spleen cells from colchicine-pretreated mice were used as accessory cells in a T

cell proliferation assay, a markedly enhanced proliferative response was found compared with the response seen with splenocytes from untreated mice. Thus, colchicine, when present during the induction phase of DTH, may stimulate accessory cell functions resulting in enhanced T cell sensitization.

The elicitation phase of DTH is believed to involve the activation of such sensitized T cells, leading to lymphokine secretion responsible for the activation and focusing of effector mononuclear cells to the inflammatory site. A single dosage of colchicine, administered at the time of antigen challenge apparently suppressed some critical stage(s) of this effector mechanism. This result paralleled that obtained in the adoptive cell-transfer experiment where it was shown that colchicine, administered at the time of cell transfer, completely prevented the development of EAE in recipients. If EAE and MS share features of a final common efferent pathway, colchicine may prove effective in limiting lesion development in the human disease.

### Summary

Colchicine was found to inhibit the clinical and histopathological manifestations of monophasic experimental allergic encephalomyelitis in mice. For inhibition of actively induced disease, inoculation of colchicine at the time of encephalitogenic challenge was found to be most effective. In adoptive transfer experiments, lymph node cells (LNC) from colchicine treated donors failed to transfer the disease. Additionally, colchicine treatment of recipients receiving an otherwise disease-inducing level of sensitized LNC prevented the development of disease. Experiments involving delayed-type hypersensitivity expression support an inhibitory role for the drug on event(s) of the efferent pathway of the cellular immune response.

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