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Immunology

Prevention of autoimmune demyelination in non-human primates by a cAMP-specific phosphodiesterase inhibitor

(multiple sclerosis/experimental allergic encephalomyelitis/tumor necrosis factor)

CLAUDE P. GENAIN*†, TIM ROBERTS‡, RICHARD L. DAVIS§, MY-HOA NGUYEN*, ANTONIO UCCELLI*, DARYL FAULDS¶, YI LI*, JOE HEDGPETH¶, AND STEPHEN L. HAUSER*

Departments of *Neurology, ‡Radiology, and §Pathology, University of California, San Francisco, CA 94143-0114; and ¶Department of Basic Research, Berlex Biosciences, Richmond, CA 94804-0099

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ABSTRACT **Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system that serves as a model for the human disease multiple sclerosis. We evaluated rolipram, a type IV phosphodiesterase inhibitor, for its efficacy in preventing EAE in the common marmoset *Callithrix jacchus*. In a blinded experimental design, clinical signs of EAE developed within 17 days of immunization with human white matter in two placebo-treated animals but in none of three monkeys that received rolipram (10 mg/kg s.c. every other day) beginning 1 week after immunization. In controls, signs of EAE were associated with development of cerebrospinal fluid pleocytosis and cerebral MRI abnormalities. In the treatment group, there was sustained protection from clinical EAE, transient cerebrospinal fluid pleocytosis in only one of three animals, no MRI abnormality, and marked reduction in histopathologic findings. Rolipram-treated and control animals equally developed circulating antibodies to myelin basic protein. Thus, inhibition of type IV phosphodiesterase, initiated after sensitization to central nervous system antigens, protected against autoimmune demyelinating disease.**

The human demyelinating disease multiple sclerosis (MS) is thought to result from an autoimmune response to one or more antigens of the central nervous system (CNS) (1, 2). In the disease model experimental allergic encephalomyelitis (EAE), immunization of susceptible animals with CNS tissue elicits an inflammatory CNS disease that bears some resemblance to human MS (3). T-lymphocyte mediation of EAE is demonstrated by the capacity of antigen-specific T cells to adoptively transfer disease when injected into a syngeneic naive animal (4, 5). An important difference between typical acute EAE and MS relates to the extent of demyelination that occurs. Demyelination is characteristic of MS, yet is scant or absent in many forms of acute EAE (3, 6). Recently, a novel MS-like model of EAE has been described in the common marmoset *Callithrix jacchus*. *C. jacchus* EAE is characterized by a relapsing remitting clinical course and by pathologic findings of perivascular inflammation, demyelination, and astrogliosis (7).

Proinflammatory cytokines are thought to participate in tissue damage in EAE (reviewed in ref. 8). Interferon γ has been proposed as one cytopathogenic factor. An additional candidate that may act synergistically with interferon γ is tumor necrosis factor α (TNF-α). TNF-α is reported to be associated with exacerbations in MS and with pathology in EAE (9–16). In rodents, the capacity of T-cell clones to adoptively transfer EAE correlates with their ability to produce TNF-α upon stimulation with myelin antigens—for example, myelin basic protein (MBP) or proteolipid protein (17, 18). Signs of EAE can be abrogated with antibodies to, or other inhibitors of, TNF-α (19–22). TNF-α enhances antigen-induced T-cell proliferation (23), lymphocyte adhesion to microvascular endothelium, and trafficking to the CNS (24–27). *In vitro*, TNF-α may also contribute to oligodendroglial cell death and demyelination (28–32). Strategies designed to inhibit this cytokine represent a potential selective approach to the treatment of demyelinating diseases.

Inhibitors of the type IV phosphodiesterase (PDE-IV) suppress the release of TNF-α from macrophages and monocytes (33–36), two cell types that express primarily the type IV isoenzyme (37). This effect is mediated via an increase in the intracellular concentration of cAMP (37–39) and results from inhibition of transcription of the TNF-α gene and release of TNF-α from its precursor molecule (38, 40–42). The selectivity for cytokines of the TNF family is emphasized by the absence of inhibitory effect on interleukin 1. Here, we evaluated the effect of rolipram, a well-characterized selective inhibitor of PDE-IV, on EAE in *C. jacchus*.

MATERIALS AND METHODS

Animals. *C. jacchus* marmosets were maintained in a primate colony at the University of California, San Francisco, and were cared for in accordance with the institutional guidelines. Blood (2.5 ml) and cerebrospinal fluid (CSF) (20–100 μl) were obtained under anesthesia with ketamine, 20 mg/kg i.m. MRI was performed under anesthesia with propofol, 20–50 mg/hr by i.v. drip.

Dose-Finding Studies. *In vitro*, the effects of rolipram were assessed using macrophages obtained from peritoneal exudates of healthy *C. jacchus* following thioglycollate/mineral water injection. TNF-α release was measured in supernatants of 5×10^5 macrophages stimulated with lipopolysaccharide (LPS; 100 ng/ml) and cultured for 18 hr in 500 μl of RPMI 1640 medium supplemented with 10% controlled processed serum replacement 2 medium (Sigma) in the presence of increasing concentrations of rolipram (0–100 μM). In preliminary studies *in vivo*, rolipram doses of 0.05 mg/kg, 0.5 mg/kg, or 5 mg/kg administered s.c. at 48-hr intervals were insufficient to produce detectable plasma concentrations. Therefore, a higher dose of 10 mg/kg was chosen for testing the effect of rolipram on EAE. Administration of rolipram s.c. induced salivation, vomiting, excessive grooming, and head twists, side effects that could be prevented by i.m. odansetron hydrochloride (0.3–0.6 mg/kg) 20 min prior to rolipram administration.

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Abbreviations: MS, multiple sclerosis; CNS, central nervous system; EAE, experimental allergic encephalomyelitis; TNF-α, tumor necrosis factor α; PDE-IV, type IV phosphodiesterase; CSF, cerebrospinal fluid; Gd, gadolinium diethylenetriaminepentaacetic acid; LPS, lipopolysaccharide; MBP, myelin basic protein; G3PDH, glyceraldehyde-3-phosphate dehydrogenase.

†To whom reprint requests should be addressed at: Department of Neurology, Box 0114, University of California School of Medicine, San Francisco, CA 94143-0114.

In vitro, odansetron had no effect on TNF-α release by peritoneal macrophages.

Induction of EAE. Six *C. jacchus* were actively immunized with normal human white matter (7). Beginning at day 7 following immunization, rolipram (10 mg/kg) dissolved in dimethyl sulfoxide (treatment group) or dimethyl sulfoxide alone (placebo group) was administered every 48 hr by s.c. injection for a total of 44–46 days or until sacrifice. Following cessation of treatment, animals were observed for an additional 7–19 days. The course of EAE was monitored by daily clinical examinations and by sequential examinations of the CSF. Plasma and CSF concentrations of rolipram were measured at 3-week intervals. MRI of the brain was performed prior to immunization and at various intervals from 2 to 58 days after immunization. Clinical observers and radiologists who interpreted MRI findings were blinded to the treatment groups.

At the end of the study, or earlier if required due to severe EAE, animals were sacrificed by exsanguination under ketamine anesthesia. Neuropathologic findings were graded according to the density of monocytic infiltration (perivascular cuffing) in the CNS white matter (+ to +++) and to the presence or absence of macrophage infiltration associated with demyelination and gliosis.

Biological Measurements. TNF-α was measured in triplicate samples of culture supernatants using the cytotoxicity assay on WEHI 164 cells (43), followed by quantitation of acid phosphatase (44). A standard curve was constructed in each assay using recombinant human TNF-α (Sigma). Plasma anti-MBP antibody titers were measured by ELISA in 96-well plates coated with 8 μg of purified human MBP per well (45), using 100 μl of *C. jacchus* serum (1:100 dilution) and 100 μl of peroxidase-conjugated anti-monkey IgG (1:500, Sigma). ELISA plates were developed with 100 μl of ABTS [2,2′-azinobis(3-ethylbenzthiazolinesulfonic acid)] peroxidase substrate per well (Kirkegaard & Perry Laboratories) and read at 405 nm. Plasma and CSF concentrations of rolipram were measured using an RIA (46).

Brain levels of TNF-α mRNA were quantitated from 3 mg of fresh frozen white matter taken at autopsy and washed with phosphate-buffered saline to remove blood contaminants. A total RNA fraction was extracted using RNA-zol B (Biotecx Laboratories, Houston). A first strand cDNA was synthetized using 2.5 μg of total RNA in a 100-μl reaction mixture containing PCR buffer (Promega), 1.5 mM $MgCl_2$, 1 unit of Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL), 1 mM dNTP, 25 nM random hexamers, and RNase inhibitor (Pharmacia). Ten microliters of cDNA was used for PCR amplification in a 34-μl reaction mixture in the presence of PCR buffer, 0.5 mM $MgCl_2$, 1 unit of *Taq* polymerase (GIBCO), and 0.5 μM (each) glyceraldehyde-3-phosphate dehydrogenase (G3PDH) and TNF-α upstream and downstream primers. The sequences of the primers were 5′-GCAATGATCCCAAAGTAGACCTGCCCAGACT-3′ (3′ TNF-α), 5′-GAGTGACAAGCCTGTAGCCCATGTTGTAGCA-3′ (5′ TNF-α), 5′-CATGTGGGCCATGAGGTCCACCAC-3′ (3′ G3PDH), and 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ (5′ G3PDH). PCR amplification was carried out on duplicate samples for 35 cycles in a DNA thermal cycler. In preliminary experiments, we were unable to reproducibly detect brain TNF-α mRNA with a lower number of cycles.

RESULTS

Dose-Finding Studies. *In vitro*, rolipram inhibited LPS-induced release of TNF-α by *C. jacchus* macrophages. The IC_{50} was 12 nM, and concentrations of 0.4 μM or above inhibited TNF-α release by 90%. In all animals receiving 10 mg of rolipram per kg, plasma concentrations were consistently within the range of the IC_{50} 48 hr after injection (1.9–18.7 nM). CSF rolipram concentration could be measured in two animals and was 40–60% of that in plasma, with a significant correlation between plasma and CSF concentrations ($n = 3$, $P < 0.05$, $r = 0.998$).

Effect of Rolipram on EAE. These data are summarized in Table 1. One control animal unexpectedly died during an MRI procedure on day 9 after immunization. In the other two controls, clinical signs of EAE developed 15 and 17 days, respectively, after immunization. One control (444-91) had a spontaneous clinical relapse on day 66. By contrast, none of the three rolipram-treated animals developed signs of acute EAE during treatment. One rolipram-treated animal (13-89) developed signs of EAE 17 days after cessation of rolipram. The two other treated animals remained asymptomatic until euthanized, 7 and 19 days after cessation of treatment.

MRI abnormalities were present in both controls at day 17 and day 30 after immunization but in none of the three rolipram-treated animals (Table 1). Findings early in the course of clinical disease consisted of small, round foci of Gd enhancement on T1-weighted sequences, indicative of blood–brain barrier disruption. On subsequent examinations, foci of increased T2-weighted signal, suggestive of edema and/or demyelination, were present. In Fig. 1, early and late MRI findings in representative control (30-92) and treated (13-89) animals are shown.

Mononuclear cell CSF pleocytosis was present before and at the onset of clinical signs in the control animals, and pleocytosis persisted until the animals were euthanized (Fig. 2). In

Table 1. Effect of rolipram on EAE in *C. jacchus*

Animal no.	Treatment*	Clinical signs of EAE*	MRI findings*	Sacrifice*	Pathology	
					Perivascular cuffing	Demyelination
13-89	Rolipram; 7–53	Lethargy, anorexia, anisocoria; 69	—; −12 —; 28 —; 58	70	+	−
612-91	Rolipram; 7–53	None	—; 2 —; 51	72	−	−
55-92	Rolipram; 7–51	None	—; 16 —; 44	58	+	−
30-92	Placebo; 7–36	Paraplegia, anisocoria; 15	—; 8 Gd enhancement; 30 Bright T2 foci; 36	36	+++	+
444-91	Placebo; 7–53	Sensory loss lower limbs; 17 Paraplegia; 66	—; 10 Gd enhancement; 17 Bright T2 foci; 31	80	+++	+

Gd, gadolinium diethylenetriaminepentaacetic acid.
*Numbers refer to days after immunization.

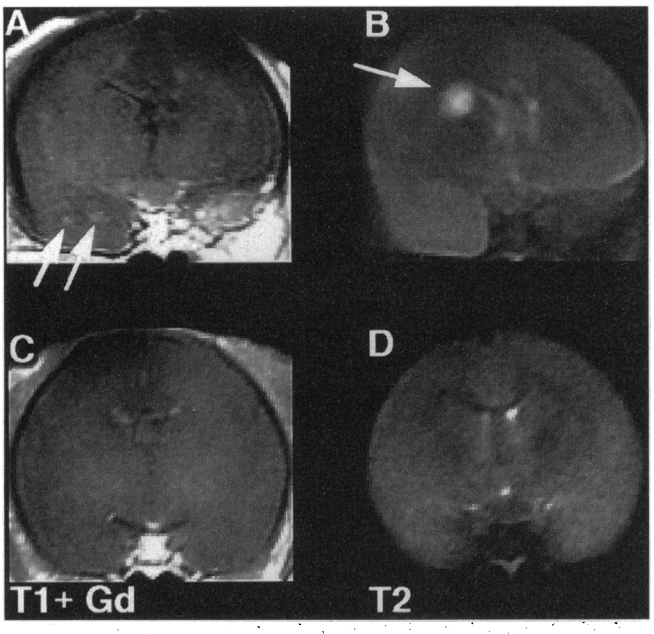

FIG. 1. Representative MRI findings in control and rolipram-treated *C. jacchus*. (*Left*) Coronal T1-weighted images (TR 400 ms, TE 12 ms) after Gd administration (0.1 mmol/kg). (*A*) Control (30-92), day 30 after immunization; two foci of Gd enhancement are present in the right temporal lobe (arrows). (*C*) Rolipram (13-89), day 28; normal study. (*Right*) T2-weighted scans (TR 2500 ms, TE 90 ms) in the same animals. (*B*) Control (30-92), day 36; a large focus of high signal intensity has developed (arrow), suggesting edema and/or demyelination. (*D*) Rolipram, day 58: the T2-weighted image is normal.

the rolipram group, CSF pleocytosis was entirely absent in two animals (13-89 and 55-92). In the third (612-91), CSF pleocytosis was delayed and transient (Fig. 2). After cessation of treatment, a second rolipram-treated animal (55-92) developed CSF pleocytosis. We were unable to collect CSF from animal 13-89 immediately prior to autopsy.

Immune Responses to MBP. All animals developed measurable antibody titers to MBP following immunization. Titers

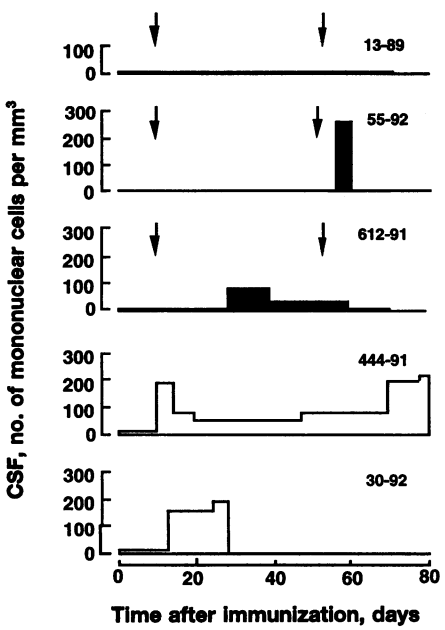

FIG. 2. Effect of rolipram on CSF pleocytosis during EAE in *C. jacchus*. Closed bars (top), animals treated with rolipram; open bars (bottom), controls treated with placebo. Arrows indicate the interval of treatment.

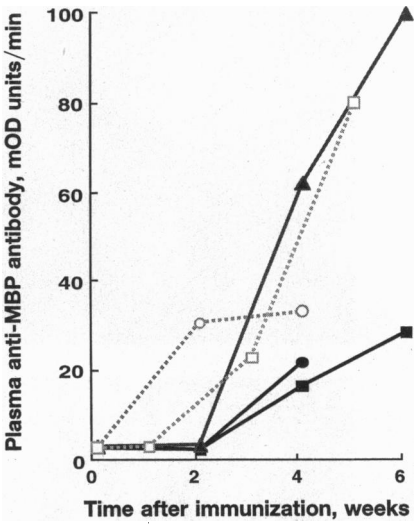

FIG. 3. Plasma anti-MBP antibody titers in controls and treated animals. Open symbols, controls (444-91 and 30-92); closed symbols, rolipram (13-89, 55-92, and 612-91).

were similar in the treatment and control groups, indicating that rolipram did not suppress development of a humoral response to the administered antigen (Fig. 3).

Neuropathologic Findings. In control animals, numerous, large (>0.6 mm) inflammatory infiltrates composed of mononuclear cells were found within periventricular white matter of the cerebral hemispheres and posterior and lateral funiculi of the spinal cord (Fig. 4). By contrast, little or no inflammation was found in rolipram-treated animals even though autopsy was performed on animals euthanized 7–19 days after cessation of treatment (Table 1, Fig. 4). Rare periventricular infiltrates of small size (<0.2 mm) were present in two animals, one of whom had clinical signs of EAE and the other in whom CSF pleocytosis was present at the time of sacrifice. The spinal cords of all three rolipram-treated animals, studied with multiple sagittal sections encompassing cervical to lumbar levels, were histologically normal. In controls many demyelinating lesions were present, characterized by macrophage infiltration, extensive and sharply demarcated myelin loss, and gliosis, all features that were uniformly absent from lesions of animals in the treatment group.

Brain Levels of TNF-α mRNA. Compared to controls, TNF-α mRNA levels were reduced in two of the treated animals that showed no clinical signs of EAE (Fig. 5). The treated animal (13-89) that developed EAE 17 days after cessation of treatment showed a level of TNF-α mRNA similar to that of the untreated controls.

DISCUSSION

Rolipram completely prevented the appearance of clinical signs of EAE following immunization, and this effect was sustained for the entire duration of treatment. The immunization regimen employed results in moderate signs of EAE, beginning 2–4 weeks after immunization, in 100% of *C. jacchus* (7). Protection from EAE in the treatment group was not due to an effect of the antiemetic odansetron, the carrier dimethyl sulfoxide, repeated handling, or s.c. injections, manipulations that were common to treated and untreated animals. Paraclinical indices confirmed the clinical evidence of a treatment effect. CSF pleocytosis occurred in both control animals but only transiently in a single animal during rolipram treatment. MRI scans were abnormal in controls but normal in the treatment group. These data indicate that rolipram prevented significant disruption of the blood–brain barrier

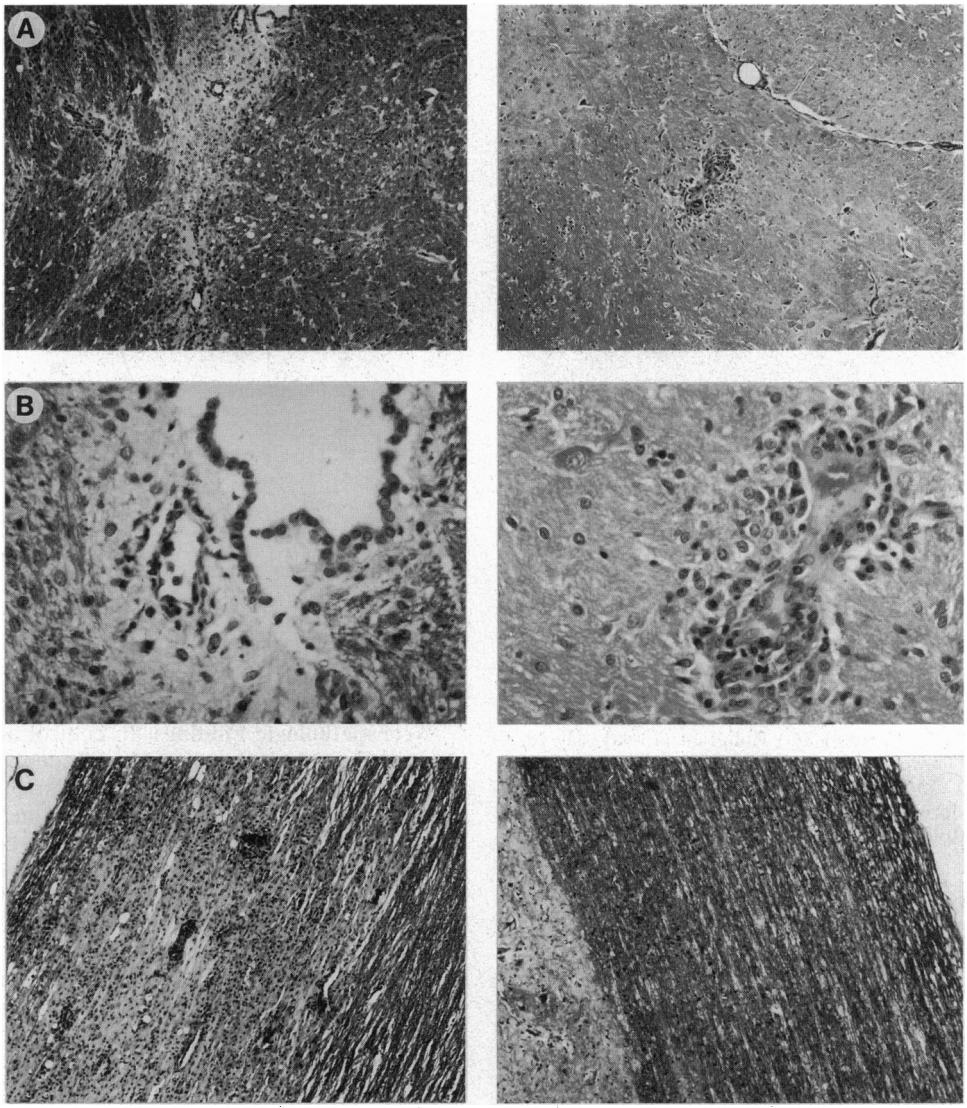

FIG. 4. Neuropathologic findings. Representative pathology in control (*Left*) and rolipram-treated (*Right*) *C. jacchus*. (*A*) Deep periventricular white matter. Note perivascular infiltration and large surrounding area of demyelination in the control (30-92). In the rolipram-treated animal (55-92) perivascular infiltration is present but no surrounding demyelination is seen. [Luxol fast blue/periodic acid Schiff (LFB/PAS); ×24.] (*B*) Higher magnifications of the same lesions, illustrating perivascular infiltration by monocytes and macrophages and gliosis in the control. (LFB/PAS; ×120.) In the rolipram-treated animal, prominent perivascular cuffing is present but gliosis is absent. (Hematoxylin/eosin; ×120.) (*C*) Sagittal sections through the lateral funiculi of the thoracic spinal cord, illustrating a large area of infiltration accompanied by extensive demyelination in the control (30-92) but no abnormality in the rolipram-treated animal (13-89). (LFB/PAS; ×24.)

and CNS invasion by inflammatory cells, a conclusion also supported by histologic examination of the CNS.

Rolipram treatment was begun on day 7 following immunization in order to induce an immune response to CNS tissue in treated animals. Antibody responses specific to MBP, indicating sensitization, developed equally in control and treatment groups. These findings suggest that the protective effect of rolipram resulted from interference with events subsequent to the induction of a primary immune response to CNS tissue. This interpretation is also consistent with earlier observations in rodents indicating that inhibition of TNF-α suppresses adoptively transferred EAE but does not prevent the induction of MBP-reactive T cells in actively immunized animals (21). In *C. jacchus*, antigen-specific T cells appear to persist during rolipram treatment, as termination of the drug was followed by the appearance of clinical signs of EAE in one animal, by CSF pleocytosis in another, and by autopsy findings of EAE in both.

In some rolipram-treated animals CNS inflammatory lesions were present at autopsy, yet demyelination and astrogliosis were uniformly absent from the vicinity of inflammatory infiltrates. Perhaps the absence of demyelination reflects an early stage of lesion development, initiated following withdrawal of rolipram. However, demyelination is a very early event in *C. jacchus* EAE and is present within 1–2 days of the onset of clinical signs (7). In the present study, one animal (612-91) had transient CNS inflammation detected as CSF pleocytosis during rolipram treatment, yet at autopsy no demyelination was present. The pathophysiology of demyelination may be mediated in part by an action of TNF-α on oligodendrocytes (27–30), an effect that may have been modified by rolipram treatment. In the CNS, TNF-α may be produced by multiple cell types, including macrophages, monocytes, astrocytes, and microglial cells (8). Levels of TNF-α mRNA were lower in brain tissue of protected *C. jacchus* than in controls with EAE, corresponding to the absence of macrophage infiltration or gliosis in treated animals. Further studies are necessary to define the effects of rolipram treatment on different CNS cell types in *C. jacchus*.

Compared to inhibitors of the other isoforms of PDE, inhibitors of PDE-IV are more potent in suppressing TNF-α

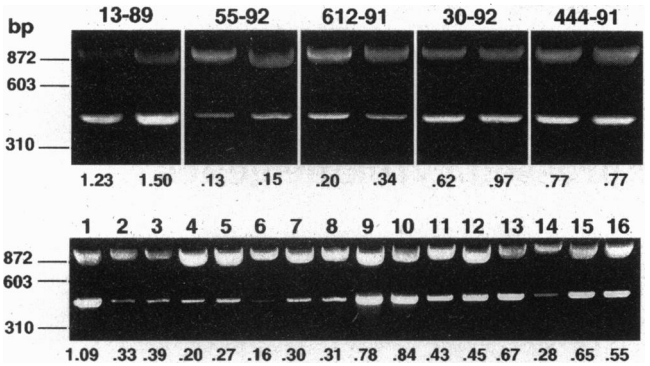

FIG. 5. Brain TNF-α mRNA levels in control and rolipram-treated animals. PCR products for TNF-α (425 bp) and G3PDH (920 bp) used as an internal standard are shown. The ratios of TNF-α/G3PDH band intensity are shown below the gels. (*Upper*) In the two controls (30-92, 444-91), the relative TNF-α product was more abundant than in two of three rolipram-treated animals (55-92, 612-91). In the third rolipram-treated animal sacrificed at the time of acute EAE, a TNF-α band of high intensity was present. (*Lower*) Relative brain TNF-α message level in representative populations of eight normal *C. jacchus* and nine *C. jacchus* with acute EAE. Animals with EAE (lanes 1 and 9–16) consistently have higher levels of TNF-α than unaffected animals (lanes 2–8).

release from mononuclear cells (33, 35–37). For example, in LPS-stimulated human peripheral blood mononuclear cells rolipram has an IC_{50} of 130 nM, >500 times lower than that of the nonspecific inhibitor pentoxifylline (35). The rolipram IC_{50} found for macrophages from *C. jacchus* (12 nM) is lower than that reported in mice but similar to that of humans. Based on dose-finding data in *C. jacchus*, a s.c. dose of 10 mg/kg was employed to test for inhibition of EAE. Side effects of treatment, notably vomiting, were immediate and of short duration (<½ hr). Interestingly, rolipram has been evaluated as an antidepressant in several human trials and the most common side effects were nausea and vomiting (47).

C. jacchus EAE is a unique model for human MS because of the clinical and pathologic similarities between the two conditions. Current data demonstrate that this model can be efficiently employed to evaluate a prospective therapy for MS that may not be testable in non-primate systems. We conclude that inhibition of PDE-IV could have major therapeutic potential, either alone or in combination with other agents that raise intracellular concentrations of cAMP, in the therapy of MS or related disorders.

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1. Waksman, B. H. (1985) *Nature (London)* **318,** 104–105.
2. Allegretta, M., Nicklas, J. A., Srinam, S. & Albertini, R. J. (1990) *Science* **247,** 718–721.
3. Alvord, E. C., Jr., Kies, M. W. & Sucklings, A. J. (1984) *Experimental Allergic Encephalomyelitis: A Useful Model for Multiple Sclerosis* (Liss, New York).
4. Paterson, P. Y. (1960) *J. Exp. Med.* **111,** 119–135.
5. Pettinelli, C. B. & McFarlin, D. E. (1981) *J. Immunol.* **127,** 1420–1423.
6. Raine, C. S. (1984) *Lab. Invest.* **50,** 608–635.
7. Massacesi, L., Genain, C. P., Lee-Parritz, D., Letvin, N. L. & Hauser, S. L. (1995) *Ann. Neurol.*, in press.
8. Benveniste, E. N. (1992) *Am. J. Physiol.* **263,** C1–C16.
9. Brosnan, C. F., Selmaj, K. & Raine, C. S. (1988) *J. Neuroimmunol.* **18,** 87–94.
10. Chung, I. Y., Norris, J. G. & Benveniste, E. N. (1991) *J. Exp. Med.* **173,** 801–811.
11. Kuroda, Y. & Shimamoto, Y. (1991) *J. Neuroimmunol.* **34,** 159–164.
12. Hofman, F. M., Hinton, D. R., Johnson, K. & Merrill, J. E. (1989) *J. Exp. Med.* **170,** 607–612.
13. Hauser, S. L., Doolittle, T. H., Lincoln, R., Brown, R. H. & Dinarello, C. A. (1990) *Neurology* **40,** 1735–1739.
14. Selmaj, K., Raine, C. S., Cannella, B. & Brosnan, C. F. (1991) *J. Clin. Invest.* **87,** 949–954.
15. Beck, J., Rondot, P., Catinot, L., Falcoff, E., Kirchner, H. & Wietzerbin, J. (1988) *Acta Neurol. Scand.* **78,** 318–323.
16. Sharief, M. K. & Hentges, R. (1991) *N. Engl. J. Med.* **325,** 467–472.
17. Powell, M. B., Mitchell, D., Lederman, J., Buckmeier, J., Zamvil, S. S., Graham, M., Ruddle, N. H. & Steiman, L. (1990) *Int. Immunol.* **2,** 539–544.
18. Kuchroo, V. K., Martin, C. A., Greer, J. M., Ju, S.-T., Sobel, R. A. & Dorf, M. A. (1993) *J. Immunol.* **151,** 4371–4382.
19. Ruddle, N. H., Bergman, C. M., McGrath, K. M., Lingenheld, E. G., Grunnet, M. L., Padula, S. J. & Clark, R. B. (1990) *J. Exp. Med.* **172,** 1193–1200.
20. Selmaj, K., Raine, C. S. & Cross, A. H. (1991) *Ann. Neurol.* **30,** 694–700.
21. Monastra, G., Cross, A. H., Bruni, A. & Raine, C. S. (1993) *Neurology* **43,** 153–163.
22. Satambrogio, L., Hochwald, G. M., Saxena, B., Leu, C. H., Martz, J. E., Powell, M. B., Mitchell, D., Lederman, J., Buckmeier, J., Zamvil, S. S., Graham, M., Ruddle, N. H. & Steiman, L. (1990) *Int. Immunol.* **2,** 539–544.
23. Yokota, S., Geppert, T. D. & Lipsky, P. E. (1988) *J. Immunol.* **140,** 531–536.
24. Hughes, C. C. W., Male, D. K. & Lantos, P. L. (1988) *Immunology* **64,** 677–681.
25. Cavender, D. E., Edelbaum, D. & Ziff, M. (1989) *Am. J. Pathol.* **134,** 551–560.
26. McCarron, R. M., Wang, L., Racke, M. K., McFarlin, D. E. & Spatz, M. (1993) *J. Neuroimmunol.* **43,** 23–30.
27. Barten, D. M. & Ruddle, N. H. (1994) *J. Neuroimmunol.* **51,** 123–133.
28. Selmaj, K. & Raine, C. S. (1988) *Ann. Neurol.* **23,** 339–346.
29. Soliven, B., Szuchet, S. & Nelson, D. J. (1991) *J. Membr. Biol.* **124,** 127–137.
30. Zajicek, J. P., Wing, M., Scolding, N. J. & Compston, D. A. S. (1992) *Brain* **115,** 1611–1631.
31. Selmaj, K. W., Farooq, M., Norton, W. T., Raine, C. S. & Brosnan, C. F. (1990) *J. Immunol.* **144,** 129–135.
32. Butt, A. M. & Jenkins, H. G. (1994) *J. Neuroimmunol.* **51,** 27–33.
33. Beavo, J. A. & Reifsnyder, D. H. (1990) *Trends Biochem. Sci.* **11,** 150–155.
34. Molnar-Kimber, K. I., Yonno, L., Hemlip, R. J. & Weichman, B. M. (1992) *Mediators Inflammation* **1,** 411–417.
35. Semmler, J., Wachtel, H. & Endres, S. (1993) *Int. J. Immunopharmacol.* **15,** 409–413.
36. Schade, F. U. & Schudt, C. (1993) *Eur. J. Pharmacol.* **230,** 9–14.
37. Torphy, T. J. & Undem, B. J. (1991) *Thorax* **46,** 512–523.
38. Taffet, S. M., Singhel, K. J., Overholtzer, J. F. & Shurtleff, S. A. (1989) *Cell. Immunol.* **120,** 291–300.
39. Endres, S., Fulle, H.-J., Sinha, B., Stoll, D., Dinarello, C. A., Gerzer, R. & Weber, P. C. (1991) *Immunology* **72,** 56–60.
40. Renz, H., Gong, J. H., Schmidt, A., Nain, M. & Gemsa, D. (1988) *J. Immunol.* **141,** 2388–2393.
41. Han, J., Brown, T. & Beutler, B. (1990) *J. Exp. Med.* **171,** 465–475.
42. Tannenbaum, C. S. & Hamilton, T. A. (1989) *J. Immunol.* **142,** 1274–1280.
43. Eskandari, M. K., Nguyen, D. T., Kunkel, S. L. & Remick, D. G. (1990) *Immunol. Invest.* **19,** 69–79.
44. Connolly, D. T., Knight, M. B., Harakas, N. K., Wittwer, A. J. & Fedre, J. (1986) *Anal. Biochem.* **152,** 136–140.
45. Deibler, G. E., Martenson, R. E. & Kies, M. W. (1972) *Prep. Biochem.* **2,** 139–165.
46. Krause, W. & Kuhne, G. (1992) *J. Chromatogr.* **573,** 303–308.
47. Horowski, R. & Sastre-Y-Hernandez, M. (1985) *Curr. Ther. Res.* **38,** 23–29.