Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 5051–5055, July 1990
Neurobiology

Diphtheria toxin mutant selectively kills cerebellar Purkinje neurons

(ataxia/cerebrospinal fluid/ricin/neurological diseases/immunotoxins)

CHARLES J. RIEDEL*, KARIN M. MURASZKO, AND RICHARD J. YOULE

Biochemistry Section, Surgical Neurology Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institutes of Health, Bethesda, MD 20896

Communicated by A. M. Pappenheimer, Jr., April 12, 1990 (received for review September 26, 1989)

ABSTRACT **CRM107 (crossreacting material 107), a double point mutant of diphtheria toxin that lacks receptor-binding activity, specifically kills cerebellar Purkinje cells *in vivo*. After injection into guinea pig cerebrospinal fluid, CRM107 (0.9 μg) and CRM107–monoclonal antibody conjugates (10 μg) kill up to 90% of the total Purkinje cell population with no detectable toxicity to other neurons. Animals exhibit ataxia, tremor, and abnormalities of posture and tone. Native diphtheria toxin, ricin, and ricin A chain do not cause ataxia and do not reduce the Purkinje cell population after intrathecal injection into guinea pigs at toxic or maximally tolerated doses. However, in rats, which will tolerate higher doses of diphtheria toxin than guinea pigs, Purkinje cells can be killed by both CRM107 and diphtheria toxin. A truncated mutant of diphtheria toxin, called CRM45, can also cause Purkinje cell killing but has additional toxicity not seen with CRM107. Animals treated with intrathecal CRM107 or CRM107 linked to antibodies may serve as models for Purkinje cell loss in a broad spectrum of human diseases and may be used to further study cerebellar physiology. Understanding the basis for the Purkinje cell sensitivity to CRM107 may illuminate other causes of Purkinje cell loss.**

The cell bodies of Purkinje neurons are arranged in a layer, one cell thick, located between the outer molecular layer and the deeper granular layer of the cerebellum. Their axons transmit the only efferent impulses from the cerebellar cortex, relaying predominantly in the deep cerebellar nuclei. Several inherited diseases in humans, such as ataxia telangectasia and certain lysosomal storage disorders, result in extensive loss of Purkinje cells (1). Loss of Purkinje cells is a prominent component of the histopathologic changes observed in cerebellar injury secondary to phenytoin and chronic ethanol intoxication (2–5). Paraneoplastic cerebellar degeneration associated with carcinomas is also characterized by widespread disappearance of Purkinje cells (6). In mice, several inherited diseases exhibit prominent loss of Purkinje cells (7). Why Purkinje cells are selectively lost in many diseases of different etiology is unknown. Clinical signs of cerebellar dysfunction commonly manifested in these conditions include ataxia and tremor.

Diphtheria toxin (DT) and ricin are potent protein toxins comprised of two disulfide-linked subunits (8, 9). The A chain is an enzyme that inhibits protein synthesis, causing cell death. The B chain binds the toxin to the cell surface and facilitates transport of the A chain into the cytosol. Cell-type-specific toxins have been made by linking ricin, ricin A chain, and DT to monoclonal antibodies. Although these antibody–toxin conjugates can be highly potent and cell-type-specific *in vitro*, numerous attempts to selectively eliminate cells *in vivo* have met with only limited success (10–12).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

A mutant of DT, crossreacting material 45 (CRM45), has a termination codon after amino acid 386, resulting in a truncated 45-kDa protein. This mutant was found to have a reduced binding activity, indicating that the C-terminal 17 kDa contained the receptor-binding site (13). However, the mutant retains three of four hydrophobic domains thought to be involved in entry into the cytosol. Another mutant of DT, called CRM107, has amino acid substitutions at positions 390 and 525 in the C-terminal region of the toxin (14). These mutations reduce the *in vitro* binding and toxicity of CRM107 10,000-fold. However, the capacity of the toxin B chain to transport the A chain into the cytosol is preserved (15).

Purkinje neurons can selectively internalize certain macromolecules from the cerebrospinal fluid (CSF) (16–18). Although the physiological significance of this uptake capacity is unknown, it may lead to death of Purkinje cells in certain disease states, such as lipid storage disorders, where the accumulation of macromolecules can be toxic. We have found that binding-deficient mutants of DT are taken up by Purkinje cells and one of these mutants, CRM107, will selectively eliminate this neuronal population.

MATERIALS AND METHODS

Toxins. DT was purchased from List Biological Laboratories (Campbell, CA), and CRM107 was purified on DEAE-cellulose as reported (14). Ricin was purified as described (19), and ricin A chain was purchased from Vector Laboratories. Immunotoxins were synthesized and purified as reported (15).

Intrathecal Injections. Toxins were injected intrathecally into female Hartley guinea pigs (300–500 g) obtained from Charles River Breeding Laboratories and anesthetized with ketamine and xylazine (30 mg/kg and 2 mg/kg, respectively). Toxin samples in 0.1 ml of phosphate-buffered saline containing 0.2% bovine serum albumin were injected into the cisterna magna after confirming needle position by the free outflow of CSF. To compare different toxins with various molecular weights, the amount of toxin is frequently expressed in molarity, defined as the initial toxin concentration assuming even distribution of the injectate in the guinea pig CSF volume of 0.5 ml. Animals were observed daily for up to 2 months for behavioral changes and were treated according to the National Institutes of Health guidelines. At various time points, animals were anesthetized and perfused with 10% neutral buffered formalin fixative. The whole brains were removed, embedded in paraffin, step sectioned, and stained with hematoxylin/eosin. Representative sections

Abbreviations: DT, diphtheria toxin; CRM, crossreacting material; CSF, cerebrospinal fluid; EDN, eosinophil-derived neurotoxin; ECP, eosinophil cationic protein.

*Present address: Department of Neurosurgery at the New York Neurological Institute, Columbia University, New York, NY 10032.

were also examined histologically with myelin and silver stains. Maximal tolerated dose is defined as the highest dose examined that did not kill any animals in the cohort.

Neuron Quantitation. Three sections from the cerebellum of each animal were counted unblinded, one each from the midline, 1 mm lateral, and 2 mm lateral. Purkinje cell nuclei were counted at ×640, and the cell layer length was measured from ×10 photographs of microscope sections by using a digitizing tablet and morphometric computer software. Values are expressed as Purkinje cells (mean ± SD) per millimeter. Granule cell density (cells per mm^2; mean ± SD) was measured under the microscope with a counting reticule, and the granule cell area was determined from ×10 photomicrographs with the morphometric software. Data were analyzed by using the SAS system for multivariate repeated measures analysis of variance (SAS Institute, Cary, NJ). In addition, Purkinje cells were counted by a modification of the procedure of Herrup (20).

RESULTS

The dose–survival curve for intrathecally injected DT is shown in Fig. 1. DT injected at maximally tolerated doses or even lethal doses did not cause any detectable cerebellar deficits in the guinea pigs. These animals grew ill over 48 hr, displaying paralysis of hind and forelimbs. There was no evidence of ataxia or gait abnormalities preceding death. Histopathological examination showed that DT had no effect on the number of Purkinje cells or granule cells at maximally tolerated doses (Fig. 2). Apparently DT kills other populations of cells, neurons and oligodendrocytes, as reported previously (21–23), that are critical for animal survival, at concentrations insufficient to kill Purkinje cells.

We then tested a point mutant of DT, CRM107, that lacks receptor-binding activity and may selectively kill cells with a high endocytosis rate. CRM107 could be safely administered at a dose 300-fold higher than the amount of native DT that produced systemic toxicity and animal death (Fig. 1). Animals treated with 930 ng of CRM107 (2×10^{-8} M initial CSF concentration) developed a characteristic behavioral abnormality between 3 and 26 days (mean time to ataxia, 9 days) after injection. Consistent behavioral changes were noted, including ataxic gait, tremor, and postural changes. An increase in muscle tone also occurred and was most pronounced in the hind limbs. Unlike classic diphtheritic neuropathy in humans and animals, CRM107-treated animals did not display paralysis or sensory loss. Animals responded appropriately to light touch and noxious stimulus. This clinical picture is more consistent with a cerebellar abnormality than with a peripheral neuropathy. The mean time of onset of ataxia is shown in Table 1. CRM107 injected intraperitoneally into guinea pigs did not cause any detectable neurological effects, neither cerebellar deficits nor peripheral paralysis at maximally tolerated doses.

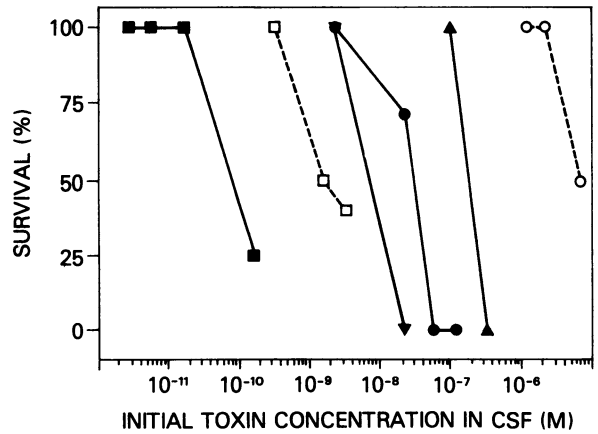

FIG. 1. Toxicity of intrathecally injected toxins. ●, CRM107; ▲, CRM107–antibody conjugates; ■, DT; □, ricin; ▼, CRM45; ○, ricin A chain. Concentrations are expressed in molarity of toxin moiety in the CSF immediately after injection assuming an even distribution in a guinea pig CSF volume of 0.5 ml. Cohort size for each dose ranged from three to seven animals except for the highest dose of CRM107–antibody conjugate, where two animals were used.

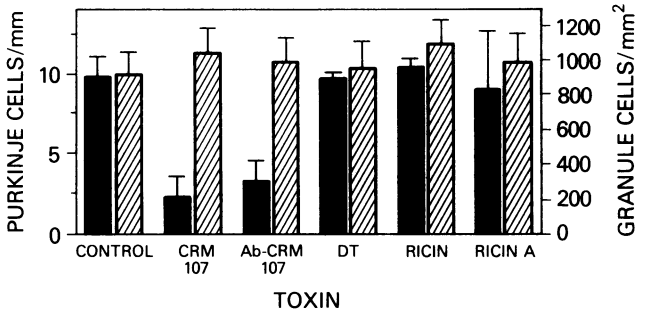

FIG. 2. Quantification of Purkinje cells and granule cells after treatment with protein toxins. Solid bars represent Purkinje cells, and hatched bars represent granule cells. CRM107- and CRM107–antibody (Ab) conjugate-treated animals had Purkinje cell numbers significantly ($P < 0.003$) different from the controls and all the other treatment groups but not significantly different from each other. However, the granule cell numbers of the CRM107- and CRM107–antibody conjugate-treated animals were not significantly different from any of the other groups. No statistically significant differences in Purkinje cell number or granule cell number were found among the animals treated with ricin, ricin A chain, or DT and untreated control animals. Animals received toxins to yield initial CSF concentrations of 2.2×10^{-8} M (CRM107), 1.1×10^{-7} M (CRM107–antibody conjugate), 1.6×10^{-11} M (DT), 3.2×10^{-9} M (ricin), and $2.2–6.7 \times 10^{-6}$ M (ricin A chain). Animals were sacrificed from 5 to 100 days after toxin injection.

Linking CRM107 to various antibodies reduces toxicity *in vitro* (15) and *in vivo* (Fig. 1). CRM107–antibody conjugates could be safely administered intrathecally at a dose 5 times higher than that of CRM107 (Fig. 1). Two different monoclonal antibodies were used, 454A12 and OKT4 (14, 24). The 454A12 monoclonal antibody, an IgG1, binds the human transferrin receptor, and OKT4, an IgG2, binds the human CD4 antigen on human T cells. Neither antibody is known to bind any guinea pig antigens, and OKT4 has been reported not to bind to Purkinje cells (24). Animals receiving CRM107 linked to either antibody developed a motor syndrome identical to animals treated with CRM107 alone. A more reproducible time of onset of ataxia was seen with the CRM107–antibody conjugate than with CRM107. At the dose of 10 μg per animal (10^{-7} M initial CSF concentration), ataxia always

Table 1. Time of onset of neurological disorders of guinea pigs injected intrathecally with toxins

Toxin injected	Dose,* M	Animals per cohort	Mean day of onset of ataxia
DT	2×10^{-11}	4	No ataxia
Ricin	2×10^{-9}	4	No ataxia
CRM107	2×10^{-9}	3	Day 19
	2×10^{-8}	7	Day 9
CRM107–antibody conjugate	1.1×10^{-7}	7	Day 6
	3.3×10^{-7}	2	Day 3
CRM45	2×10^{-8}	4	Day 3†
Ricin A chain	2×10^{-6}	3	No ataxia

*The dose represents the initial CSF concentration assuming even distribution of the toxin in a guinea pig CSF volume of 0.5 ml.
†Ataxia developed in only three of the four animals. In addition to ataxia, two of the four animals developed forelimb paralysis.

developed by the seventh day after injection (Table 1). In addition to the reproducible time of onset, this dose never caused animal death in contrast to a CRM107 concentration of 2×10^{-8} M, which killed 29% of the animals (Fig. 1). The neurological deficits caused by CRM107 and the CRM107–antibody conjugate persisted for up to 6 months or more in severely affected animals. Considerable improvement in coordination often occurred within weeks in moderately afflicted animals.

Histopathological examination of the brains of animals treated with CRM107 alone (data not shown) or conjugated to antibody (Fig. 3) revealed massive loss of Purkinje neurons from the cerebellar cortex. Four other groups of neurons present within the cerebellar cortex appeared unaffected in number and morphology: stellate and basket cells within the molecular layer and granule and Golgi cells occupying the granular layer (Figs. 2 and 3; data not shown). The dentate nucleus was histologically normal. On occasion, vacuolization in cerebellar white matter was seen, but this could not be reliably discriminated from fixation artifacts. Glial abnormalities were limited to proliferation of Bergmann glia adjacent to areas of Purkinje cell loss. This type of reaction is known to accompany Purkinje cell loss in other disease processes. Neither CRM107 nor CRM107–antibody conjugates had any detectable effect on the histology of the cerebrum, brain stem, spinal cord, choroid plexus, or meninges.

We quantitated the effects of these toxins on both Purkinje cells and granule cells. CRM107 at 2×10^{-8} M eliminates an average of 77%, and in some animals 90%, of the total cerebellar Purkinje cells (Fig. 2). The CRM107–antibody conjugate at 1.1×10^{-7} M eliminates an average of 67% of the total Purkinje cells, which is not statistically different from the results with CRM107 alone. Quantitation of the total number of cerebellar Purkinje cells performed on two animals by a different procedure (20) showed a 78% reduction in Purkinje cells with 1.1×10^{-7} M CRM107–antibody conjugate. In contrast, the number of granule cells in the animals treated with CRM107 or CRM107–antibody conjugates was not significantly different than the number in untreated animals (Fig. 2).

Regional differences in the extent of Purkinje cell depletion were observed. Superficial folia, adjacent to the cisterna magna and more exposed to the cerebrospinal fluid, often showed nearly complete depletion (Fig. 3*b*). Deep within folds, Purkinje cell loss was less pronounced. Similar distribution has been described for the uptake of fluorescent dyes (16). This regional difference in the extent of Purkinje cell depletion is consistent with the model that access of the Purkinje cell layer to CSF increases susceptibility to the toxins.

CRM45, like CRM107, lacks the receptor-binding activity of DT and also lacks other regions of the B chain, such as the cationic region and the fourth hydrophobic domain that are retained in CRM107 (13, 25). CRM45 injected intrathecally into guinea pigs caused marked neurological deficits distinct from CRM107. Injection of 0.5 μg (an initial CSF concentration of 2×10^{-8} M) of CRM45 intrathecally into guinea pigs initially caused ataxia and tremor similar to CRM107. Half of the animals subsequently developed a flaccid paralysis of the forelimbs (Table 1). Five microgram doses of CRM45 rou-

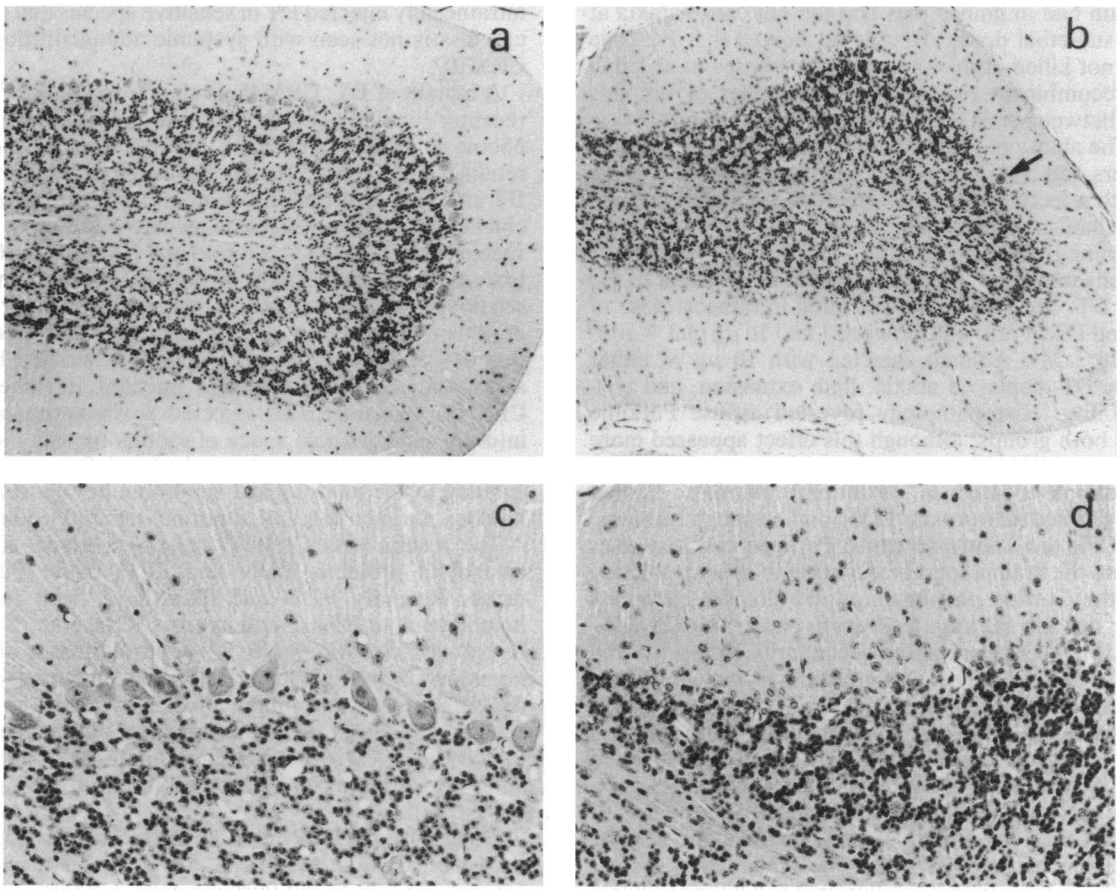

FIG. 3. Photomicrography of cerebellar folia from a control animal and from an animal treated with a CRM107–antibody conjugate. Low (×81) (*a*) and high (×164) (*c*) magnification of a control animal injected intrathecally with 66 μg (initial concentration in the CSF of 5.5×10^{-7} M) of CRM107–antibody conjugate that was pretreated with anti-DT globulin, which neutralizes the *in vitro* cytotoxicity of the conjugate. Low (*b*) and high (*d*) magnification of an animal injected intrathecally with 13 μg of CRM107–antibody conjugate (initial concentration in the CSF of 1.1×10^{-7} M). The arrow in *b* shows the sole remaining Purkinje cell in this section of the folia. Animals were sacrificed 35 days after toxin injection.

tinely resulted in flaccid paralysis in guinea pigs and in rats. On a molar basis, CRM45 is slightly more toxic than CRM107 when injected intrathecally into guinea pigs (Fig. 1). Histological examination revealed extensive loss of Purkinje cells in the cerebellum but also vacuolization of the molecular and granular layers and regional cell loss within the granular layer. In addition to the cerebellar effects, the dorsal aspect of the spinal cord was vacuolated in the CRM45-treated animals. This spinal cord effect may explain the paralysis seen in these animals. CRM45 is apparently taken up by Purkinje cells as is CRM107, indicating that the C-terminal 17-kDa region present in CRM107 is not required either for the recognition of Purkinje cells or for the entry of the toxin into the cell. Hydrophobic domains exposed in CRM45 (26) and presumably not exposed in CRM107 may mediate adhesion of CRM45 to other cells in the central nervous system, causing the cell death not found with CRM107.

We examined whether other protein toxins, ricin and ricin A chain, would selectively kill Purkinje cells. After injection of ricin into the guinea pig CSF, systemic toxicity with alopecia, weight loss, and animal death occurred at concentrations that caused no loss of Purkinje cells (Figs. 1 and 3). Ricin A chain, like CRM107, lacks receptor-binding activity and was tolerated by animals at a dose almost 10,000 times higher than that of native ricin (Fig. 1). Interestingly, Purkinje cells remained unaffected at maximally tolerated doses of ricin A chain, despite a concentration 100 times higher than that of CRM107, which killed 77% of the Purkinje cells (Fig. 3). We also examined the toxicity of recombinant ricin A chain after intrathecal injection into rats (27). Recombinant ricin A chain had a similar level of toxicity in rats as native ricin A chain had in guinea pigs and did not cause ataxia at lethal and sublethal doses. Histology showed that Purkinje cells were not killed at maximal nonlethal doses or at lethal doses of recombinant ricin A chain. Whether or not this difference between ricin A chain and the two DT mutants is related to the ability of CRM107 and CRM45 to interact with lipid bilayers and translocate the enzymatic A chain into the cytosol or to selective properties inherent to the Purkinje cell uptake mechanism remains unknown.

We examined whether CRM107 and DT would deplete Purkinje cells in rats, which are known to be resistant to the toxicity of DT. The maximally tolerated intrathecal dose of CRM107 and DT in rats was between 1 and 10 μg (3.3×10^{-8} to 3.3×10^{-7} M). Animals injected with 10 μg of either CRM107 or DT displayed ataxia, limb extension, and postural instability. Histopathology revealed diffuse Purkinje cell loss in both groups, although this effect appeared more pronounced in CRM107-treated rats.

Two proteins located in eosinophil granules, named eosinophil-derived neurotoxin (EDN) and eosinophil cationic protein (ECP), are known to cause Purkinje cell loss after injection into the brain as part of a syndrome of neurotoxicity known as the Gordon phenomenon (28, 29). We examined whether or not the Purkinje cell death caused by CRM107 could be explained as occurring secondarily to a provoked eosinophilia. CSF taken from several animals that developed Purkinje cell loss showed only occasional lymphocytes and no eosinophilia. No animals showed any infiltration of eosinophils into brain tissue. In addition, anti-DT globulin injected into the CSF along with CRM107–antibody conjugates completely blocked the behavioral abnormalities and the loss of Purkinje cells (Fig. 3 *a* and *c*). We see no evidence that the Purkinje cell loss reported here could be explained by eosinophilia and show directly that the Purkinje cell killing requires DT activity.

DISCUSSION

DT and ricin have been used previously to selectively kill neuron populations after injection into peripheral nerves (30, 31). The toxin is routed to the corresponding cell bodies by retrograde axonal transport, where the toxin kills the neuron. We have found that intrathecal injection of a DT mutant will specifically eliminate most cerebellar Purkinje cells. In contrast to the previous work on intraneural injection, the Purkinje cells are likely taking up the toxins by means of dendrites in the molecular layer that project toward the CSF. Regions of the Purkinje cell layer more exposed to the CSF are more sensitive to CRM107. Purkinje neurons can selectively extract other macromolecules from the CSF, including lectins and antibodies (16–18). The mechanism of uptake and the basis for selectivity are poorly understood, but blocking studies indicate that it is an active process dependent on microtubule function (16).

Systemic delivery of DT can cause sensory and motor neuropathy in humans and in animals (32). The neuropathy results from a classic segmental demyelination of nerve fibers, leaving the axon intact (33). Consistent with these *in vivo* observations, Schwann cells and oligodendrocytes are found to be exceptionally sensitive to DT *in vitro* (21, 22). However, the central nervous system appears normal in diphtheritic neuropathy, apparently protected from the toxin by the blood–brain barrier (23). CRM107 and CRM107 conjugates injected into the CSF fluid did not appear to cause a peripheral neuropathy because both sensory and motor function were preserved. The neurologic symptoms produced by intrathecal CRM107 were most compatible with a cerebellar syndrome and consistent with the histologic findings of Purkinje cell loss. The Purkinje cell toxicity seen with CRM107 is unlikely to play a role in human diphtheritic neuropathy because (*i*) Purkinje cell loss is not seen with intrathecally injected DT in sensitive species and (*ii*) Purkinje cell loss is not seen with systemic administration of DT or CRM107.

A mutant of DT, CRM45, is similar to CRM107 in that the receptor-binding activity is absent and three of four hydrophobic domains thought to be involved in cytoplasm entry are retained (13). CRM45 is >20- to 40-fold less toxic than native DT after intracerebral injection into a sensitive species, the chicken, and equally as toxic as native DT in an insensitive species (21). CRM45 is 200-fold and CRM107 is 400-fold less toxic than native DT after intrathecal injection into another sensitive species, the guinea pig. Pappenheimer *et al.* (21) note that the type of paralysis seen with intracerebral injection of CRM45 was variable. Similarly we find both ataxia and paralysis in guinea pigs injected intrathecally with CRM45. Waksman (23) injected toxin–antitoxin mixtures into the subarachnoid space of rabbits by using an injection method similar to ours. A cerebellar deficit may have contributed to the neurological syndrome he reports; however, he does not mention any abnormalities in Purkinje cells.

The results with CRM107 suggest a model for how the eosinophil proteins, EDN and ECP, cause Purkinje cell death. Recently EDN and ECP have been found to be homologous to RNase and express RNase activity (34, 35). Rybak and coworkers (36, 37) reported that a ribonuclease homologous to ECP and EDN will enzymatically cleave ribosomal RNA and inhibit protein synthesis in a cell-free system. In analogy to what is known about the mechanism of toxicity of CRM107, we suggest that EDN and ECP may enter the Purkinje cell cytosol, inhibit protein synthesis, and kill the cell.

How EDN and ECP enter the Purkinje cell cytosol remains unexplained. Ricin A chain has a low capacity to enter the cell cytosol and fails to kill Purkinje cells. CRM107 and CRM45, however, express an activity that allow them to enter the cell cytosol, and they kill Purkinje cells. We speculate that the membrane translocation activity found in CRM45 and CRM107 and absent in ricin A chain may be important for Purkinje cell entry and that a corresponding activity in EDN

and ECP is required for Purkinje cell killing. In this regard, ECP has been recently reported to form pores in membranes (38).

Among neurons intrinsic to the central nervous system, Purkinje cells exhibit the greatest uptake of certain CSF-borne macromolecules. This exceptional uptake activity possibly mediates the cell-type-specific toxicity of intrathecally injected CRM107. How and why Purkinje cells selectively extract molecules from the CSF and what endogenous substances they normally take up remain unanswered questions. One intriguing possibility is that this uptake may serve as a common mechanism for Purkinje cell death in a number of diseases of different etiology.

Note Added in Proof. A recent paper (39) reports Purkinje cell loss in rats injected intraventricularly with an anti-neuronal monoclonal antibody, anti-Thy 1.1, coupled to the plant toxin saporin.

We thank Drs. Virginia Johnson and Susanna Rybak for thoughtful criticism of the manuscript and Dr. Pappenheimer for scientific discussions and for supplying CRM45. We are grateful to Drs. Raffi Fass and Jossi Shiloach for supplying CRM107, Cetus for supplying 454A12 and recombinant ricin A chain, and Dr. David Katz for consultation on neuropathology. We thank Don McMahon and Jeffery Benedict for assistance with statistical analysis and Stuart Walbridge for technical help. We also thank the referees for their thoughtful suggestions concerning the manuscript.

1. Gilman, S., Bloedel, J. R. & Lechtenberg, R. (1981) *Disorders of the Cerebellum* (Davis, Philadelphia).
2. Ghatak, N. R., Santoso, R. A. & McKinney, W. M. (1976) *Neurology* **26,** 818–820.
3. Kokenge, R., Kutt, H. & McDowell, F. (1965) *Neurology* **15,** 823–829.
4. Victor, M., Adams, R. D. & Mancall, E. L. (1959) *Arch. Neurol.* **1,** 579–588.
5. Pentney, R. J. (1982) *Brain Res.* **249,** 397–401.
6. Anderson, N. E., Cunningham, J. M. & Posner, J. B. (1987) *CRC Crit. Rev. Neurobiol.* **3,** 245–299.
7. Sidman, R. L. (1987) in *Encyclopedia of Neuroscience*, ed. Adelman, G. (Birkhaeuser, Boston), Vol. 2, pp. 828–832.
8. Pappenheimer, A. M., Jr. (1977) *Annu. Rev. Biochem.* **46,** 69–94.
9. Olsnes, S. & Pihl, A. (1982) in *Molecular Action of Lectins and Viruses*, eds. Cohen, P. & van Heynigen, S. (Elsevier/North-Holland Biomed., Amsterdam), pp. 51–83.
10. Vitetta, E. S., Fulton, R. J., May, R. D., Till, M. & Uhr, J. W. (1987) *Science* **238,** 1098–1104.
11. Pastan, I., Willingham, M. C. & Fitzgerald, D. J. P. (1986) *Cell* **47,** 641–648.
12. Youle, R. J. & Colombatti, M. (1986) *Monoclonal Antibodies and Cancer*, ed. Roth, J. (Futura, Mount Kisco, NY), pp. 173–213.
13. Uchida, T., Pappenheimer, A. & Greany, R. (1973) *J. Biol. Chem.* **248,** 3838–3844.
14. Greenfield, L., Johnson, V. G. & Youle, R. J. (1987) *Science* **238,** 536–539.
15. Johnson, V. G., Wilson, D., Greenfield, L. & Youle, R. J. (1988) *J. Biol. Chem.* **263,** 1295–1300.
16. Borges, L. F., Elliot, P. J., Gill, R., Iversen, S. D. & Iverson, L. L. (1985) *Science* **228,** 346–348.
17. Borges, L. F. & Busis, N. A. (1985) *Arch. Neurol.* **42,** 690–694.
18. Karpiak, S. E. & Mahadik, S. P. (1987) *Exp. Neurol.* **98,** 453–457.
19. Youle, R. J. & Neville, D. M., Jr. (1982) *J. Biol. Chem.* **257,** 1598–1601.
20. Herrup, K. (1986) *Dev. Biol.* **115,** 148–154.
21. Pappenheimer, A. M., Jr., Harper, A. A., Moynihan, M. & Brockes, J. P. (1981) *J. Infect. Dis.* **145,** 94–102.
22. Murray, K. & Nobel, M. (1985) *J. Neurol. Sci.* **70,** 283–293.
23. Waksman, B. H. (1961) *J. Neuropathol. Exp. Neurol.* **20,** 31–77.
24. Garson, J. A., Beverley, P. C. L., Coakham, H. B. & Harper, E. I. (1982) *Nature (London)* **298,** 375–377.
25. Johnson, V. G. & Youle, R. J. (1990) in *Intracellular Trafficking of Proteins*, eds. Steer, C. J. & Hanover, J. A. (Cambridge Univ. Press, Cambridge, U.K.), in press.
26. Boquet, P., Silverman, M., Pappenheimer, A. & Vernon, W. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 4449–4453.
27. Piatak, M., Lane, J. A., Laird, W., Bjorn, M. J., Wang, A. & Williams, M. (1988) *J. Biol. Chem.* **263,** 4837–4843.
28. Turner, H., Jackson, H. & Parker, F. (1938) *Am. J. Med. Sci.* **195,** 27–32.
29. Durack, D. T., Sumi, M. & Klebanoff, S. J. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 1443–1447.
30. Wiley, R. G., Blessing, W. W. & Reis, D. J. (1982) *Science* **216,** 889–890.
31. Jacobs, J. M., Cavanaugh, J. B. & Mellick, R. S. (1966) *Br. J. Exp. Pathol.* **47,** 507–517.
32. Fisher, C. M. & Adams, R. D. (1956) *J. Neuropathol. Exp. Neurol.* **15,** 243–268.
33. Webster, H. deF., Spiro, D., Waksman, B. & Adams, R. D. (1961) *J. Neuropathol. Exp. Neurol.* **20,** 5–34.
34. Gleich, G. J., Loegering, D. A., Bell, M. P., Checkel, J. T., Ackerman, S. J. & McKean, D. J. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 3146–3150.
35. Silfman, N. R., Loegering, D. A., McKean, D. J. & Gleich, G. J. (1986) *J. Immunol.* **137,** 2913–2917.
36. Rybak, S. M. & Vallee, B. L. (1988) *Biochemistry* **27,** 2288–2294.
37. St. Clair, D. K., Rybak, S. M., Riordan, J. F. & Vallee, B. L. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 8330–8334.
38. Young, J. D., Peterson, C. G. B., Venge, P. & Cohn, Z. A. (1986) *Nature (London)* **321,** 613–616.
39. Davis, T. L. & Wiley, R. G. (1989) *Brain Res.* **504,** 216–222.