posits, L. B. Ebert, Ed. (Plenum, New York, 1985), pp. 303–376.
16. L. M. Stock and R. S. Willis, *J. Org. Chem.* **50**, 3566 (1985).
17. D. E. Bergbreiter and J. M. Killough, *J. Am. Chem. Soc.* **100**, 2126 (1978).
18. In this analysis of a reductive methylation involving ^{13}C-enriched methyl groups, I am ignoring that in a reductive alkylation the carbon atom attached to the added methyl group goes from sp^2 to sp^3 hybridization. In the case of reductively methylated perylene, these rehybridized atoms appear at 38.6 and 41.7 δ in ^{13}C NMR (*12*). To address this neglect, I performed an additional experiment in which methyl iodide of normal isotopic abundance was used in the alkylation step. CP/MAS NMR at 50 MHz of the resultant filtered solid showed an sp^2-hybridized carbon content of 81%. Although the regions of CH_3-O and rehybridized aromatic carbon (RAC) did not show resolved peaks, a partitioning of the sp^3 region of 1 CH_3-O to 4.4 RAC to 4.4 CH_3-C would suggest that 55% (= 5.4/9.8) of the sp^3 region arises from added methyl groups. This would indicate a level of methylation higher than that found in the first experiment with ^{13}C-enriched methyl groups and again consistent with the idea that soot behaves more as molecules than as graphite.
19. N. A. Sethi, R. J. Pugmire, J. C. Facelli, D. M. Grant, *Anal. Chem.* **60**, 1574 (1988).
20. J. W. Childers and R. A. Palmer, *Am. Lab.* **17**, 22 (March 1986).
21. R. Schlogl, G. Indlekofer, P. Oelhafen, *Angew. Chem. Int. Ed. Engl.* **26**, 309 (1987).
22. W. C. Herndon, in *Polynuclear Aromatic Compounds*, L. B. Ebert, Ed. (American Chemical Society, Washington, DC, 1988), pp. 1–12.
23. I thank C. Clausen for the gift of the soot, K. Rose and C. Pictroski for technical assistance with the NMR, and J. Scanlon, L. Gebhard, and J. Kelliher for other technical assistance.

17 October 1989; accepted 10 January 1990

Thymic Epithelium Tolerizes for Histocompatibility Antigens

JOSSELYNE SALAÜN, ANTONIO BANDEIRA, IBRAHIM KHAZAAL, FRANÇOISE CALMAN, MONIQUE COLTEY, ANTONIO COUTINHO, NICOLE M. LE DOUARIN

The role of thymic epithelium in the establishment of tissue tolerance was analyzed with a murine chimeric system. All T cells differentiated from birth onward in a thymus comprising allogeneic epithelium and syngeneic hematopoietic cells. Embryonic thymic rudiments that contained no hematopoietic cells from C3H (H-2^k) donors were grafted to newborn athymic (nude) BALB/c (H-2^d) mice. Chimeras that had normal T cell numbers and function rejected third-party skin grafts, but permanently accepted grafts syngeneic to the thymic epithelium. In vitro functional assays did not always correlate with the state of tolerance in vivo. Thus, pure thymic epithelium induces tolerance to histocompatibility antigens.

THE THYMUS HAS A KEY ROLE IN THE development of the immune system. Intrathymic differentiation and selection of T lymphocytes seem to result in the establishment of self major histocompatibility complex (MHC) restriction and in tolerance to self antigens. Two types of thymic stroma cells, endodermally derived epithelial cells and accessory cells of hematopoietic origin, are implicated in these processes and are attributed different functions. Tolerance and clonal deletion (negative selection) of autoreactive lymphocytes are thought to be mediated by the hematopoietically derived dendritic cells of the medulla, whereas selection leading to MHC-restriction (positive selection) may be mediated by contact of the differentiating lymphocytes with epithelial cells (EC) (*1*).

Previous studies aimed at inducing allotolerance were carried out in the adult mouse by grafting fetal thymuses depleted of hemapoietic cells (HC) either by deoxyguanosine treatment or by culture at low temperature; the results were controversial. Deoxyguanosine-treated thymuses, although expressing donor MHC antigens, were not rejected when implanted into normal or nude mice, and yet did not induce allotolerance [as tested in mixed lymphocyte reaction (MLR) and cytolytic activities (*2*)]. However, T cells from deoxyguanosine-treated thymus grafts showed H-2–restricted specificity for antigen (*1*). It has also been found that thymic grafts depleted of HC can tolerize precursors of cytolytic T cells, but only for the minor histocompatibility antigens carried by the thymic epithelial stroma (*3*). In contrast, thymuses cultured at low temperature before grafting into an athymic nude mouse induced tolerance in both intrathymic and spleen cells, as determined by MLR tests (*4*). In none of these experiments was tolerance assessed through grafts of tissues other than the thymus itself, particularly since deoxyguanosine-treated thymus is not rejected even by immunocompetent allogeneic hosts (*2*).

In the experimental protocols above, it was impossible to ascertain that all the HC-derived cells (lymphocytes and medullary dendritic cells) of the thymus were completely eliminated. Moreover, T cells developing in those chimeras arose into the immunologically mature, though deficient, environment of an adult animal. We, therefore, investigated the roles of the endodermal epithelium and the HC-derived dendritic cells in tolerance induction by grafting the early embryonic thymic epitheliomesenchymal rudiment before its invasion by HC. If such an early allogeneic thymic anlage is introduced into a nude mouse at birth, all the thymic-dependent T cells that develop in the animal would have differentiated by contact with the MHC alloantigens of the donor thymus at nearly physiologic developmental stages. Their ability to recognize these alloantigens as self could then be tested in vivo with skin grafts or with in vitro assays.

We studied thymus development in birds by the construction of quail and chick chimeras. A chick that receives a xenogeneic (quail) graft of either a limb or a bursa of Fabricius at embryonic day 4 (E4) acutely rejects it after birth, when its immune system is mature (*5*). This could be prevented, however, if the epithelial thymic rudiment from the donor was also implanted; thus, thymic EC can present self antigens to the differentiating T cells in a tolerogenic manner. In the mouse thymus, development proceeds from the endoderm of the third branchial pouch, which starts to grow and is colonized by HC from E11 onward. At E10 the presumptive thymic rudiment is devoid of HC and is unable to develop into a lymphoid thymus if cultured in the absence of a source of HC (*6*). Neither the lymphoid nor the macrophage or dendritic cells labeled with appropriate Thy-1 and MHC class II markers, respectively, were ever found to be of donor type (*7*). We have, therefore, investigated whether the thymic epithelial component has the same effect on the induction of tolerance to tissue grafts in the mouse as it has in birds (*8*). Fully allogeneic thymic epithelium reconstituted the T cell compartment of athymic nude mice and induced tolerance to skin grafts of the donor strain. Therefore, thymic EC in the absence of MHC-matched thymic HC ensured the state of tolerance.

Between 1 and 13 days after birth we grafted 10 to 15 third branchial arch regions

J. Salaün, I. Khazaal, F. Calman, M. Coltey, N. M. Le Douarin, Institut d'Embryologie cellulaire et moléculaire du CNRS et du Collège de France, 49bis, Avenue de la Belle-Gabrielle, 94736 Nogent-sur-Marne Cédex, France.
A. Bandeira and A. Coutinho, Unité d'Immunobiologie, Institut Pasteur, URA 359, Paris, France.

from E10 C3H (H-2^k) embryos into BALB/c (H-2^d) nude mice (n = 84). At this stage (25 to 34 somites), the thymic epithelium is not yet colonized by HC (*6*). Sixty-seven of these mice died before the age of 5 months, a death rate consistent with unmanipulated nude mice in our colonies. Seventeen animals (20%) survived and were found to have T cell function, a percentage similar to that obtained with syngeneic E10 thymic grafts (*8*). Fifteen of the 17 animals, all in good health at 5 months of age, received tail skin grafts from host (BALB/c), donor (C3H), and third-party (C57BL/6) mice. The C57BL/6 skin was rejected in 9 to 30 days, whereas these grafts were accepted in control nude mice that had no thymic transplant. Syngeneic (BALB/c) and thymic epithelium donor (C3H) skin grafts were accepted in every case (Fig. 1). Thus, when the T cell compartment was reconstituted through C3H thymic epithelial grafts, C3H skin grafts were tolerated, whereas C3H and C57BL/6 skin grafts were rejected in pseudochimeras (BALB/c thymic rudiments grafted into BALB/c nude mice) (*8*). Although most of the experiments have involved C3H thymic grafts, C57BL/6 (E10) branchial pouches were implanted on BALB/c nude mice. Ten of 30 chimeric mice had a reconstituted T cell compartment and accepted C57BL/6 skin grafts but rejected C3H skin grafts after 13 to 20 days.

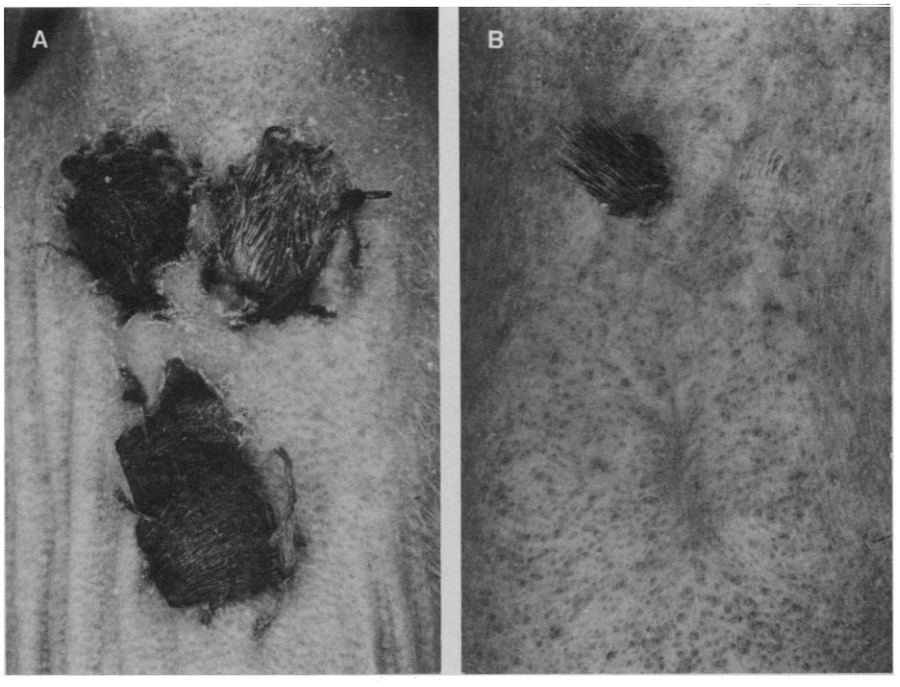

Fig. 1. Tolerance to allogeneic skin grafts induced by thymic epithelium. BALB/c nude mice that had received C3H thymic epithelium transplants at birth were tested for their ability to reject skin grafts at 5 to 6 months of age. Skin grafts were performed as described (*8*). The three tail skin grafts were from BALB/c (recipient haplotype), C3H (donor thymic epithelium haplotype), and third-party (C57BL/6) mice. Skin grafts were considered as accepted when they were healthy for at least 3 months. Controls included BALB/c nude mice that were untreated (no grafts rejected) or reconstituted with syngeneic thymic epithelium (both allogeneic grafts rejected). (**A**) Skin grafts 12 hours and (**B**) 3 months after implantation. Only C3H (left) and BALB/c (right) grafts are tolerated; the third-party graft, C57BL/6, (bottom) was rapidly rejected.

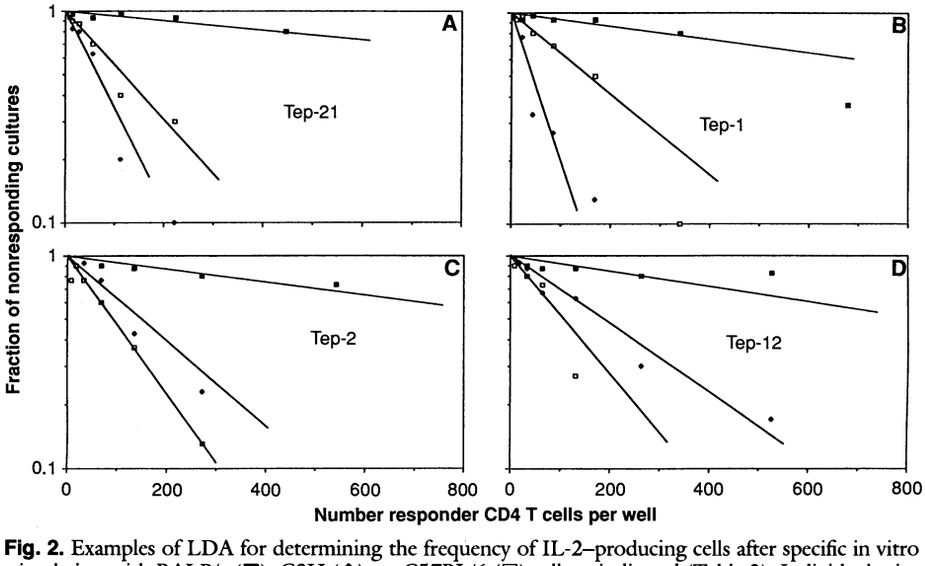

Fig. 2. Examples of LDA for determining the frequency of IL-2–producing cells after specific in vitro stimulation with BALB/c (■), C3H (◆), or C57BL/6 (□) cells as indicated (Table 2). Individual mice are shown in each panel (Tables 1 and 2 and Fig. 3). Splenic responder cells were analyzed for the expression of CD4, and this value was used to determine the number of specifically stimulated cells in each well (*9*).

Thirteen BALB/c nude mice that had C3H thymic epithelium grafts were killed 3 to 5 months after receiving the skin graft. The mice were 9 to 12 months old and all were in good health. Thymic grafts from beneath the skin in the neck were comprised of cartilaginous nodules (developed from branchial arch mesenchyme) and at least one well-colonized thymus, as described in syngeneic experiments (*8*). Immunofluorescence labeling with monoclonal antibodies (MAb) to MHC class II antigen confirmed the donor (H-2^k) phenotype of the epithelium and the exclusive recipient (H-2^d) origin of the bone marrow–derived cells. The spleen and lymph nodes from these animals were fully reconstituted with the major lymphocytes subsets (Table 1). When compared to normal euthymic mice, some of the animals had fewer T cells in the spleen or lymph nodes, but there were no statistically significant differences between nude mice reconstituted with syngeneic or allogeneic thymic rudiments. Expression of some V_β families in peripheral $CD3^+$ T cells was similar in mice with grafts from either syngeneic or allogeneic thymic epithelium; again, if compared to euthymic animals, some of the mice showed a limited representation of one or another family. Proliferative responses to the T cell mitogen concanavalin A (Con A) were at a reasonable level (59 to 98% and 54 to 110% of control responses in nude mice that had syngeneic or allogeneic thymic epithelium grafts, respectively), which allowed us to investigate functional T cell reactivities. Allo (or self)-reactivities of splenic lymphocytes from chimeric mice and euthymic animals were studied in vitro for production of interleukin-2 (IL-2). These responses were quantitated by limiting dilution analyses (LDA) in a system that detects every IL-2–producing T cell, independent

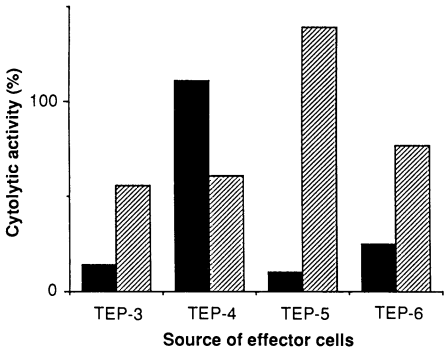

Fig. 3. The specific cytolytic activity of titrated splenic lymphocytes from nude animals with grafts and from normal euthymic BALB/c mice, stimulated for 5 days with irradiated (2000 rads) C3H (solid bars) or C57BL/6 (hatched bars) stimulator spleen cells, respectively, was studied in ^{51}Cr-release assays (*18*). Cytolytic units were calculated from each responder-target titration curve and the response is presented as the percent of those obtained in each experiment with euthymic BALB/c mice.

Table 1. Allogeneic (C3H) thymic epithelium grafts promote repopulation of the T cell compartment of nude mice. Spleen and lymph node cells from mice prepared as in Fig. 1 were analyzed by flow cytometry (FACScan, Becton and Dickinson) for the indicated markers under conditions and with antibodies as described (*17*). Only spleen cell analyses are shown, but similar results were obtained with lymph node lymphocytes. Standard deviations are in parenthesis. ND, not determined. For the normal mice, $n = 3$.

Mice	Spleen cell marker							
	% of total spleen cells			% of CD3$^+$ cells				
	CD3$^+$	CD4$^+$	CD8$^+$	V$_\beta$6	V$_\beta$8.1	V$_\beta$8.2	V$_\beta$8.3	V$_\beta$11
				Ungrafted				
BALB/c *nu/nu*	<1	ND	ND	ND	ND	ND	ND	ND
BALB/c normal	24.9 (±8.4)	18.5 (±4.2)	5.7 (±1.0)	8.1 (±2.7)	8.3 (±2.8)	14.8 (±2.1)	14.5 (±5.3)	2.0 (±1.0)
C3H normal	23.1 (±1.9)	18.3 (±1.2)	6.0 (±0.7)	13.3 (±1.6)	8.0 (±1.0)	12.5 (±0.7)	12.3 (±2.4)	2.4 (±0.4)
				C3H nu/nu *(allogeneic) recipients*				
Tep-1	21.6	16.9	5.0	ND	ND	ND	ND	ND
Tep-2	20.3	13.7	4.4	4.6	5.0	10.3	24.9	3.4
Tep-3	16.8	12.7	3.8	0.6	4.7	8.5	5.3	ND
Tep-7	17.6	13.2	5.5	8.4	12.5	14.0	0.1	ND
Tep-8	10.1	7.1	5.3	13.1	6.1	11.0	17.2	2.3
Tep-11	18.6	14.1	3.4	5.3	6.1	13.2	12.2	1.4
Tep-12	22.4	13.1	7.8	4.9	4.6	13.2	9.2	2.0
				BALB/c (syngeneic) recipients				
Tep-20	17.7	11.1	5.2	8.1	4.6	10.3	8.5	2.7
Tep-21	19.2	13.4	5.8	ND	ND	ND	ND	ND
Tep-22	24.1	13.5	6.4	4.1	6.6	15.1	11.4	1.2

Table 2. LDA of IL-2–producing cells. The frequencies of IL-2–producing CD4$^+$ cells after specific stimulation were determined in an assay that detects single cells irrespective of clonal amplification in vitro (*9*). Limiting numbers of responder splenic cells were cocultured with irradiated (2000 rads) cells from nude mice T cell–depleted peritoneal cells (20 μl) in wells of round-bottom microtiter plates. Thirty-six hours later, plates were irradiated (2000 rads) and an average of 50 IL-2–dependent CTLL/2 cells (in 5 μl) were added to each well. Forty-eight hours later, saturating amounts of Con-A–induced rat spleen cell supernatants were added to cultures as a source of IL-2. Incorporation of [^{3}H]thymidine was measured after a 16-hour incubation with label from day 3 to day 4. Four to six responder cell concentrations, corresponding to 30 wells per point, were analyzed. Wells were positive when counts per minute were higher than the mean plus five times the standard deviation of cultures containing no responder cells. Frequency estimates were calculated as before, on the basis of chi-square minimizations (*9*). For the normal mice, $n = 3$.

Mice	Stimulator cells		
	BALB/c	C57BL/6	C3H
	Ungrafted normal		
BALB/c*	1:1491–1667	1:96–402	1:129–208
	Grafted C3H		
Tep-1	<1:1000	1:220	1:70
Tep-2	<1:1000	1:140	1:220
Tep-3	1:985	1:252	<1:1000
Tep-7	<1:1000	1:161	1:143
Tep-8	<1:1000	1:207	1:245
Tep-11	ND	1:67	1:189
Tep-12	<1:1000	1:156	1:273
	Grafted BALB/c		
Tep-21	<1:1000	1:182	1:100
Tep-22	1:480	1:188	1:70
Tep-23	1:442	1:96	1:182

*Ranges are shown for the ungrafted control only.

of proliferation (*9*). Six of the seven tolerant mice that we analyzed had similar frequencies of IL-2–producing T cells specific for C3H or C57BL/6 (Table 2 and Fig. 2). In contrast, IL-2–producing cells were infrequent after stimulation with host BALB/c cells.

In vitro cytotoxicity tests (legend to Fig. 3) revealed that some tolerant animals, for example, the animal identified as Tep-4, may retain the ability to generate cytotoxic T lymphocytes (CTLs) that kill C3H targets as well as third-party, C57BL/6 allogeneic cells. If compared to CTL responses of euthymic BALB/c mice, however, most nude mice that had C3H thymus grafts showed reduced cytolytic responses to donor targets, but had killer activity to third-party antigens. Two of the animals (Tep-3 and Tep-5) had little in vitro response to C3H stimulator cells in either IL-2 production or CTL assays. These animals also did not reject C3H skin grafts: thus, they were made fully tolerant to antigens by the thymic epithelium transplant. We conclude that the peripheral T cell compartment of the chimeric nude mice that permanently tolerated donor skin grafts is not always depleted of T cell clones directed against the tolerized MHC antigens.

Our findings show that pure thymic epithelium attracts allogeneic hematopoietic precursors that then differentiate and repopulate the peripheral lymphoid organs. The immune system in these animals rejects third-party allogeneic tissues, whereas there is a long-lasting state of in vivo tolerance both to the thymic epithelium itself and to donor skin grafts. Since no autoimmune disorders were so far recorded in any of our chimeric nude mice with functional T cells, we must also conclude that host bone marrow–derived components ensure tolerance to the host's self antigens. It would appear, therefore, that in tolerance induction both lineages of thymic stroma cells may serve equivalent functions. This conclusion is at variance with the currently dominant notion that induction of tolerance by clonal deletion is a unique function of HC in thymic medulla (*1*) and that differentiating T cells ignore MHC antigens of thymus epithelium

(*2, 3*). As pointed out above, however, there is less discrepancy in the results than in the conclusions, for in these experiments lymphocyte activities were studied in vitro, whereas we have assessed tissue tolerance in vivo. Results similar to ours were obtained in several other systems (*10*), including manipulations of amphibian embryos (*11*), also tested by tissue grafts. The fact that nude mice engrafted with an allogeneic thymic epithelium may produce T cells that respond to donor lymphocytes, yet simultaneously accept skin grafts (referred to as "split tolerance"), also suggests that a complete clonal deletion is not necessary for inducing physiological tolerance to tissue grafts. Split tolerance, which extends to divergent reactivities in proliferative versus cytolytic tests (*12*), has been frequently observed, and the very use of the term indicates the unjustified comparison of heterogeneous categories of phenomena, as well as our current limitations in the understanding of these processes. "Peripheral tolerance" without thymic clonal deletion has been shown in transgenic mouse models (*13*) and embryonic allogeneic grafts (*14*). All of this evidence indicates that, in addition to the well-established process of clonal deletion in the thymus (*15*), other mechanisms, such as clonal "anergy" or "suppression," exist that do not involve the thymus. These prevent autoimmune aggression by self-reactive clones that escaped thymic negative selection.

Our experimental model is a complement to other systems that address the problem of self-nonself descrimination. Thus, it allows for a quasi-normal ontogeny of immunocompetent cells, exposing them to allogeneic antigens that are exclusively expressed by thymic epithelium. Transgenic mouse models targeting expression of MHC antigens to selected tissues have shown some degree of "leakiness" (*16*) and are limited to the genes isolated thus far. In contrast, the appropriate choice of thymic epithelium donor and nude recipient strain combinations provides a means for addressing any antigenic differences. Furthermore, by varying the age of euthymic recipients of E10 thymic epithelium, it is possible to test the importance of developmental state of the host immune system on the ability of thymic epithelial grafts to induce tolerance. Finally, interactions between lymphocytes that have developed in different MHC thymic environments can also be investigated by multiple allogeneic thymic epithelial grafts into nude mice.

REFERENCES AND NOTES

1. D. Lo and J. Sprent, *Nature* **319**, 672 (1986); J. Sprent, D. Lo, E.-K. Gao, Y. Ron, *Immunol. Rev.* **101**, 171 (1988).
2. H. von Boehmer and K. Schubiger, *Eur. J. Immunol.* **14**, 1048 (1984); E. J. Jenkinson *et al.*, *ibid.* **12**, 583 (1982); A. R. Ready *et al.*, *Nature* **310**, 231 (1984).
3. H. von Boehmer and K. Hafen, *Nature* **320**, 626 (1986).
4. R. K. Jordan *et al.*, *ibid.* **314**, 454 (1985).
5. N. M. Le Douarin and F. Jotereau, *J. Exp. Med.* **142**, 17 (1975); F. Jotereau, E. Houssaint, N. M. Le Douarin, *Eur. J. Immunol.* **10**, 620 (1980); F. V. Jotereau and N. M. Le Douarin, *J. Immunol.* **129**, 1869 (1982); N. M. Le Douarin, F. P. Guillemot, P. D. Oliver, B. Péault, in *Progress in Immunology*, V. Y. Yamamura and T. Tada, Eds. (Academic Press, New York, 1983), pp. 613–631; F. P. Guillemot, P. D. Oliver, B. Péault, N. M. Le Douarin, *J. Exp. Med.* **160**, 1803 (1984); N. M. Le Douarin, F. Dieterlen-Lièvre, P. D. Oliver, *Am. J. Anat.* **170**, 261 (1984); H. Ohki *et al.*, *Science* **237**, 1032 (1987); M. Belo *et al.*, *Int. Immunol.* **1**, 105 (1989).
6. D. Metcalf and M. A. S. Moore, in *Haemopoietic Cells* (North-Holland, Amsterdam, 1971), pp. 225–242; J. Fontaine, F. M. Calman, C. Kaplan, N. M. Le Douarin, *J. Immunol.* **126**, 2310 (1981); M. Good, K. Pike, G. Nossal, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 3045 (1983).
7. J. Salaün *et al.*, *Eur. J. Immunol.* **16**, 523 (1986).
8. I. Khazaal *et al.*, *Cell Diff. Dev.* **26**, 211 (1989).
9. B. Rocha and A. Bandeira, *Scand. J. Immunol.* **27**, 47 (1988); C. Taswell, *J. Immunol. Methods* **72**, 29 (1984).
10. H. H. Wortis *et al.*, *J. Exp. Med.* **134**, 681 (1971); F. Loor and B. Kindred, *ibid.* **138**, 1044 (1973); B. Kindred and F. Loor, *ibid.* **139**, 1215 (1974); D. D. Isaak, *J. Reticuloendothel. Soc.* **23**, 231 (1978); A. Singer, K. Hathcock, R.-J. Hodes, *J. Exp. Med.* **155**, 339 (1982); F. Loor and L. B. Hagg, *Cell. Immunol.* **29**, 200 (1977); R. M. Zinkernagel *et al.*, *J. Exp. Med.* **151**, 376 (1980); R. Hong *et al.*, *ibid.* **149**, 398 (1979); L. J. Jenski and R. Hong, *J. Immunol.* **135**, 947 (1985); H. J. Schuurman *et al.*, *ibid.* **137**, 2440 (1986); H. J. Schuurman *et al.*, *Scand. J. Immunol.* **26**, 129 (1987); L. J. Jenski *et al.*, *Thymus* **11**, 135 (1988); L. J. Jenski and B. A. Miller, *ibid.*, p. 151; F. Furukawa *et al.*, *ibid.* **12**, 11 (1988).
11. M. F. Flajnick, L. Du Pasquier, N. Cohen, *Eur. J. Immunol.* **15**, 540 (1985).
12. J. Sprent, H. von Boehmer, M. Nabholz, *J. Exp. Med.* **142**, 321 (1975).
13. G. Morahan *et al.*, *Nature* **339**, 622 (1989); D. Lo *et al.*, *J. Exp. Med.* **170**, 87 (1989).
14. C. Corbel *et al.*, *Int. Immunol.* **2**, 33 (1990).
15. B. J. Fowlkes *et al.*, *Nature* **334**, 620 (1988); H. Hengartner *et al.*, *ibid.* **336**, 388 (1988); P. Kisielow *et al.*, *Nature* **333**, 742 (1988); J. W. Kappler, N. Roehm, P. Marrack, *Cell* **49**, 273 (1987); J. W. Kappler *et al.*, *Nature* **332**, 35 (1988); H. R. Mac Donald *et al.*, *ibid.*, p. 40; H. R. Mac Donald *et al.*, *J. Exp. Med.* **167**, 2005 (1988).
16. W. van Ewijk *et al.*, *Cell* **53**, 357 (1988).
17. L. Forni, in *Immunological Methods*, I. Lefkovits and B. Pernis, Eds. (Academic Press, New York, 1979), p. 151; H. Heitzmann and F. M. Richards, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3537 (1974); antibody to CD3 (145-2C11): O. Leo *et al.*, *ibid.* **84**, 1374 (1987); antibody to CD4 (G.K.1-5): D. P. Dialynas *et al.*, *J. Immunol.* **131**, 2445 (1983); antibody to CD8 (53.6.72): J. A. Ledbetter and L. A. Herzenberg, *Immunol. Rev.* **47**, 63 (1979); antibody to $V_\beta 6$ (44.22.1): J. Payne *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7695 (1988); antibody to $V_\beta 8$ (F23.1): U. D. Staerz, H.-G. Rammensee, J. Benedetto, M. J. Bevan, *J. Immunol.* **134**, 3994 (1985); antibody to $V_\beta 8.2$ (F23.2): J. W. Kappler, U. Staerz, J. White, P. Marrack, *Nature* **332**, 35 (1988); antibody to $V_\beta 8.1$ and $V_\beta 8.2$ (KJ16): K. Haskins *et al. J. Exp. Med.* **160**, 452 (1984); antibody to $V_\beta 11$ (RR3-15): J. Bill, O. Kanagawa, D. L. Woodland, E. Palmer, *J. Exp. Med.* **169**, 1405 (1989).
18. A. Beretta, P. Langlade-Demoyen, E.-L. Larsson, *Scand. J. Immunol.* **24**, 643 (1986).

19. We acknowledge the help of M. Vincent for mouse breeding and O. Burlen-Defranoux, E. Bourson, S. Gournet, and Y. Rantier for manuscript preparation. This work was supported by the CNRS, INSERM and by grants from the European Economic Community Association Française Contre les Myopathies. I.K. is the recipient of a fellowship from AFM.

18 July 1989; accepted 1 December 1989

The Calcium Channel Blocker Nifedipine Attenuates Slow Excitatory Amino Acid Neurotoxicity

J. H. WEISS, D. M. HARTLEY, J. KOH, D. W. CHOI*

High concentrations of potent *N*-methyl-D-aspartate (NMDA) agonists can trigger degeneration of cultured mouse cortical neurons after an exposure of only a few minutes; in contrast, selective non-NMDA agonists or low levels of NMDA agonists require exposures of several hours to induce comparable damage. The dihydropyridine calcium channel antagonist nifedipine was used to test whether this slow neurotoxicity is mediated by a calcium influx through voltage-gated channels. Nifedipine had little effect on the widespread neuronal degeneration induced by brief exposure to high concentrations of NMDA but substantially attenuated the neurotoxicity produced by 24-hour exposure to submaximal concentrations of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate, kainate, or quinolinate. Calcium ion influx through dihydropyridine-sensitive, voltage-dependent calcium channels may be an important step in the neuronal injury induced by the prolonged activation of NMDA or non-NMDA glutamate receptors.

EXPOSURE TO EXCESS GLUTAMATE OR related compounds can destroy neurons in the central nervous system (*1*) and may be responsible for neuronal loss in some neurological diseases (*2*). High concentrations of glutamate or selective NMDA agonists can trigger neuronal degeneration after an exposure of only a few minutes (*3–5*), possibly due to excess Ca^{2+}

Department of Neurology and Neurological Sciences, H-3160, Stanford University Medical School, Stanford, CA 94305.

*To whom correspondence should be addressed.