tion, we subjected crude IL-1 preparations to chromatofocusing and gel filtration. The activity on thymocytes (costimulator assay) and on vascular cells (6-keto-$PGF_{1\alpha}$ production) was evaluated in the various fractions in parallel. After separation by size (Sephacryl S-200) or charge (chromatofocusing), no appreciable dissociation was found between costimulator activity on thymocytes and modulatory activity on vascular cell arachidonic acid metabolism. After fractionation with Sephacryl S-200, both the costimulator activity on thymocytes and the modulation of arachidonic acid metabolism eluted as single peak corresponding to an apparent molecular mass of approximately 15,000 daltons (Fig. 1). As expected (*18*), costimulator activity on chromatofocusing was heterogenous with three peaks. The capacity to induce 6-keto-$PGF_{1\alpha}$ production in endothelial cells showed a similar pattern (Fig. 2). Possibly because of the lower sensitivity of aortic smooth muscle cells (Table 1), no consistent activity was found in the more acidic *p*H region when these cells were used as vascular indicators (data not shown).

The results discussed so far indicate that IL-1, or a monocyte product closely associated with it, induced 6-keto-$PGF_{1\alpha}$ production in vascular cells. We used culture supernatants of stimulated mononuclear cells for original observations on lymphokine modulation of arachidonic acid metabolism in vessel walls (*8*). In view of the variety of mononuclear cell products present in such preparations, we investigated the possibility that human lymphokines other than IL-1 might interact with vessel wall cells. Recombinant IL-2 (1 to 10 U/ml), IFN-γ and IFN-α (recombinant A and hybrid A/D), and natural IFN-β (at 500 to 1000 U/ml) had no effect on 6-keto-$PGF_{1\alpha}$ production by human endothelial or rat aortic smooth muscle cells (data not shown). These results are in agreement with those of Eldor *et al.* (*19*), who did not observe stimulation of the spontaneous release of prostacyclin by human IFN-β on bovine endothelial cells.

Our results show that IL-1, or a material closely associated with this lymphokine, induces PGI_2 synthesis in human umbilical vein endothelial cells and in rat aortic smooth muscle cells. Modulation of vascular cell arachidonic acid metabolism was found with both crude and highly purified IL-1 preparations. Moreover, after chromatofocusing and gel filtration, there was copurification of IL-1 activity, as detected in the thymocyte costimulator assay, and of IL-1 activity as detected by modulation of PGI_2 synthesis in cells of the vessel walls. Thus induction of PGI_2 production adds to the list of nonimmune effects of IL-1 (*18, 20*). Elucidation of the molecular basis of the pleiotropic effects of IL-1 will involve cloning of the gene encoding this mediator, or family of mediators, and the production of large quantities of homogenous material.

The in vivo relevance of the modulation of vessel wall arachidonic acid metabolism by IL-1 is unclear. Since vessel wall PGI_2 plays a major role in the regulation of important biological functions such as platelet aggregation and vascular tone (*9*), IL-1–induced PGI_2 secretion might contribute to vascular changes at sites of delayed hypersensitivity reactions (*7*). Inasmuch as various cell types can produce IL-1 (*18, 20*), this mediator could serve as a communication signal between tissues and blood vessels. Considerable effort has been devoted to designing pharmacological approaches to thrombosis by selectively inhibiting arachidonic acid metabolism in platelets as opposed to that in vascular cells (*9, 21*). Elucidation of the "slow" pathway through which IL-1 induces PGI_2 synthesis in vascular cells may offer clues for the development of new strategies in antithrombotic therapy.

References and Notes

1. W. M. Baldwin III, *Immunol. Today* **3**, 267 (1982).
2. B. M. Martin *et al.*, *J. Immunol.* **126**, 1510 (1981).
3. P. J. Polverini *et al.*, *Nature (London)* **269**, 804 (1977).
4. M. C. Cohen *et al.*, *Science* **215**, 301 (1982).
5. G. C. Bagby, Jr., *et al.*, *Blood* **62**, 663 (1983).
6. J. S. Pober *et al.*, *J. Exp. Med.* **157**, 1339 (1983).
7. P. J. Polverini, R. S. Cotran, M. M. Sholley, *J. Immunol.* **118**, 529 (1977).
8. E. Dejana *et al.*, *Blood* **64**, 1280 (1984).
9. S. Moncada and J. R. Vane, *Pharmacol. Rev.* **30**, 293 (1978).
10. F. Colotta *et al.*, *J. Immunol.* **132**, 936 (1984).
11. I. Gery *et al.*, *Cell. Immunol.* **64**, 293 (1981).
12. L. J. Rosenwasser and C. A. Dinarello, *ibid.* **63**, 134 (1981).
13. G. Scala *et al.*, *Nature (London)* **309**, 56 (1984).
14. E. Dejana *et al.*, *Biochim. Biophys. Acta* **750**, 261 (1983).
15. M. Busacca *et al.*, *Am. J. Obstet. Gynecol.* **148**, 1127 (1984).
16. E. Vicenzi *et al.*, *Clin. Exp. Immunol.* **57**, 385 (1984).
17. C. Chiabrando *et al.*, *J. Chromatogr.* **279**, 581 (1983).
18. C. A. Dinarello, *Rev. Infec Dis.* **6**, 51 (1984).
19. A. Eldor *et al.*, *J. Clin. Invest.* **73**, 251 (1984).
20. M. P. Bevilacqua *et al.*, *J. Exp. Med.* **160**, 618 (1984).
21. G. de Gaetano, C. Cerletti, V. Bertelé, *Lancet* **1982-II**, 974 (1982).
22. P. Travo, G. Barrett, G. Burnstock, *Blood Vessels* **17**, 110 (1980).

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Inhibition of Interferon-Gamma May Suppress Allograft Reactivity by T Lymphocytes in Vitro and in Vivo

Abstract. *The addition to mixed-leukocyte reactions of monoclonal antibodies to interferon-γ abrogated alloantigen recognition and induction of cytotoxic T lymphocytes by inducing early and highly effective suppressor T lymphocytes. This inhibitory activity was not confined to in vitro models, since daily injection of the antibodies into CBA/J mice blocked the usual rejection of allogenic tumor cells.*

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T-lymphocyte activation requires both presentation of the antigen in the context of the proper major histocompatibility complex (MHC) products and non–antigen-specific signals provided by interleukins (*1*). Of these, immune interferon (IFN-γ), mainly released by helper T lymphocytes on activation by alloantigens (*2*), seems to be endowed with many immunoregulatory effects besides its antiviral activity (*3*). IFN-γ obtained by recombinant DNA technology is a potent inducer of MHC antigen expression on a great variety of cells (*4*), a helper and maturation factor in B-cell antibody production (*5*), and an activator of macrophage functions (*6*). It may therefore play a critical role in triggering antigen recognition and allograft rejection.

To investigate its possible contribution to the mixed-leukocyte reaction (MLR) and allograft rejection, we performed a functional ablation of IFN-γ by using the

monoclonal antibody (MoAb) AN-18.17.24, which neutralizes IFN-γ but not all the other mouse lymphokines. It immunoprecipitates biosynthetically labeled murine IFN-γ and allows the construction of affinity columns for the purification of murine IFN-γ (*7*).

When a 1:10 dilution of AN-18.17.24 (1 μg/ml) was added at the beginning of an MLR between responder C57BL/6 (B6) mouse (H-2^b) and irradiated (stimulator) DBA/2 mouse (H-2^d) spleen cells, both proliferation and cytotoxicity were abrogated more than 90 percent. Moreover, a linear relation was found between MoAb concentration and inhibition of both responses. In contrast, when the MoAb was added after 48 to 96 hours of culture, both functions remained at the control levels. Since the same inhibition pattern was found with AN-18.17.24 $(Fab)_2$ fragments, a nonspecific cytotoxic effect mediated by the MoAb molecule can be ruled out. IFN-γ titers in the supernatants decreased from 160 IU/ml (untreated control MLR) to 3 IU/ml (MoAb added at the beginning of the MLR or after 96 hours). This, however, cannot be considered an indication of inhibition of IFN-γ release, since the excess MoAb present may have neutralized the IFN-γ produced. Finally, a 1:10 dilution of an unrelated MoAb (AN-37; 1 μg/ml), added at time 0, had no effect on these functions (Table 1).

The early steps of the MLR consist of alloantigen recognition and acquisition of the ability to respond to interleukins by precursor T lymphocytes (*1*). To clarify the nature of the inhibition observed, B6 spleen cells primed in vitro for 14 days against DBA/2 stimulator spleen cells were restimulated with the specific alloantigen or supplemented with exogenous interleukin-2 (IL-2) in the presence or absence of antibody to IFN-γ MoAb. IL-2 induces the proliferation of primed T lymphocytes by overcoming the additional need for stimulator cells (*8*). As shown in Table 2, the antibody to IFN-γ MoAb suppressed the alloantigen-stimulated but not the IL-2–dependent T-lymphocyte proliferation. These results show that, once IL-2 is produced, antibody to IFN-γ MoAb can no longer affect the subsequent proliferation of T lymphocytes and suggest that IFN-γ is directly involved in the early phases of alloantigen recognition.

Many mechanisms could be responsible for this inhibition, including induction of suppressor cells (*9*). To test this hypothesis, an MLR between responder B6 and stimulator DBA/2 spleen cells was set up in the presence or absence of

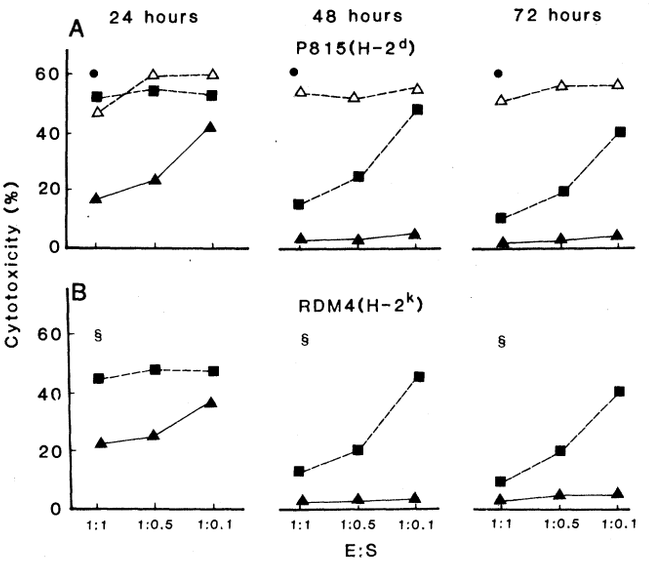

Fig. 1. Suppressor activity of B6 spleen cells harvested at 24, 48, and 72 hours of culture from MLR's with or without antibody to IFN MoAb. In vitro generation of suppressor cells was carried out by coculturing 3×10^6 B6 responder and 5×10^6 DBA/2 stimulator spleen cells in 24-well tissue plates (Nunclon). At intervals the DBA/2-primed B6 cells were harvested and transferred as third-party cells to new primary MLR's at various effector-to-suppressor (E:S) ratios. These primary MLR's were set by culturing 3×10^6 B6 responder with 5×10^6 DBA/2 (H-2^d) or CBA/J (H-2^k) stimulator spleen cells for 5 days in identical wells. The cytotoxic activity generated was then tested against P815 (H-2^d) and RDMA (H-2^k) target cells in a 4-hour ^{51}Cr release assay. The percentage of cytotoxic activity at an effector-to-target ratio of 25 to 1 is shown for cells from an MLR not supplemented with third-party cells (●, §); supplemented with third-party cells from an MLR without antibody to IFN MoAb (■); supplemented with third-party cells from an MLR with antibody to IFN MoAb (▲); or supplemented with antibody to Thy 1.2 MoAb and C-treated (*10*) third-party cells from an MLR with antibody to IFN MoAb (△). The third-party cells from an MLR without antibody to IFN-γ MoAb displayed cytotoxicity against H-2^d ranging from 4 to 8 percent and no cytotoxicity against H-2^k target cells. Those from an MLR with antibody to IFN-γ MoAb did not display any significant cytotoxicity.

Table 1. Results of an MLR between 3×10^5 B6 responder and 5×10^5 stimulator spleen cells in the presence of various dilutions of AN-18.17.24 MoAb and AN-37 MoAb. The cells were cultured in RPMI 1640 medium (supplemented with 5 percent fetal bovine serum and $5 \times 10^{-5} M$ β-mercaptoethanol) in flat-bottomed microtiter plates at 37°C in humidified air containing 5 percent CO_2; these were the culture conditions for all the in vitro experiments reported, except where otherwise specified. After 96 hours 1 μCi (2 Ci/mmol) of [^{3}H]thymidine was added, and 6 to 8 hours later its uptake was measured (*14, 15*). The uptake by unstimulated cultures was 8.1×10^3 count/min. Standard deviations did not exceed 10 percent of the mean and are not included. The stimulation index (*14, 15*) is shown in parentheses. For generation of cytotoxic lymphocytes, 3×10^6 B6 responder plus 5×10^6 stimulator DBA/2 spleen cells were cultured for 6 days in 24-well Nunclon tissue plates. Cytotoxic activity against P815 mastocytoma (H-2^d) target cells was evaluated in a 4-hour ^{51}Cr release assay. The percentage of specific lysis (*14, 15*) at an effector-to-target cell ratio of 50 to 1 is reported. Supernatants from the MLR established for the generation of cytotoxic cells were tested for IFN activity by microtitration of the cytopathology of vesicular stomatitis virus (*7*). Titers are expressed in international units by using the NIH reference standard G-002-9045511. The IFN titer of unstimulated culture supernatants was less than 3 IU. The experiments were repeated five times with similar results; the results of one experiment are reported.

MoAb dilution	Time of MoAb addition (hours)	Uptake of [^{3}H]thymidine (count/min $\times 10^{-3}$)	Cytotoxicity (%)	IFN titer (IU)
No MoAb		90.3 (11.1)	82.3	160
		AN-18.17.24		
1:10	0	8.4 (1.1)	2.5	3
1:40	0	46.3 (5.7)	39.1	81
1:160	0	88.4 (10.5)	71.9	160
1:10	48	51.9 (6.1)	35.3	3
1:40	48	85.8 (10.5)	65.4	81
1:160	48	89.6 (11.0)	79.8	160
1:10	96	104.1 (12.8)	86.7	3
1:40	96	99.5 (12.2)	81.1	81
1:160	96	91.6 (11.3)	78.5	160
		AN-37		
1:10	0	95.0 (11.7)	80.3	160
1:40	0	92.9 (11.4)	83.4	160

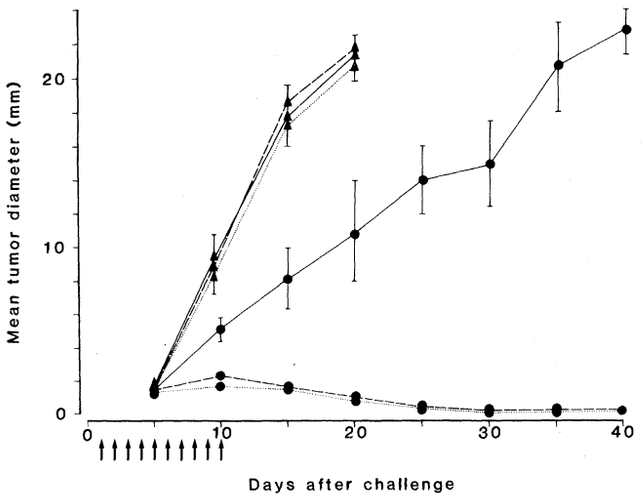

Fig. 2. Effect of the injection of MoAb to IFN-γ MoAb on the rejection of allogeneic tumor cells. Shown are the growth and rejection patterns of CE-2 (H-2^d) tumor cells injected in groups of six female mice untreated (- - - - - -), injected with control AN.37 MoAb (· · · · · ·), or injected with antibody to IFN-γ MoAb (——). Animals were syngeneic BALB/c mice (▲) and allogeneic CBA/J (H-2^k) mice (●). The arrows show the times when 0.4 ml of a 1:10 dilution of MoAb was injected subcutaneously at the tumor challenge site (*15*). The mice were palpated every 5 days to pinpoint the moment of tumor appearance, after which neoplastic masses were measured with calipers in the two perpendicular diameters to ascertain the average diameter (*13*).

antibody to IFN-γ MoAb. The cells were harvested at intervals and added to a new primary MLR between responder B6 and stimulator DBA/2 or CBA/J (H-2^k) spleen cells as third-party cells. The third-party cells from the MLR in the presence of antibody to IFN-γ MoAb abrogated the generation of cytotoxic B6 lymphocytes specific to both alloantigens (Fig. 1). Suppression was already evident with third-party cells harvested after 24 hours of culture in the presence of antibody to IFN-γ MoAb and increased with cells harvested after 48 and 72 hours (Fg. 1A). When antibody to IFN-γ MoAb was omitted, however, significant suppression was displayed only by cells harvested after 48 and 72 hours of culture at an effector-to-suppressor ratio of 1 to 0.5.

Suppressor third-party cells from the MLR in the presence of antibody to IFN-γ MoAb belong to the T-lymphocyte lineage, since their activity was removed by treatment with antibody to Thy 1.2 MoAb and C (Fig. 1A) (*10*). They also suppressed the MLR between responder B6 and stimulator CBA/J spleen cells and thus are non–antigen-specific (Fig. 1B) (11). A possible conclusion is that antibody to IFN-γ MoAb inhibits MLR's by inducing early and highly effective T-suppressor lymphocytes that may counteract the activity of helper-inducer T lymphocytes in the early phases of alloantigen recognition. Alternatively, it may directly interfere with inducer T-cell activity. In any case, these findings permit a more definitive interpretation of the role of IFN-γ in the generation of cytotoxic T lymphocytes in the MLR, as envisaged by Farrar *et al.* (*12*).

Because the MLR is regarded as the in vitro counterpart of allograft rejection in vivo (*1*), we investigated the ability of antibody to IFN-γ MoAb to inhibit such rejection by using a methylcholanthrene-induced fibrosarcoma (CE-2) of the BALB/c strain (*13–15*). Subcutaneous injection of 1×10^7 tumor cells always killed syngeneic BALB/c mice in less than 20 days, whereas it resulted in quick and total rejection in allogeneic CBA/J mice, which differ from BALB/c mice in both minor and major histocompatibility alloantigens (Fig. 2). By contrast, tumor outgrowth and death of recipient CBA/J mice took place when ten daily subcutaneous injections of 0.4 ml of a 1:10 dilution of antibody to IFN-γ MoAb were also given at the challenge site (Fig. 2) (*15*). Injection of unrelated AN-37 antibody had no effect (Fig. 2).

These results show that antibody to IFN-γ MoAb can inhibit MLR's in vitro and allograft rejection in vivo. Antigen presentation with interruption of the lymphokine cascade appears to drive the immune response toward T-suppressor circuits. This may constitute a hitherto unknown pathway of tolerance induction.

These conclusions may have important implications, since the presence of highly active suppressor T lymphocytes in tumor-bearing individuals and in tolerance has been repeatedly demonstrated (*1, 11, 16*). In light of our findings, it can be argued that tumor antigens as well as tolerogenic antigen signals may lack the ability to switch on the lymphokine cascade, thus inducing tolerance instead of an effective immune response. New models of immunotherapy and immunoprotection offering fresh perspectives on tumor and transplantation immunology may therefore be envisaged.

Table 2. Effect of antibody to IFN-γ MoAb on the proliferation of DBA/2-primed T lymphocytes restimulated with DBA/2 alloantigens or IL-2. B6 spleen cells were primed in vitro for 14 days against stimulator DBA/2 spleen cells. Primed lymphocytes (1×10^5 and 1×10^4) were then cultured again in the presence of 5×10^5 stimulator DBA/2 spleen cells or 20 U of IL-2 produced by EL-4 cells stimulated with phorbol myristic acetate ($10^{-8}M$) and partially purified on a Sephacryl S-200 column. The cells were cultured in the presence of various dilutions of MoAb in flat-bottomed microtiter plates. After 48 hours [^{3}H]thymidine uptake was titrated. The background radioactivity was 3.7×10^3 and 4.6×10^2 count/min for DBA/2-stimulated and IL-2–stimulated cultures, respectively. Standard deviations did not exceed 10 percent of the mean and are not reported. Each experiment was repeated four times with similar results; the results of one experiment are reported.

MoAb dilution	Uptake of [^{3}H]thymidine (count/min $\times 10^{-3}$)
DBA/2	
No MoAb	94.3
1:10	19.4
1:40	73.5
1:160	89.9
IL-2	
No MoAb	19.5
1:10	20.6
1:40	18.9
1:160	21.1

References and Notes

1. W. E. Paul, Ed., *Fundamental Immunology* (Raven, New York, 1984); S. Cohen, E. Pick, J. J. Oppenheim, Eds., *The Biology of the Lymphokines* (Academic Press, New York, 1979).
2. S. Landolfo *et al.*, *J. Interferon Res.* **1**, 339 (1981); S. Landolfo *et al.*, *Eur. J. Immunol.* **12**, 295 (1982); S. Landolfo *et al.*, *Immunogenetics* **9**, 245 (1979).
3. S. Baron, F. Dianzani, G. J. Stanton, Eds. *Tex. Rep. Biol. Med. No. 41* (1982).
4. P. S. Steeg *et al.*, *J. Exp. Med.* **156**, 1780 (1982); T. Y. Basham and T. C. Merigan, *J. Immunol.* **130**, 1492 (1983); J. S. Pober *et al.*, *J. Exp. Med.* **157**, 1339 (1983).
5. H. J. Leibson, M. Gefter, A. Zlotnick, P. Marrack, J. W. Kappler, *Nature (London)* **309**, 799 (1984); C. L. Sidman, J. D. Marshall, L. D. Shultz, P. W. Gray, H. M. Johnson, *ibid.*, p. 801.
6. J. L. Pace *et al.*, *J. Immunol.* **130**, 2011 (1983); F. Cofano *et al.*, *ibid.* **133**, 3102 (1984).
7. M. Prat *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4515 (1984); G. Gribaudo *et al.*, *J. Interferon Res.* **5**, 199 (1985); S. Landolfo *et al.*, *J. Immunol.* **128**, 2807 (1982).
8. G. Moller, Ed., *Immunol. Rev.* **63**, I (1982).
9. S. S. Rich and R. R. Rich, *J. Exp. Med.* **140**, 1588 (1974); T. Hirano and A. A. Nordin, *J. Immunol.* **116**, 1115 (1976); R. J. Hodes, L. M. Nadler, K. S. Hathcoch, *ibid.* **119**, 961 (1977).
10. T-lymphocyte depletion was obtained by using MoAb to Thy-1.2 MoAb (NEN) and guinea pig C twice consecutively. Residual populations contained less than 0.5 percent Thy-1.2 cells, as determined by immunofluorescence.
11. Nonspecific suppressor T lymphocytes have also been observed by others [C. W. Pierce and J. A. Kapp, in *The Lymphokines*, J. W. Hadden and W. E. Stewart, Eds. (Humana, Clifton, N.J., 1981), pp. 207–230].
12. W. L. Farrar, H. M. Johnson, J. J. Farrar, *J. Immunol.* **126**, 1120 (1981).
13. The growth in syngeneic BALB/c mice of CE-2

fibrosarcoma can be hindered by tumor-specific T-helper cells or lymphokines (*14, 15*).
14. G. Forni and M. Giovarelli, *J. Immunol.* **132**, 527 (1984).
15. G. Forni *et al.*, *ibid.* **134**, 1305 (1985).
16. S. Fujimoto, M. I. Greene, A. H. Sehon, *J. Immunol.* **116**, 791 (1976); L. L. Perry *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 920 (1979); C. D. Mills and R. J. North, *J. Exp. Med.* **157**, 1448 (1983).
17. Supported by grants from the National Research Center, Italy (PFCMI 83.00663.52 and PF Oncologia 84.00582.44) and from the Ministry of Public Education (40 percent).

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Diazepam-Binding Inhibitor: A Neuropeptide Located in Selected Neuronal Populations of Rat Brain

Abstract. *An endogenous polypeptide of rat brain has been identified that is capable of displacing 1,4-benzodiazepines and the esters of the 3-carboxylic acid derivatives of β-carbolines from their specific synaptic binding sites. This polypeptide was termed diazepam-binding inhibitor (DBI). Previous studies have shown that DBI injected intraventricularly in rodents elicits "proconflict" responses and antagonizes the "anticonflict" action of benzodiazepines. An antiserum to this peptide, directed toward an immunodeterminant near its amino terminus, makes it possible to detect, measure, and study the neuronal location of this peptide in rat brain. In the rat cerebral cortex, DBI immunoreactivity is located in neurons that are not GABAergic (GABA, γ-aminobutyric acid); in the cerebellum and hippocampus, however, it might be present also in GABAergic neurons.*

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Anxiety can be lessened or precipitated in humans by various chemicals that bind with high affinity to a specific recognition site for benzodiazepines. The occupancy of these sites by various derivatives of 1,4-benzodiazepines facilitates GABAergic (GABA, γ-aminobutyric acid) transmission, enhances the threshold to convulsions elicited by drugs that impair GABAergic transmission, prolongs the opening bursts of chloride ion channels elicited by GABA, and relieves episodes of anxiety in humans (*1*). In contrast, occupancy of these sites by esters of β-carboline 3-carboxylic acid negatively modulates GABAergic transmission, lowers the convulsion threshold to drugs that impair GABAergic transmission, depresses chloride ion conductance evoked by GABA, and precipitates panic anxiety in humans (*2*). The question then arises as to whether the benzodiazepine recognition sites are physiologic receptor sites or drug acceptors. In demonstrating that a drug recognition site has physiological significance, it is relevant to show that an endogenous ligand exists. Identifying this ligand may also help to characterize the biochemical abnormalities associated with symptoms, such as anxiety, convulsions, and sleep disorders, that are ameliorated by benzodiazepine treatment.

From homogenates of rat and human brain, we have isolated and purified to homogeneity a neuropeptide that displaces ^{3}H-labeled β-carboline 3-carboxylic acid (median inhibitory concentration, 1 μ*M*) bound to high-affinity recognition sites in crude synaptic membranes (*3*). This peptide was termed diazepam-binding inhibitor (DBI) to indicate its ability to displace specific ligands from benzodiazepine recognition sites (*3*). DBI has 104 amino acid residues and contains two identical octadecapeptide chains with the following amino acid sequence: NH_2-QATVGDVNTDRPGLLDLK-OH(*4*). DBI probably functions as a precursor of this octadecapeptide, which might act as an endogenous ligand of benzodiazepine recognition sites. DBI belongs to a new class of brain polypeptides that presumably takes part in the regulation of GABAergic transmission. The sequence of 52 of the 104 amino acid residues of the DBI structure is as follows: (52)TQPTDEEMLFIYSHFKQATVGDVNTDRPGLLDLKGKXIMKTYVEKVEELKKKY-OH(104). This sequence does not resemble any known mammalian neuropeptide (*3*).

When injected intraventricularly into rats subjected to the "conflict-punishment" behavioral model proposed by Vogel *et al.* (*5*), DBI antagonizes the "anticonflict" action of diazepam and facilitates "conflict" behavior; that is, it enhances the shock-induced suppression of drinking in thirsty animals by lowering the threshold for the response (*3*). These actions of DBI are antagonized by the imidazobenzodiazepine RO 15 1788 (*3*), which is a specific benzodiazepine antagonist. This suggests that the brain contains a specific ligand for benzodiazepine recognition sites that is operative in GABA receptor modulation in a manner similar to esters of β-carboline 3-carboxylic acid and is capable of facilitating the onset of conflict in the Vogel model. We infer that a modulation of specific GABAergic synapses might be operative because DBI added to primary cultures of spinal cord neurons decreases the

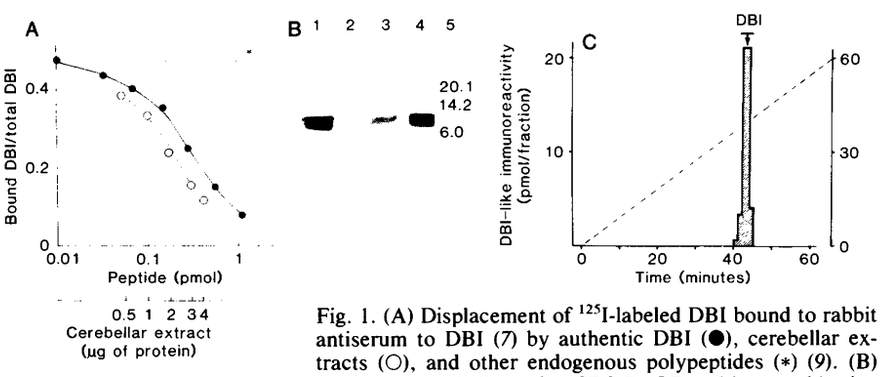

Fig. 1. (A) Displacement of ^{125}I-labeled DBI bound to rabbit antiserum to DBI (*7*) by authentic DBI (●), cerebellar extracts (○), and other endogenous polypeptides (*) (*9*). (B) Characterization of DBI-like material in rat cerebellum after SDS-PAGE and immunoblotting with DBI antiserum. Cerebellar extracts were subjected to 15 percent SDS-PAGE (*3*) and blotted onto nitrocellulose paper. The paper was probed with antiserum (1:1000) and then visualized by the peroxidase method (*14*). (Lane 1) Standard DBI (10 μg). (Lane 2) 1*N* acetic acid cerebellar extract (24 μg of protein). (Lane 3) 0.1 percent SDS cerebellar extract (100 μg of protein). The sample was homogenized in 0.1 percent SDS–3 percent β-mercaptoethanol and 15 percent glycerol in 50 m*M* tris buffer (*p*H 7.4), boiled for 5 minutes, and centrifuged at 48,000*g* for 20 minutes. The supernatant was applied to the gel. (Lane 4) Comparative staining of DBI (10 μg) by Coomassie blue after SDS-PAGE. (Lane 5) Molecular weight markers (in thousands). (C) Characterization of DBI-like immunoreactivity in rat cerebellar extract after reversed-phase HPLC. Acetic acid cerebellar extract (~300 μg of protein) was chromatographed on a reversed-phase HPLC column equilibrated with 0.1 percent TFA-H_2O (TFA, trifluoroacetic acid). The column was developed with a 0 to 60 percent linear gradient of 0.1 percent TFA-acetonitrile over 60 minutes at a flow rate of 2 ml/min. Fractions were lyophilized before the radioimmunoassay.