mals analyzed [seven of seven backcrosses, three of three intercrosses (typing of eight animals is shown in Fig. 2)] were homozygous for the NOD 9.5-kb band ($P < 0.0001$, binominal distribution), suggesting a recessive contribution to diabetes susceptibility. Because the incidence of diabetes in backcross females was only 15.6 percent, we hypothesize that there is at least one and probably two or more susceptibility genes in addition to the MHC-linked diabetogenic gene.

It is interesting that the diabetogenic histocompatibility gene functions in a "recessive" manner. The recessive nature of the MHC contribution to the development of diabetes is analogous to the results obtained from breeding studies in the BB rat, which also develops type I diabetes (*15*). Among these animals the incidence of diabetes in individuals homozygous for the MHC (RT1-u) is ten times greater than that in heterozygous individuals in crosses with Lewis, Brown Norway, and BBN RT1-A rats (*15, 16*). In human type I diabetes there is a higher incidence of concordance in diabetic siblings sharing both histocompatibility regions (20 percent) than in those sharing one histocompatibility antigen haplotype (5 percent) (*17*).

Class I and class II MHC-linked gene products are expressed in a codominant fashion on the cell surface. Responsiveness to foreign antigens controlled by class II genes is inherited as a dominant trait (*18*). The recessiveness of the diabetogenic influence of the NOD MHC genes contrasts with the "dominant" inheritance of class II–determined responsiveness to foreign antigens and can be explained by at least three mechanisms. First, if the MHC-linked diabetogenic gene is a class II immune-response gene, a lack of diabetes in heterozygotes may be due to decreased cell-surface expression of a specific α:β class II heterodimer on antigen-presenting cells, lymphocytes, or possibly the target tissue. Since class II molecules are α:β heterodimers, the α and β chains can associate in mice heterozygous at I-A loci, which would reduce the expression of the parental-type heterodimers (*19*). Such decreased parental heterodimer expression can cause a proportional decrease in the responsiveness of MHC-restricted T-cell clones (*14*). Second, the anti-islet immune response involved in type I diabetes of NOD mice may be controlled by a class II immune "suppressor" gene. In such a system, low responsiveness is inherited as a dominant trait and high responsiveness is inherited as a recessive trait (*20*). Third, the MHC-linked gene involved in the pathogenesis of type I diabetes mellitus may involve a deletion (for example, lack of an I-E chain gene). Congenital adrenal hyperplasia results from a deficiency in a 21-hydroxylase enzyme and is inherited as an MHC-linked autosomal recessive trait (*21*). Further breeding studies and analyses of the unique MHC of the NOD mouse should allow direct testing of the three hypotheses.

REFERENCES AND NOTES

1. S. Makino *et al.*, *Exp. Anim. (Tokyo)* **29**, 1 (1980).
2. M. Hattori *et al.*, *Endocrinology* **110** (suppl.), 177 (1982).
3. M. Hattori *et al.*, *Diabetes* **32** (suppl. 1), 149 (1983).
4. S. Makino, Y. Muraoka, Y. Kishimoto, Y. Hayashi, *Exp. Anim. (Tokyo)* **34**, 425 (1985).
5. M. Harada and S. Makino, paper presented at the 12th Congress of the International Diabetes Federation, Madrid, 1985.
6. K. Moriwaki, T. Sagai, K. Ohkawa, M. Hattori, S. Makino, *Exp. Anim.*, in press.
7. C. David, D. L. Shreffler, J. A. Frelinger, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2509 (1973).
8. C. A. Janeway *et al.*, *J. Immunol.* **132**, 662 (1984).
9. G. J. Hammerling, U. Hammerling, H. Lemke, *Immunogenetics* **8**, 433 (1979).
10. K. Ozato, N. Mayer, D. H. Sachs, *J. Immunol.* **124**, 533 (1980).
11. J. W. Kappler, B. Skidmore, J. White, P. Marrack, *J. Exp. Med.* **153**, 1198 (1981).
12. A. Bhattacharya, M. E. Dorf, T. A. Springer, *J. Immunol.* **127**, 2488 (1981).
13. D. J. Mathis, C. Benoist, V. E. Williams II, M. Kanter, H. O. McDevitt, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 273 (1983).
14. L. Glimcher and E. M. Shervach, *J. Exp. Med.* **156**, 640 (1982).
15. R. A. Jackson *et al.*, *ibid.* **159**, 1629 (1984).
16. J. B. Buse *et al.*, *ibid.*, **162**, 444 (1985).
17. A. N. Gorsuch *et al.*, *Diabetes* **31**, 862 (1982).
18. B. Benacerraf and H. O. McDevitt, *Science* **175**, 273 (1972).
19. D. B. Murphy, P. P. Jones, M. R. Loken, H. O. McDevitt, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 5404 (1980).
20. P. Debre, C. Waltenbaugh, M. Dorf, B. Benacerraf, *J. Exp. Med.* **144**, 272 (1976).
21. P. C. White, M. I. New, B. Dupont, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 7505 (1984).
22. J. B. Buse *et al.*, *Diabetes* **33**, 700 (1984).
23. E. Choi, K. McIntyre, R. N. Germain, J. G. Seidman, *Science* **221**, 283 (1983).
24. J. J. Hyldig-Nielsen *et al.*, *Nucleic Acids Res.* **11**, 5055 (1983).
25. M. Kronenberg *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5704 (1983).

26. We thank C. David and M. Loken for provision of FIa1,2,3,7 (A.TH anti-A.TL), AF6-120, and 25-9-17; P. Cronin for secretarial assistance, and Kyoto University Radioisotope Center and the Experimental Animal Center for maintaining our NOD mouse colony before it was moved to the Joslin Diabetes Center. Supported by NIH grants AM32083-03 and AM07009-01 and the Juvenile Diabetes Foundation.

5 July 1985; accepted 15 November 1985

Pinealocyte Projections into the Mammalian Brain Revealed with S-Antigen Antiserum

H.-W. KORF, A. OKSCHE, P. EKSTRÖM, I. GERY, J. S. ZIGLER, JR., D. C. KLEIN

Neural processes from mammalian pinealocytes have been discovered in several brain areas. These processes were visualized immunocytochemically in the Djungarian hamster, *Phodopus sungorus*, with an antiserum against bovine retinal S-antigen and traced as far as the region of the posterior commissure and habenular nuclei. This result indicates that pineal-to-brain connections exist in the mammal, and that the mammalian pineal gland, currently thought of only as a neuroendocrine organ, may communicate directly with select brain regions by way of these projections. The existence of mammalian pinealocyte projections is consistent with the view that these cells are not of glial origin but are derivatives of photoreceptor cells of the pineal complex of lower vertebrates that transmit signals to the brain by neural projections.

THE PINEAL ORGAN HAS UNDERgone remarkable structural and functional modification during evolution (*1*). In poikilotherms it has a prominent photosensory apparatus and sends neural projections to the brain (*2*). In contrast, the mammalian pineal gland is not directly photosensitive, but is regulated by light acting on the lateral eyes and a circuit of central and peripheral neural structures (*3*); in addition, it is generally thought that the mammalian pineal gland acts as a neuroendocrine organ which controls target tissue exclusively through the circadian release of melatonin and perhaps other hormones into the circulation (*4*). However, we now present evidence that mammalian pinealocytes send projections into the brain, pointing to the possibility that some mammalian pinealocytes might function as neurons.

A group of 13 Djungarian hamsters were studied (*5*). In this species, a superficial pineal gland is connected to a deep pineal gland by a pineal stalk (Fig. 1). The superficial pineal gland was surgically removed from three animals 1 week before fixation

H.-W. Korf, A. Oksche, P. Ekström, Department of Anatomy and Cytobiology, Justus-Liebig-University of Giessen, D-6300 Giessen, Federal Republic of Germany.
I. Gery and J. S. Zigler, Jr., Laboratory of Vision Research, National Eye Institute, National Institutes of Health, Bethesda, MD 20892.
D. C. Klein, Section of Neuroendocrinology, Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892.

(*6*). Serial coronal brain sections were examined immunocytochemically using a rabbit antiserum against purified bovine retinal S-antigen (*7, 8*). The S-antigen is a highly antigenic protein found only in retinal photoreceptors and pinealocytes belonging to the sensory line, which includes pineal photoreceptors of poikilotherms, modified pineal photoreceptors of sauropsids and mammalian pinealocytes (*7, 9*); it has not been detected in other tissues. In this study immunoreactions were not seen when the antiserum had been absorbed with highly purified S-antigen (*8*) or when it was replaced by nonimmune rabbit serum.

Immunoreactive material was identified in the superficial, stalk, and deep portions of the pineal organ, and within a small number of cells in the medial habenular nucleus (Fig. 2, a–c); surrounding brain tissue was unreactive. As described in other species (*7*), marked cell-to-cell variation in the intensity of the immunoreaction was also found in the Djungarian hamster pineal organ. However, the majority of cells were clearly labeled, and the shape of immunoreactive cells resembled that of typical pinealocytes; this observation, together with the knowledge that pinealocytes form the major cellular component of the pineal organ and that only 5 to 10 percent of the cells are astrocytic glia (*10, 11*), indicates that the labeled cells in the Djungarian hamster pineal gland are pinealocytes.

Cells located in the deep portion of the pineal organ gave rise to immunoreactive processes with varicosities (Fig. 2, d–k) that penetrated deep into the medial habenular nucleus and the region of the posterior commissure. Bifurcations of these processes were occasionally identified within these regions. Examination of serial sections indicated that processes originated from S-antigen–labeled pinealocytes. The issue of whether these processes might in part represent sympathetic neurons secondarily labeled as they passed from the superficial pineal gland and through the deep pineal gland (*12, 13*) was tested by examining three animals after the superficial pineal gland was removed. This procedure cuts the sympathetic innervation of the deep pineal. However, this treatment did not eliminate or seem to reduce S-antigen–labeled processes in the habenular and posterior commissure regions of the brain, which indicated that the processes were not largely sympathetic neurons. The possibility that these processes represent "conventional" intrapineal neurons is remote because such neurons are extremely rare in rodents (*10*). Also, the possibility that these processes represent glial processes is remote because the S-antigen has not been identified in glia in nonpineal regions and because only pinealocytes appear to contain this marker in the pineal gland (*7*). Thus, it would appear from these findings that pinealocytes send projections into the brain in mammals as is true of lower vertebrates and that the general morphological organization of the epithalamus in the mammal is fundamentally similar to that in lower vertebrates. The projections identified in this study may explain the electrophysiological evidence of neural communication between the pineal organ and brain (*14*).

These findings bear on the question of the evolution of the mammalian pineal cell. The

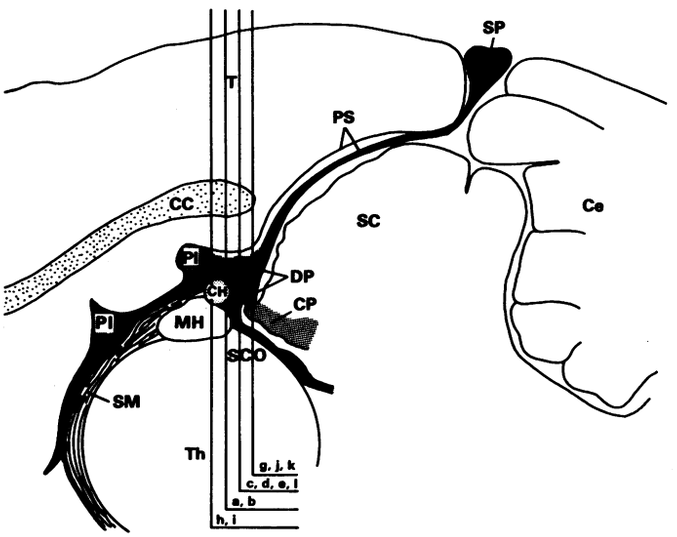
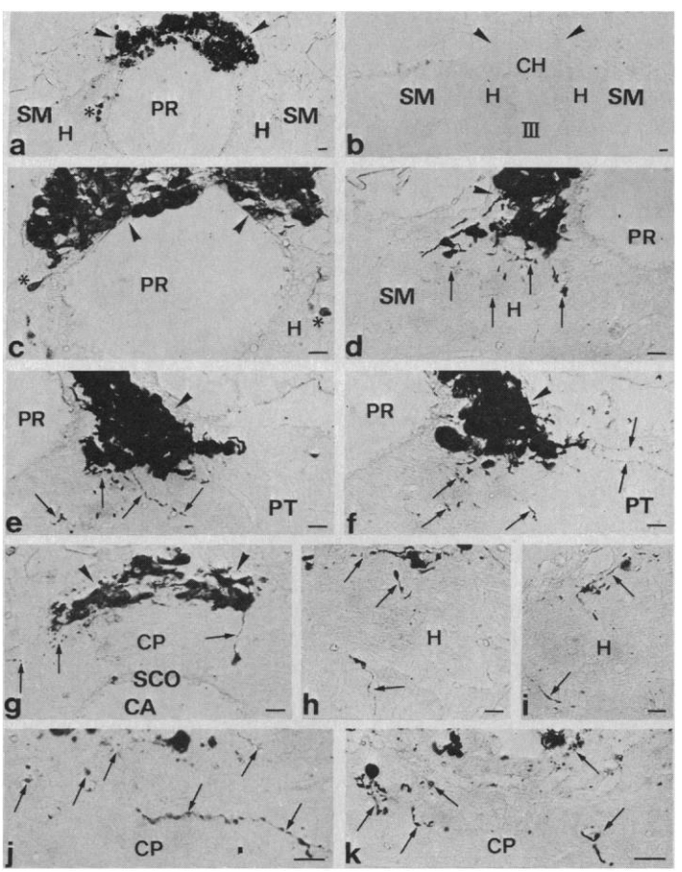


Fig. 1 (left). Sagittal section through the brain of the Djungarian hamster, *Phodopus sungorus*, close to the midsagittal plane. Lines represent the planes of section of micrographs in Fig. 2; letters correspond to the individual micrographs. Abbreviations: T, telencephalon; CC, corpus callosum; Pl, plexus choroideus of the III ventricle; SM, stria medullaris; MH, medial habenular nucleus; CH, habenular commissure; Th, thalamus; DP, deep pineal organ; PS, pineal stalk; SP, superficial pineal organ; CP, posterior commissure; SCO, subcommissural organ; SC, superior colliculus; Ce, cerebellum. Fig. 2 (right). Retinal S-antigen–like reactivity in the deep portion of the pineal organ, the epithalamic and mesencephalic region of the Djungarian hamster, *Phodopus sungorus*. Coronal paraffin sections. Arrowheads show the deep portion of the pineal organ. Abbreviations: H, habenular nucleus; CH, habenular commissure; CP, posterior commissure; PR, pineal recess of the third ventricle; III, third ventricle; SCO, subcommissural organ; SM, stria medullaris; PT, pretectal area; CA, cerebral aqueduct. Asterisks indicate scattered immunoreactive cells in the habenular nucleus and arrows the immunoreactive processes of pinealocytes. Bars, 20 μm. (a) Survey of the epithalamic region; deep pineal, comprising numerous strongly immunoreactive pinealocytes. Scattered immunoreactive cells in the habenular nucleus. (b) Control region corresponding to that shown in (a).



No immunoreaction is seen in the deep pineal and the habenular nuclei after incubation of the section with the diluted antibody (1:2000) to which 100 nmol of the purified bovine retinal S-antigen was added. (c to i) Immunoreactive processes of pinealocytes in the habenular nucleus. (j and k) Immunoreactive processes in the region of the posterior commissure.

existence of mammalian pinealofugal projections adds an anatomical entry to a growing list of features, primarily biochemical (*7, 15*), establishing that the mammalian pinealocyte in the sensory cell line evolved from the photosensitive pinealocyte of poikilotherms (*1*).

These findings also challenge current concepts of how the mammalian pineal sends messages. Substantial evidence supports the general belief that it communicates exclusively by releasing melatonin into the blood (*4*). Our evidence leads to the speculation that pinealocyte processes may deliver chemical messages directly to specific target sites in the central nervous system.

An obvious question to address is whether any function of the mammalian pineal gland might depend on central pinealocyte projections. The answer might lie in the body of reports on effects of the administration of melatonin. Many of these studies have been ignored because they use doses of melatonin that are considered high, on the basis of the physiological concentration of the compound in blood (0.1 n*M*). However, the concentration of melatonin outside a pinealocyte process might be several orders of magnitude higher than the normal blood concentration because melatonin within the pineal gland can be 10,000 n*M* and because as a highly lipophilic compound it can readily cross the plasma membrane. Along the same lines, it seems reasonable that other pineal products could be released from these processes. Taurine, for example, is a transmitter substance that is rapidly released from pineal cells through an adrenergic–cyclic AMP–regulated mechanism (*16*).

This development makes the mammalian pinealocyte a candidate for a role as neuron. It will now be important to characterize mammalian pinealocyte processes, to determine if and where they make functional contacts, and to establish their contents and whether they have a physiological function. A more complete understanding of the physiological role of the mammalian pineal gland could result as investigators try to uncover a functional relationship between the pineal organ and areas of the brain receiving pineal projections. Perhaps these connections transmit information about daily rhythmicity.

REFERENCES AND NOTES

1. J. P. Collin, in *The Pineal Gland*, G. E. W. Wolstenholme and J. Knight, Eds. (Churchill-Livingstone, Edinburgh, 1971), pp. 79–125; A. Oksche, *ibid.*, pp. 127–146; L. Vollrath, in *Handbuch der mikroskopishen Anatomic des Menschen*, A. Oksche and L. Vollrath, Eds. (Springer, Berlin, 1981), vol. 4, p. 170.
2. H. Meissl and E. Dodt, *Dev. Endocrinol.* **14**, 61 (1981); A. Oksche and H. G. Hartwig, *Prog. Brain Res.* **52**, 113 (1979); W. D. Eldred, T. E. Finger, J. Nolte, *Cell Tissue Res.* **211**, 215 (1980); H. W. Korf and U. Wagner, *ibid.* **219**, 567 (1981); P. Ekstrom, *J. Comp. Neurol.* **226**, 321 (1984).
3. R. Y. Moore and D. C. Klein, *Brain Res.* **71**, 1 (1974); D. C. Klein and R. Y. Moore, *ibid.* **174**, 245 (1979).
4. S. Clark, R. Short, V. Mark, Eds., *Photoperiodism, Melatonin and the Pineal* (Pitman, London, 1985).
5. K. Hoffman, *Dev. Endocrinol.* **14**, 123 (1981); H. Illnerova, K. Hoffmann, J. Vanecek, *Neuroendocrinology* **38**, 226 (1984). This species has been used to study the role of the pineal gland in photoperiodism.
6. The animals were deeply anesthetized by intraperitoneal injection of pentobarbital (40 mg per kilogram of body weight). They were perfused with physiological saline containing 15,000 I.U. heparin per liter followed by Bouin's fixative. After dissection, the brains were fixed in Bouin's fluid for 48 hours, dehydrated, and embedded in paraffin. They were then cut into serial frontal sections (5 μm thick).
7. C. M. Kalsow and W. B. Wacker, *Invest. Ophthamol. Vis. Sci.* **16**, 181 (1977); *Int. Arch. Allergy Appl. Immunol.* **44**, 11 (1973); H. W. Korf *et al.*, *Cell Tissue Res.* **239**, 81 (1985); M. Mirshahi *et al.*, *Biol. Cell* **52**, 195 (1985); C. Pfister *et al.*, *Science* **228**, 891 (1985).
8. Highly purified bovine retinal S-antigen [(J. S. Zigler, M. Mochizuki, T. Kuwabara, I. Gery, *Invest. Ophthalmol. Vis. Sci.* **259**, 77 (1984)] was emulsified in complete Freund's adjuvant, and each rabbit received a total of approximately 0.5 mg injected subcutaneously at multiple sites at four biweekly intervals. S-Antigen–like immunoreactivity was demonstrated with the use of the peroxidase-antiperoxidase (PAP) method of L. A. Sternberger [*Immunocytochemistry* (Wiley, New York, 1979)]. The sections were incubated (72 hours at 4°C) with the antiserum (NEI 040111184) diluted 1:2000 with phosphate-buffered saline (PBS) (*p*H 7.2), rinsed in PBS (30 minutes), incubated (1 hour at room temperature) with porcine immunoglobulins to rabbit immunoglobulin G (Dako, Copenhagen) diluted 1:20 in PBS, rinsed in PBS (30 minutes), and then incubated (1 hour at room temperature) with the PAP complex (Dako, Copenhagen) diluted 1:80 in PBS. For histochemical demonstration of peroxidase activity, the sections were incubated in 0.25 percent diaminobenzidine containing 0.003 percent peroxide. Controls were prepared by (i) replacing the primary antiserum by normal nonimmune rabbit serum and (ii) incubating the sections with the diluted antibody (1:2000) to which 100 n*M* of the purified bovine retinal S-antigen was added.
9. With the antiserum used in this study, we have demonstrated S-antigen–like immunoreactivity in retinal photoreceptors and in typical photoreceptors of fish and amphibia, in modified pineal photoreceptors of sauropsids, and in pinealocytes of mammals including humans. [H.-W. Korf *et al.*, in *Pineal and Retinal Relationships*, D. C. Klein and P. O'Brien Eds. (Academic Press, New York, in press)]. Immunoreactivity was also found in invertebrate photoreceptors (T. van Veen *et al.*, unpublished results).
10. L. Vollrath, in (*1*); H.-W. Korf and M. Møller, *Pineal Res. Rev.* **2**, 41 (1984).
11. M. Møller, A. Ingild, E. Bock, *Brain Res.* **140**, 1 (1978).
12. A. Björklund, Ch. Owman, K. A. West, *Z. Zellforsch. Mikrosk. Anat.* **127**, 570 (1972).
13. J. T. Nielsen and M. Møller, *Cell Tissue Res.* **187**, 235 (1978); M. Møller, J. T. Nielsen, T. van Veen, *ibid.* **201**, 1 (1979); M. Møller and T. van Veen, in *The Pineal Gland: Anatomy and Biochemistry*, R. J. Reiter, Ed. (CRC Press, Boca Raton, FL, 1981), vol. 1, p. 69.
14. P. Semm, in *The Pineal Gland and Its Endocrine Role*, J. Axelrod, F. Fraschini, G. P. Velg, Eds. (Plenum, New York, 1983), p. 437.
15. R. L. Somers and D. C. Klein, *Science* **226**, 182 (1985).
16. G. H. T. Wheler and D. C. Klein, *Biochem. Biophys. Res. Commun.* **90**, 22 (1979); *Brain Res.* **187**, 155 (1980).

1 October 1985; accepted 18 November 1985

High Titers of Autoantibodies to Topoisomerase I (Scl-70) in Sera from Scleroderma Patients

J. H. SHERO, B. BORDWELL, N. F. ROTHFIELD, W. C. EARNSHAW*

Patients with rheumatic diseases often have circulating autoantibodies to nuclear components. The clinical significance of the antibodies is controversial, although in some cases they are valuable in the diagnosis of the disease. This report presents results of a study of Scl-70, an autoantigen recognized by sera of many patients with the most severe form of progressive systemic sclerosis. It was possible to show, by three independent criteria, that Scl-70 is the abundant nuclear enzyme DNA topoisomerase I. Therefore, antibody probes of high titer and high affinity are now available for the study of this important nuclear enzyme.

RHEUMATIC DISEASES SUCH AS SYSTEMIC lupus erythematosus and scleroderma are often associated with the presence of antinuclear autoantibodies (*1*). With few exceptions (*2*) the cellular identity of these antigens is unknown. We have, therefore, recently begun the detailed characterization of the major chromosomal autoantigens recognized by sera of scleroderma patients (*3, 4*).

Scl-70, a nuclear autoantigen reported to have a molecular weight of 70,000 (70K) (*5, 6*), is recognized by sera from certain scleroderma patients (particularly those with diffuse disease). However, when we used the immunoblotting method to characterize human Scl-70 (Fig. 1), the antigen was found to be significantly larger than previously estimated (*5, 7*). We observed a single

J. H. Shero and W. C. Earnshaw, Department of Cell Biology and Anatomy, Johns Hopkins School of Medicine, Baltimore, MD 21205.
B. Bordwell and N. F. Rothfield, Division of Rheumatic Diseases, Department of Medicine, University of Connecticut Health Sciences Center, Farmington 06032.

*To whom correspondence should be addressed.