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CHAPTER 20

Autoimmune hypothalamic diabetes insipidus ("autoimmune hypothalamitis")

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Introduction

Diabetes insipidus (DI) is a syndrome characterized by hypotonic polyuria and polydipsia. In hypothalamic DI, also known as central, neurogenic or hypothalamic DI, the disorder results from decreased secretion of osmoregulated arginine vasopressin (AVP) hormone which is synthesized in the perikarya of large neurosecretory cells located mainly in the supraoptic and paraventricular nuclei of the hypothalamus. Hypothalamic DI is a rare disorder which may be inherited as either a dominant (Repaske et al., 1990) or recessive (Forssmann, 1955) trait or it may be acquired at any age. Once the diagnosis of hypothalamic DI has been established, every effort should be undertaken to find out its underlying cause which may require specific therapy.

Table I gives a list of possible causes of hypothalamic DI. In many cases no underlying disease is found, and for lack of better insight this form of the disorder is called idiopathic. Idiopathic hypothalamic DI comprised 30 – 45% of all spontaneously acquired cases, even if unrecognized diseases were excluded at long-term follow-up investigations (Blotner, 1958; Moses, 1985). By definition, this form had been ill-defined due to a lack of any specific markers. This matter was fortunately changed by the observation of the case of a patient with a polyendocrine autoimmune syndrome and central DI which led us to the search for and the detection of circulating autoantibodies to hypothalamic vasopressin cells in cases of idiopathic DI (Scherbaum and Bottazzo, 1983). Our findings suggest that idiopathic hypothalamic DI consists of pathogenetically heterogeneous entities. I shall here try to provide an update of the data on autoimmune hypothalamic DI and to give an outlook for possible further research into autoimmune hypothalamitis.

General features of autoimmune diseases are listed in Table II. Autoimmune diseases are characterized by lympho-plasmacellular infiltration of the affected organ and by the detection of humoral and cellular autoimmune reactions directed towards the respective autoantigen(s). Ideally, one should be able to experimentally induce the disease in a healthy animal by the transfer of cells or antibodies from an animal with the disease (Milgrom and Witebsky, 1972).

In most endocrine autoimmune diseases of peripheral glands, production of antibodies, specific cellular infiltration as well as secondary atrophy of the organs have been experimentally induced by immunization with extracts from these glands together with complete Freund's adjuvant (Rose and Witebsky, 1956). Immunization with the respective hormones appears to be less harmful to the animals. An animal model for experimental hypothalamitis has not been established so far. When antibodies to vasopressin were raised in rabbits by injecting the hormone together with complete Freund's adjuvant, Swaab and colleagues (Swaab et al., 1975)

observed transient diabetes insipidus. However, the hypothalamo-neurohypophyseal system was intact on histology with signs of increased hormone synthesis.

Only a few cases of hypothalamic DI have been described where the histopathology of the hypothalamus was studied (Gaupp, 1941; Blotner, 1958; Green et al., 1958; Braverman et al., 1965). In both, idiopathic as well as in familial hypothalamic DI there was a marked loss of neurons as well as gliosis in the supraoptic nuclei. Similar, but less pronounced changes were also seen in the paraventricular nuclei. This is in parallel with the findings in atrophic autoimmune thyroiditis (primary myxoedema), autoimmune Addison's disease and premature ovarian failure with Addison's disease where a loss of specific hormone-producing cells and replacement by fibrous tissue are seen at histopathology (Doniach et al., 1982).

TABLE I

Causes of acquired hypothalamic diabetes insipidus

Trauma
Hypophysectomy, radiation therapy, skull fracture
Tumour
Craniopharyngioma, germinoma, pinealoma, suprasellar cysts, leukaemic infiltrates, metastases (carcinoma of breast, lung)
Granulomatous diseases
Histiocytosis X, sarcoidosis, tuberculosis, syphillis
Encephalitis, meningitis
Vascular lesions
Cerebral thrombosis, haemorrhagia, aneurysm, post-partum necrosis
Autoimmune
Idiopathic

TABLE II

General features of autoimmune diseases

No evident cause for precipitation of diseases
Associated autoimmune disease
Familial trait
Improve during pregnancy
Local infiltration with mononuclear cells
Specifically sensitized lymphocytes
Specific autoantibodies in the serum

Although the brain is traditionally considered to be "immunologically privileged", immune and autoimmune responses can occur. The lack of conventional lymphatic drainage of the brain impedes transport of neural antigens to the lymphoid organs (Bradbury, 1981). However, antigen can be carried from the brain via the CSF.

Under normal conditions, the brain is protected from a variety of circulating cellular and humoral components of the blood by the blood-brain barrier. This barrier consists of a capillary and endothelial cell layer with tight junctions, a dense basement membrane and an almost complete layer of surrounding astrocytes with their protruding foot processes (Reese and Karnovsky, 1967; Brightman and Reese, 1969), so that passage of molecules and cells is only possible after penetration of the luminal and anteluminal cell membrane. This prevents the passive entry of immunoglobulins, large immunomodulators, and immunocompetent cells. However, in the circumventricular organs, such as the organum vasculosum laminae terminalis of the hypothalamus as well as at the median eminence, such a barrier does not exist (Weindl, 1973), so that an exchange can take place between the peripheral immune system and hypothalamic structures. It has also been shown that pathological clinical conditions such as encephalitis and primary or secondary brain tumors (Hirano et al., 1971; Long, 1979) as well as a number of experimental conditions such as drug-induced seizures (Lorenzo et al., 1972), intra-arterial infusions of hypertonic solutions or intracerebral injection of arachidonic acid (Westergaard, 1980; Chan and Fishman, 1984) may cause shrinking of endothelia, disconnection of tight junctions and reversible opening of the blood-brain barrier.

It has also been shown that leukocytes may leave the blood vessels in the brain in response to specific homing molecules or chemotactic factors and then secrete degradative enzymes to cleave a passage

through tissue (Naparstek et al., 1984). Thus, the normal absence of leukocytes from the brain, and their occurrence under pathological conditions such as multiple sclerosis and viral encephalitis (Hafler and Weiner, 1987) as well as in experimental models (Matsumoto et al., 1986) may represent changes in the expression of homing molecules as much as in the state of the physical blood-brain barrier (Lampson, 1987).

In addition to the immunological isolation of the brain, both neurons and glia normally lack major histocompatibility class I and class II antigens on their surface, which are essential for the ability of cells to interact with T lymphocytes. Thus, normal neural tissues cannot respond to immunocompetent T lymphocytes even if they have entered the brain (Lampson and Hickey, 1986). However, HLA class I expression can be induced in neuronal cells after exposure to, e.g., interferon (Wong et al., 1985). Cultured glial, but not neuronal cells are able to express HLA class II molecules which can present antigens in vitro (Fontana et al., 1984).

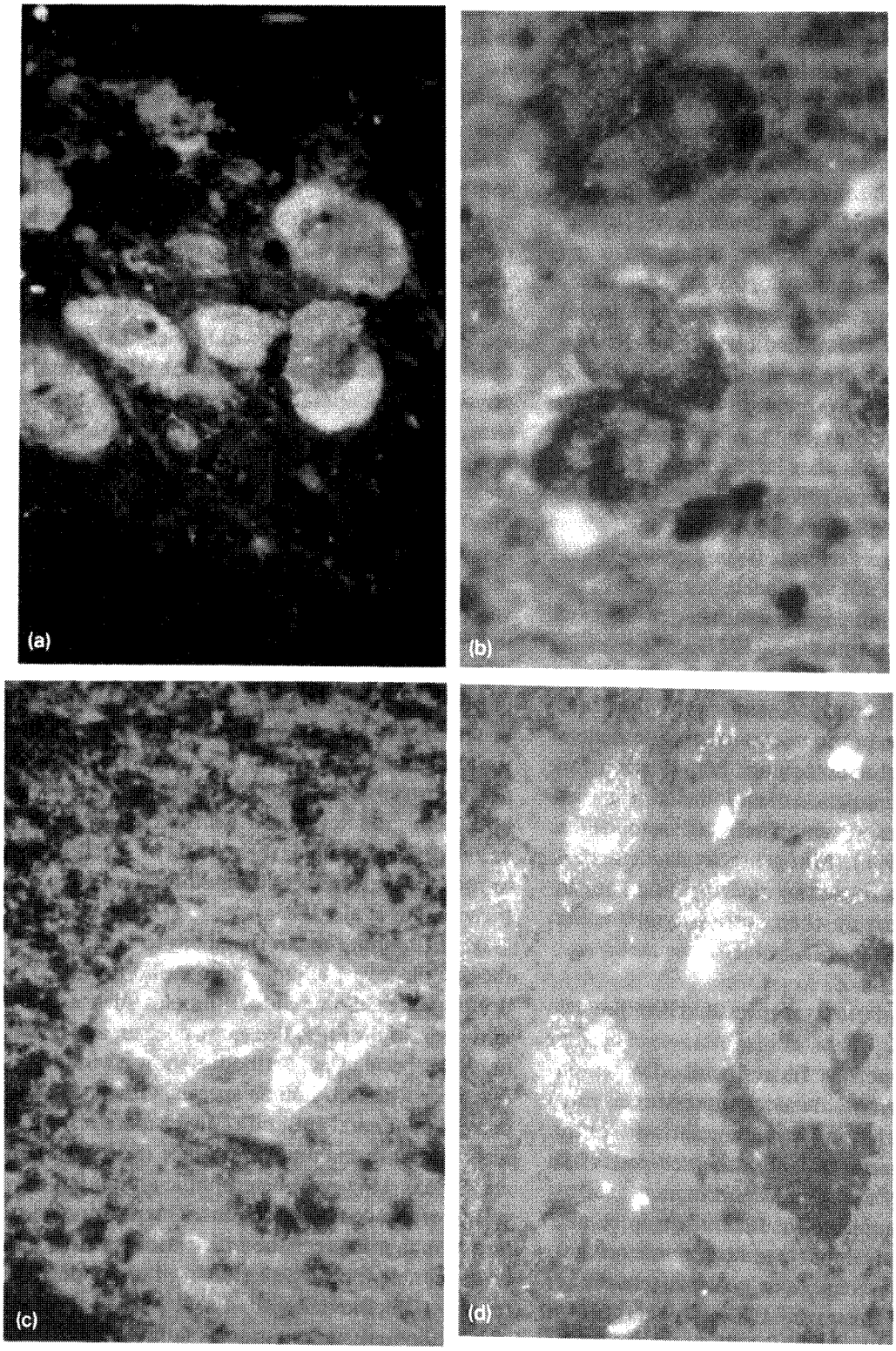
A further immunological protection of the brain is provided by the fact that lymphocytes are much less adhesive for brain microvessel endothelium than extracerebral endothelium, but adhesion can be increased by stimulation of endothelial cells with interferon gamma or tumor necrosis factor-alpha (Male et al., 1990a). Mitogen-activated lymphocytes or T cells activated by their specific antigen bind more effectively than resting lymphocytes (Male et al., 1990b).

Detection of cytoplasmic vasopressin cell antibodies

Like other cell-type specific autoantibodies within complex organs hypothalamic vasopressin cell antibodies are detected by indirect immunofluorescence testing on organ sections. Unlike the well-preserved neurotransmitters and peptide hormones such as vasopressin (Emson et al., 1982; Rossor et al., 1982), the autoantigen of these vasopressin cell antibodies is very susceptible to post-mortem decay (Scherbaum and Bottazzo, 1983), so that fresh, shock-frozen tissue has to be used in the assay. Post-fixation of cryostat sections with acetone does not influence the antibody reactivity whereas fixatives like Bouin's or formaldehyde lead to unpredictable results, often giving false-positive or false-negative reactions (own unpublished observation).

Human fetal hypothalamus has been applied as a source of antigen in all previous investigations. It should be mentioned, however, that the suitability of such specimens from prostaglandin-induced abortions is uncertain, and it should now be recommended to limit the use of hypothalamic tissues to those specimens obtained within 8 h after induction of abortion when hypothalamic cells still respond to physiological stimuli in perifusion systems (Rasmussen et al., 1986). In our obstetrical unit, however, the abortions are induced by gradually increasing doses of prostaglandins which cause a minimum of side effects to the women, but lead to delayed abortions, so that the time of fetal death in utero cannot be clearly denominated. Therefore, vasopressin cell antibody positive sera have always to serve as controls to test the suitability of a given tissue. Human adult post-mortem tissue would be a preferable source of antigen, but post-mortem delay of more than 2 h renders this tissue unsuitable for the test (Scherbaum, 1987). Also tissues from older donors are unsuitable since the autofluorescence of lipofuscin granules within neurosecretory cells of such individuals impair the reading of specific reactions in the indirect immunofluorescence test (Scherbaum et al., 1985a).

By definition, human tissue has to be applied in the first place when human autoantibodies are to be demonstrated. Some autoantibodies such as anti-mitochondrial antibodies, anti-nuclear antibodies or gastric parietal cell antibodies show a wide cross-reactivity with non-human species. Others, such as thyroid or adrenal antibodies give a more restricted reactivity with mammalian tissues (Scherbaum et al., 1986a). However, heterophilic antibodies may still give false-positive reactions. This also applies to non-human primate tissue which has been shown to be unsuitable for the detection of pituitary autoantibodies (Glück and Scherbaum, 1990). The suitability of baboon tissue as a source of antigen to test for


(a)
(b)
(c)
(d)

vasopressin cell antibodies has not been extensively studied. In the limited number of comparative tests we have performed so far, the number of false-negative reactions has not been evaluated. However, eight out of ten cases which had been positive for vasopressin cell antibodies on human tissue, were also positive when baboon hypothalamus was used (Scherbaum et al., 1985a).

Vasopressin cell antibodies give a coarse cytoplasmic staining pattern of vasopressin cells. The titers are very low, so that undiluted sera have to be applied in the first place. The antibodies may be of the IgG and/or IgA and/or IgM class so that the serum reaction has to be visualized with a polyvalent anti-IgG/IgA/IgM anti-human immunoglobulin conjugate. Vasopressin cell antibodies are equally frequent of the IgG and IgA class, but IgM antibodies rarely occur. Some of the vasopressin-containing sera also react with oxytocin cells, but others are strictly cell type-specific (Scherbaum and Bottazzo, 1983). About half the vasopressin cell antibodies fix complement. The complement-fixing ability of antibodies detected in the sera of healthy individuals has been shown to indicate high risk for rapid progression to clinical disease in islet cell antibody-positive non-diabetic individuals (Tarn et al., 1988) as well as in adrenal antibody-positive individuals without Addison's disease (Scherbaum and Berg, 1982). Healthy individuals with vasopressin cell antibodies in their sera have not been detected so far so that the impact of complement-fixing vasopressin cell antibodies cannot be determined. However, no correlation has been found between the complement-fixing ability of vasopressin cell antibodies and a distinct subgroup of patients with hypothalamic DI, nor with the severity of disease (Scherbaum, 1987).

Reactivity of the specific vasopressin cell autoantigen

While the autoantigens corresponding to a number of autoantibodies to peripheral endocrine glands have been characterized, the biochemical nature of the autoantigen reacting with vasopressin cell antibodies is still unknown. It is evident from absorption experiments that the autoantigen is distinct from the hormones arginine vasopressin, oxytocin, or their corresponding neurophysins NPII and NPI. Preincubation with an excess of the above mentioned substances does not affect the vasopressin cell reactivity of positive sera. Also all vasopressin cell antibody positive and negative patients' sera tested so far were negative for circulating vasopressin or neurophysin antibodies when tested by a sensitive direct radiobinding assay with iodinated vasopressin and neurophysin (Scherbaum et al., 1985b). This is in line with other cytoplasmic autoantibodies to endocrine glands detected by immunofluorescence testing, such as thyroid microsomal antibodies which are not directed to the hormones, but to cytoplasmic enzymes associated with the function of the respective cells (Czarnocka et al., 1985).

Fig. 1. *a.* Autoantibodies to hypothalamic vasopressin cells. An unfixed 7 μm cryostat section of human hypothalamus at the level of the supraoptic nucleus (SON) was incubated with native serum from a patient with idiopathic hypothalamic diabetes insipidus and stained with FITC-labeled anti-human-IgG. Note that the cytoplasm of large cells is stained. It could be shown by the four-layer double-fluorochrome immunofluorescence test with anti-vasopressin in the second sandwich that vasopressin cells were stained (magnification: × 250). *b.* The same area of the SON as in Fig. 1*a* incubated with normal human serum and FITC-labeled polyvalent anti-human immunoglobulin. Note that the background is brighter than the dark neurosecretory cell bodies (magnification: × 400). *c.* The same area of the SON as in Fig. 1*a* incubated with the serum of a patient with systemic lupus erythematosus containing the rare anti-ribosomal antibodies visualized by FITC-labeled anti-human IgG which may in a very rare case disturb the detection of vasopressin cell antibodies. Note the coarsely granulated cytoplasmic staining of the two large cell bodies (magnification: × 400). *d.* 7 μm cryostat section of human hypothalamus at the level of the SON. The specimen was obtained from a donor aged 50. The section was incubated with normal human serum and FITC-labeled polyvalent anti-human immunoglobulin. The autofluorescent lipofuscin deposits in the cell bodies of large neurosecretory cells hamper the evaluation of test results (magnification: × 250).

In order to account for a possible functional role, vasopressin cell antibodies should theoretically bind to structures represented on the surface of the cells, so that they can react with their respective autoantigens in vivo. Such a cell surface reactivity has been detected with the thyroid microsomal (Khoury et al., 1981), the adrenocortical (Khoury et al., 1980) and the gastric parietal cell antigen (Masala et al., 1980) as well as with the 64-kDa islet cell autoantigen glutamate decarboxylase GAD (Christie et al., 1990). Similar to the above observations we have shown in selected cases that vasopressin cell antibodies react with the surface membrane of cultured human fetal hypothalamic vasopressin cells in vitro (Scherbaum et al., 1985c).

Vasopressin cell antibodies in different forms of diabetes insipidus

The symptoms of hypothalamic DI can now easily be cured by the substitution with vasopressin analogs. However, it is clinically essential to search for a specific disease underlying hypothalamic DI. The detection of such diseases often depends on the sensitivity of the diagnostic procedures and on the duration of follow-up investigations. Among 124 patients with hypothalamic DI published by Blotner (1958), 45% were classified as idiopathic in origin. Computed tomography, nuclear magnetic imaging and sensitive pituitary function tests may now reveal, e.g., germinomas, cranial sarcoidosis or tumour metastases long before clinical symptoms become apparent. In a more recent series by Moses (1985) 30% of the cases of spontaneously acquired hypothalamic DI were classified as idiopathic.

The detection of vasopressin cell antibodies now helps to distinguish patients with possible autoimmune hypothalamic DI from other forms of idiopathic DI. One third of patients with idiopathic hypothalamic DI are positive for these antibodies. The percentage is about the same in adults (Scherbaum and Bottazzo, 1983) and in children (Scherbaum et al., 1985b) with this diagnosis. The sera from mixed hospital controls, cases of familial DI or from patients with nephrogenic DI were all negative. So were the sera from patients with DIDMOAD, a syndrome including *d*iabetes *i*nsipidus, *d*iabetes *m*ellitus, *o*ptic *a*trophy and *d*eafness. As shown in Table III, there was one important group of patients with hypothalamic DI who were frequently positive for vasopressin cell antibodies, but there were only individual positive cases in other forms of symptomatic DI.

Vasopressin cell antibodies have been detected in about half the cases of hypothalamic DI, secondary to histiocytosis X (Scherbaum et al., 1986b). Thymic abnormalities and antibodies to autologous erythrocytes have been observed in histiocytosis X

TABLE III

Prevalence of vasopressin cell antibodies in different forms of diabetes insipidus (DI)

Form of diabetes insipidus	Number tested	Number positive AVP cell antibodies
Idiopathic hypothalamic DI	52	20 (38%)
Histiocytosis X with DI	11	6 (54%)
Craniopharyngioma	11	1 (9%)
Other forms of symptomatic hypothalamic DI*	55	0 (0%)
Familial hypothalamic DI	6	0 (0%)
Nephrogenic DI	15	0 (0%)

*Other forms of symptomatic hypothalamic DI included: pituitary adenomas with post-operative DI (20), germinomas (7), DIDMOAD (diabetes insipidus, diabetes mellitus, optic atrophy and deafness, 10), metastatic tumours (5), traumatic DI (4), sarcoidosis with DI (3), post-irradiation (2), acute myeloid leukaemia (2), malformation of the hypothalamus (2).

(Nesbit et al., 1981). Clinical observations with spontaneous remissions also suggest that autoimmunity is involved at least in a subgroup of such patients (Broadbent et al., 1984). Histiocytosis X cells may invade the hypothalamus (Cline and Golde, 1973) and break the blood-brain barrier through a local inflammatory reaction. Like antigen presenting cells of the macrophage type, histiocytosis X cells bear HLA class II antigens on their surface (Murphy et al., 1981) which render them readily active in presenting locally disrupted antigen to preexisting lymphocytes. Non-processed antigen is well recognized by B lymphocytes (Lanzavecchia, 1985) which may be stimulated to proliferate and produce a specific immune response to hypothalamic tissues. A similar phenomenon has also been described in the case of a patient with histiocytosis X invading the thyroid gland, where high titers of thyroid autoantibodies were observed (Sinisi et al., 1986). Determination of vasopressin cell antibodies in cases of histiocytosis X may allow to recognize hypothalamic infiltration before the onset of clinical symptoms of hypothalamic DI which may prove to be helpful in the therapeutic management of patients with histiocytosis X.

Polyendocrine autoimmunity and hypothalamic diabetes insipidus

The coexistence of established autoimmune diseases with an idiopathic endocrine disorder suggests the possibility of an autoimmune pathogenesis of the latter. Patients with polyendocrine autoimmune diseases have provided the best source for the detection of new autoantibody specificities in endocrine diseases of unknown origin, and such a case was also the basis of our original hypothesis to suggest an autoimmune variant of hypothalamic diabetes insipidus. Two years later, an interesting further case was reported with hypothalamic diabetes insipidus associated with hypopituitarism, type 1 (insulin-dependent) diabetes mellitus, pernicious anemia and circulating antibodies to the thyroid gland, adrenal cortex, gastric parietal cells and pancreatic islet cells (Bhan and O'Brien, 1982).

Polyendocrine autoimmune syndromes are classified into three groups (Neufeld et al., 1980): the rare type 1 with primary hypoparathyroidism and recurrent mucocutaneous candidiasis which appears during the first years of life and where Addison's disease mostly develops later in childhood; type 2 polyendocrine autoimmunity centered around autoimmune Addison's disease which is associated with autoimmune thyroid diseases and/or type 1 diabetes mellitus; and type 3 polyendocrine autoimmunity characterized by either autoimmune thyroid disease or/and type 1 diabetes mellitus which is associated with another autoimmune disorder such as vitiligo, pernicious anemia or autoimmune myasthenia gravis.

Out of 39 cases of idiopathic hypothalamic DI studied, one or more autoimmune diseases were associated in 11 cases (28%). In two additional cases, autoantibodies to thyroid microsomes or to thyroglobulin were detected in the serum, suggesting subclinical autoimmunity. The list of autoimmune diseases and autoantibodies associated with idiopathic hypothalamic DI in our series is given in Tables IV and V. Thyroid autoimmunity is the main association, but it was not uncommon to observe more than one endocrine disease occurring together with DI. When a series of children with

TABLE IV

Associated organ-specific autoimmune diseases in 39 patients with idiopathic hypothalamic diabetes insipidus

M 22	Primary hypoparathyroidism, alopecia totalis, mucocutaneous candidiasis, pernicious anemia
F 73	Addison's disease, primary myxoedema, pernicious anemia
M 53	Addison's disease, Hashimoto's thyroiditis
F 62	Type 1 diabetes mellitus, Hashimoto's thyroiditis
M 61	Primary myxoedema, Sjögren's syndrome
M 21	Type 1 diabetes mellitus (and family history of type 1 diabetes)
F 24	Hashimoto's thyroiditis
F 39	Hashimoto's thyroiditis
F 52	Graves' thyrotoxicosis
F 37	Graves' thyrotoxicosis
M 60	Alopecia totalis, myasthenia gravis

TABLE V

Prevalence of endocrine autoantibodies in patients with idiopathic and secondary diabetes insipidus

	Form of diabetes insipidus	
	Idiopathic (n = 39)	Secondary (n = 81)
Autoantibodies to:		
Thyroid microsomes	6	4
Thyroglobulin	3	1
Gastric parietal cells	2	2
Intrinsic factor	1	–
Adrenal cortex	2	–
Gonadal steroid cells	2	–
Pancreatic islet cells	2	–
Pituitary prolactin cells	3	–
Number of cases with organ-specific antibodies	11 (28%)	6 (7%)

idiopathic hypothalamic DI was investigated in a separate study (Scherbaum et al., 1985b), polyendocrine cases were rare. This may reflect a distinct time course of polyendocrine autoimmunity where associated endocrine autoimmune diseases may develop later in life.

Conclusions and outlook

After the description of an autoimmune form of hypothalamic diabetes insipidus the spectrum of endocrine autoimmune diseases now extends to the hypothalamus. Our findings suggest that autoimmunity may also play a role in other hypothalamic endocrine defects which have hitherto been regarded as idiopathic. It is theoretically possible that some cases of corticotropin deficiency, hypothyroidism, hypogonadism or growth hormone deficiency are related to cell type-specific hypothalamic autoimmunity. The inaccessability of hypothalamic tissue in vivo and the poor availability of fresh human post-mortem hypothalamus, however, remain a major obstacle in further investigations. It would be interesting to assess the functional role of vasopressin cell antibodies by applying them in perifusion studies of human post-mortem hypothalamus explants. It is also unclear if vasopressin cell surface antibodies detected in the here described binding studies exert a cytotoxic effect which may be releated to the specific cell loss in the supraoptic and paraventricular nuclei. This matter as well as the question whether complement fixing vasopressin cell antibodies participate in the destructive process may be answered by cell culture studies. Will it be possible to establish an animal model of experimentally induced or even of spontaneous autoimmune hypothalamitis? Animal models like the spontaneously diabetic non-obese (NOD) mouse have provided new insights into the pathogenesis of autoimmune diseases and have allowed to develop new therapeutic strategies. What is the role of T lymphocytes in autoimmune DI? Is it possible to detect specifically sensitized T lymphocytes in the peripheral blood of patients with autoimmune DI? To answer these questions, one should get hold of the responsible autoantigen(s) in the hypothalamus. The availability of new techniques such as the screening of a human cDNA library with the autoantibodies may now allow to proceed in this field.

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Discussion

D.F. Swaab: One of the observations in other autoimmune diseases is that they might improve during pregnancy. What has been reported on such changes during pregnancy for idiopathic diabetes insipidus (DI) in literature and what is your experience? Secondly, do you think that the vasopressin cell autoantibodies are responsible for the destruction of the vasopressin neurons in autoimmune hypothalamic diabetes insipidus?

W.A. Scherbaum: This is a very important point which is true for Graves' thyrotoxicosis, autoimmune myasthenia gravis, systemic lupus erythematosus, and probably also for autoimmune hypothalamic DI. We have observed a patient with hypothalamic DI due to histiocytosis X who was also positive for vasopressin cell antibodies. In this patient the severe histiocytic skin lesions and lymph node swellings as well as DI remitted at about the 28th week of gestation and recurred after delivery. This is very remarkable in light of the fact that vasopressinase activity increases during pregnancy so that patients with symptomatic hypothalamic DI usually require larger doses of DDAVP during pregnancy.

Although this matter has not been studied in hypothalamic DI, I would not suspect that the vasopressin cell antibodies cause the disease. There are just a few autoimmune diseases, such as autoimmune myasthenia gravis or Graves' thyrotoxicosis, where specific receptor antibodies may directly interfere with the function of the corresponding organ. In other autoimmune diseases, such as experimentally induced autoimmune encephalomyelitis (EAE) or insulin-dependent diabetes mellitus in the non-obese diabetic (NOD) mouse, this matter could be studied by transfer experiments of serum or lymphocytes from diseased animals to unaffected individuals. There it could be shown that the antibodies apparently do not cause functional defects or cellular lesions. Such lesions, however, are caused by the transfer of (antigen-specific, cytotoxic) T-lymphocytes which thus appear to be more directly involved in the effector mechanisms.

G.J. Boer: It is very interesting to see that the autoantibodies you described are so specific for the vasopressin cells. I have two questions in this respect: (1) have you obtained evidence that human serum containing the autoantibodies can stain the HNS of other species? Using a sample of one of the sera we obtained from you a couple of years ago, Fred van Leeuwen and I failed to find staining in rat brain.

(2) Have you checked whether the nerve terminals of the VP cells also stain, in order to see if the staining is only at the level of the perikarya?

W.A. Scherbaum: (1) Vasopressin cell autoantibodies are higly species-specific so that I am not surprised you did not obtain a positive reaction when you applied our positive control serum on rat hypothalamus.

(2) We looked at the perikarya because we expected the autoantigen to be located there. We have not looked at nerve terminals.

H.M. Charlton: Why don't you use a rat vasopressin cell line to induce experimental hypothalamic DI in rats?

W. A. Scherbaum: To my knowledge nobody has raised such cell lines so far.

D.F. Swaab: Tixier-Vidal and De Vitry (1979) have described a vasopressin-containing cell line.

V. Geenen: (1) Did you characterize auto-antigen(s) in autoimmune DI?

(2) Have you completely eliminated the hypothesis that the autoantigens could be members of the neurohypophyseal peptide family?

W.A. Scherbaum: (1) This is a difficult task since we would need a large amount of such human vasopressin cells on the one hand, and B-lymphocyte clones producing vasopressin cell antibodies, isolated from patients with idiopathic cranial DI to immunoprecipitate the autoantigens. Screening of a human brain-specific library would be another way.

(2) There are arguments supporting the idea that the vasopressin cell antigen reacting in our own assay is different from the hormones vasopressin and oxytocin or their corresponding neurophysins:

(a) Patients with hypothalamic DI who have been treated with vasopressin for a long time are usually negative in our assay.

(b) We have done absorption studies with the above substances and the reactivity of our autoantibodies remained unchanged. Also there were no such antibodies detected in a direct radiobinding assay to elucidate this issue.

(c) In no system – islet cell antibodies/insulin, thyroid microsomal antibodies/thyroid hormones, and others – was the method of indirect immunofluorescence sensitive enough to detect the respective anti-hormone antibodies which are detected by other assays.

E. Braak: Have you been able to demonstrate components of the complement system with immunocytochemistry in the hypothalamus of patients with DI?

W.A. Scherbaum: What we have done is to incubate patients' sera with normal human hypothalamus, and we looked for the complement-fixing ability of autoantibodies by anti-complement conjugates. Here, about half the vasopressin cell antibodies were able to fix complement. What one wants to know, of course, is if there are immune complex deposits in the hypothalamus of such patients. Such deposits which are shown by direct immunofluorescence and staining, e.g., with anti-IgG or anti-complement antibodies, have indeed been shown in the pancreatic islets of patients who died with newly diagnosed type 1 diabetes mellitus and on the basement membranes of goitres removed from patients with Hashimoto's thyroiditis. Patients with idiopathic hypothalamic DI live a long life and do not usually die of their disease, so that no such immunocytochemical studies are available from patients with idiopathic DI.

H.P.H. Kremer: Were you able to demonstrate CSF abnormalities, i.e., antibodies in the CSF or oligoclonal bands in CSF or an increase in mononuclear cells?

W.A. Scherbaum: We have not tested CSF from these people since in most of our cases the diagnosis of DI had been already established, so that collection of CSF was not justifiable. Therefore we just looked at serum. Nevertheless, investigation of CSF in cases of DI would be very interesting indeed.

E. Fliers: (1) In type 1 diabetes mellitus (IDDM) it has been shown that asymptomatic NOD mice have antibodies against Langerhans islets long before they develop diabetes. Do you have data on the prevalence of anti-VP cell antibodies in subjects without DI?

(2) Might autoimmune hypothalamitis be treated with corticosteroids?

W.A. Scherbaum: (1) It would certainly be very interesting to investigate sera from normal individuals for vasopressin cell antibodies and thus for preclinical autoimmune hypothalamitis. However, hypothalamic DI and its autoimmune variant are very rare, so that I guess one would have to test thousands of individuals to pick up a positive not yet affected individual. As you can imagine, this would be impossible with the time-consuming vasopressin cell antibody test and the shortage of appropriate tissues.

(2) Theoretically, the autoimmune reaction should be ameliorated by immunosuppresive drugs. However, this is not justifiable from the clinical standpoint, since replacement therapy of vasopressin with its analogs is very easy and safe, without known side effects when appropriately applied. On the other hand you have to consider that at the time when complete hypothalamic DI is diagnosed, it is likely that already 90% of the vasopressin neurons are destroyed. Since the repair capacity of the adult brain is limited, you will just deal with the remaining 10% of cells. From type 1 diabetes mellitus we know that this is – at least in the long run – a point of no return, and all individuals will eventually, require full hormone replacement therapy even when they are treated with potent immunosuppressive drugs, such as cyclosporin A.

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