AUTOIMMUNITY TO ANTERIOR PITUITARY CELLS AND THE PATHOGENESIS OF INSULIN-DEPENDENT DIABETES MELLITUS

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Summary An immunofluorescence study using unfixed cryostat sections of human pituitary glands was carried out on sera from patients with type-Ia (juvenile-onset) diabetes (61 recent onset, 48 longstanding). 63 of their selected high-risk first-degree relatives and 117 patients with type-Ib ("polyendocrine") diabetes were tested for comparison. Healthy controls included 48 sera from laboratory staff and students. Pituitary-cell antibodies were found in none of the controls, in 2% of patients with longstanding diabetes, in 16-6% of patients with diabetes of recent onset, and in 36-6% of genetically predisposed relatives with islet-cell antibodies in their sera (of whom 7 became diabetic during a 3-year follow-up period, 4 of them reacting with pituitary cells for 1-3 years before the onset of diabetes). Thus pituitary antibodies tended to disappear after onset of symptoms. Many of the sera reacted with multiple anterior-pituitary cell types. These findings suggest a wider involvement of the endocrine-organ system in the pathogenesis of insulin-dependent diabetes and are in accordance with clinical observations showing excess growth in prepubertal boys at onset of diabetic symptoms and with the results of experiments on virus-induced diabetes in mice. The connection of these pituitary antibodies with autoimmune lymphocytic hypophysitis is at present unknown.

Introduction

The pathological hallmark of type-I (insulin-dependent) diabetes mellitus is a selective loss of pancreatic beta-cells. In young-onset cases (type-Ia), 1 which represent the majority, beta-cell destruction is probably initiated by a number of environmental insults (chemical toxins, insulitropic viruses) in genetically predisposed subjects. 2 It has been suggested that these people are more likely to inherit the HLA-DR3, B8-B8-Cw7-A1 haplotype, one of the two haplotypes which are now firmly established as contributing a major susceptibility to the disease. 3 Most population studies in children show an excess of 12-25% of boys and it is of interest that boys are more prone to some virus infections—e.g., polio and coxsackie B meningitis. These clinical observations are underlined by experiments in mice which show a striking differential effect of sex hormones on the development of diabetes and insulin in susceptible strains. 4

In type-Ib diabetes the autoimmune phenomena (islet-cell antibodies (ICA), abnormal leucocyte-migration inhibition, and raised islet levels) tend to be transient and may be entirely secondary to the islet damage. In contrast, in the later-onset more "primary autoimmune" type-Ib diabetes, there is a striking female preponderance and a tendency for lasting humoral and cell-mediated autoimmune features. 5 This might be compatible with a defect of antigen-specific suppressor T lymphocytes allowing unopposed immune reactions to persist. 6 Prospective studies have provided strong evidence for long prediabetic intervals between the initiation of beta-cell destruction, the development of biochemical decompensation, and the onset of overt diabetes. 7 The persistence of complement-fixing islet-cell antibodies (CFA-ICA) during this long interval reinforces the concept that these antibodies are useful monitors of active immune-mediated beta-cell impairment. 8 This long symptom-free latent period could be a time when abnormalities in other endocrine glands might also occur. A number of reports have described children with unusually tall stature presenting with type-I diabetes. The potential importance of this phenomenon is highlighted in a study of 77 newly diagnosed patients. 9 Boys, particularly those of prepubertal age, were tall at onset, and skeletal maturity was significantly advanced in both sexes, suggesting a prediabetic metabolic or hormonal abnormality. Thus pituitary and other endocrine effects may play a fundamental part in the early pathogenesis of type-I diabetes.

In view of these observations and despite the previous finding of pituitary antibodies mainly in polyendocrine autoimmunity, 10 we decided to look for these markers in patients with recently diagnosed diabetes and in their genetically predisposed relatives during the latency period.

Subjects

Sera were collected from the following groups:

Diabetics

(a) 61 patients with symptoms starting up to 1 year previously. These included 32 males and 29 females aged between 3 and 33 years (mean 18).
(b) 48 patients with longstanding type-1 diabetes (duration 3–23 years). These included 28 males and 20 females aged 8–43 (mean 16) selected from the prospective Barts-Windsor family study. From the 168 families in that study, 24 probands with persistent islet-cell antibodies were selected. Such patients are unusual among diabetic children, and this might indicate a more extensive immunological defect. The other 24 probands selected were ICA-negative, having presumably lost their islet-cell antibodies before recruitment to the study.

(c) 117 patients with type-1 diabetes of varying duration and having other evidence of endocrine autoimmunity, whose sera were received in the laboratory from various hospitals since 1976. These patients were older, and the sex ratio was 3F:1M.

Predisposed First-degree Relatives

We also tested on pituitary 63 first-degree relatives selected from a total of 648 from the Barts-Windsor prospective family study to determine the predictive value of pituitary antibodies in genetic predisposed diabetes, assessed by HLA-genotyping and by close follow-up of islet-cell and other antibodies. 33 were chosen because their sera contained ICA: 13 of these were complement-fixing (CF-ICA), and 20 were demonstrable only with anti-IgG antibodies (ICA-IgG). The other 30 relatives were free of islet-cell antibodies (15 from families with one member having pituitary antibodies and 15 chosen randomly). The 63 relatives were 38 males and 25 females aged 3–45 (mean age 18). The overall sex ratio for the type-1 diabetics and their relatives was 1:3M:1F. All relatives were non-diabetic in 1978, but the disease developed in 7 during the follow-up period. Tests on pituitary were done on between 6 and 10 specimens for each case. All sera were stored at −20°C until used.

Controls

48 sera from laboratory staff and medical students were tested as controls on pituitary gland but were not matched for age and sex with the diabetics (age 22–35; sex ratio 2.5M:IF).

Methods

The substrate for the immunofluorescence (IFL) tests was human pituitary glands obtained from hypophysectomies for pituitary tumours or breast carcinoma. Unfixed 4 μm cryostat sections were used for the studies, and each block was first checked for presence and distribution of the different cell types, using the appropriate horse anti-sera. The patients’ sera were all screened undiluted in the sandwich test with anti-IgG, anti-IgM, and anti-IgA fluorescein-isothiocyanate conjugates. Some pituitary-positive sera were rerested with specific conjugate to human C3. Islet-cell antibodies were always detected on unfixed group O pancreas by two methods—the sandwich test and the immunofluorescent complement-fixation test in which a third layer consists of fresh human serum added as a source of complement. Other antibodies were looked for by IFL, except for thyroglobulin and thyroid microsom antibodies, which were detected with the Wellcome THY-M & M kits.11

The particular endocrine pituitary cells reacting with patients’ sera were identified with the four-layer double-fluorochrome IFL technique,12 using mouse hybridoma monoclonal antibodies to human prolactin, growth hormone, and luteinising hormone.13 Monoclonal anti-thyrotropin and anti-follicle-stimulating hormone antibodies have not yet been applied. Autoantibodies to the corticotropin (ACTH) cells cannot be studied without splitting the immunoglobulin molecules with papain to produce Fab fragments, since nearly all normal human immunoglobulins react with ACTH cells in the IFL test through their Fc backbone.14

Results

Prevalence of Pituitary Antibodies

The overall prevalence of pituitary antibodies in the 337 sera tested is shown in table 1. The 48 normal staff and medical students were all negative on pituitary gland. Of the remaining 289 sera there were a total of 33 positive reactions. Among the young-onset cases, the 24 positive sera showed an equal sex distribution, whereas in the polyendocrine diabetes series the sex ratio was 2F:1M. Of the recent-onset diabetics, 10(16–6%) reacted with one or more pituitary endocrine cells—1 serum with prolactin cells, the other 9 with multiple cells. All the patients positive on pituitary (4 males and 6 females aged 17–33 years) also had islet-cell antibodies which were CF-ICA in 9 out of 10 cases. Among the selected Barts-Windsor probands with longstanding type-1 diabetes, only 1 male patient (2·2%) reacted with pituitary prolactin and growth-hormone cells. Both his parents had antinuclear antibodies, and 1 of his unaffected sisters had persistent ICA-IgG in her serum, though on pituitary she gave equivocal staining, which was counted as negative.

The most striking results were obtained with the sera of the genetically predisposed relatives: 13 out of 63 (20%) had pituitary antibodies, including 1 father, 2 mothers, 7 brothers, and 3 sisters. Of the still more highly selected group of ICA-positive subjects, over 36% reacted by IFL on anterior pituitary, most of them with multiple endocrine

<table>
<thead>
<tr>
<th>Groups tested</th>
<th>No. tested</th>
<th>Total positive</th>
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</thead>
<tbody>
<tr>
<td>Type-1 juvenile IDDM: Duration up to 1 yr: ICA positive</td>
<td>51</td>
<td>10 (19–6%)</td>
</tr>
<tr>
<td>ICA negative</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Duration 3–23 yr: ICA positive</td>
<td>24</td>
<td>1 (2–28%)</td>
</tr>
<tr>
<td>ICA negative</td>
<td>24</td>
<td>0</td>
</tr>
<tr>
<td>First-degree relatives: ICA positive</td>
<td>33</td>
<td>12 (36–1%)</td>
</tr>
<tr>
<td>ICA negative</td>
<td>30</td>
<td>1 (3–3%)</td>
</tr>
<tr>
<td>Type-B polyendocrine IDDM</td>
<td>117</td>
<td>9 (7–7%)</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>48</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Characterisation by double immunofluorescence</th>
<th>Single cells</th>
<th>Multiple cells</th>
<th>Unidentified cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH /TSH /Others</td>
<td>PRL /GH /LH /PRL + GH /Others</td>
<td>PRL /GH /Others</td>
<td></td>
</tr>
</tbody>
</table>

IDDM = insulin-dependent diabetes mellitus. PRL = prolactin. GH = growth hormone. LH = luteinising hormone. FSH = follicle-stimulating hormone. TSH = thyrotrophin.

*One serum also showed PRL-cell antibodies.
†Proband and relatives from Barts-Windsor IDDM family study.
cells. Among the ICA-negative relatives, only 1 female sib, aged 20, reacted with pituitary. She was healthy, without other detectable antibodies, and belonged to 1 of 2 families showing 2 members with pituitary antibodies (F006, fig. 1). The HLA-DR frequencies in those first-degree subjects with pituitary antibodies showed the expected distribution of DR3 and DR4 observed in children with typical type-1 diabetes.

The 117 patients with polyendocrine diabetes included 9 pituitary-positive patients; 3 males (of whom the youngest was 8 years old) and 6 females. 8 of the 9 reacted with prolactin cells and 1 with at least 2 cell types—an overall frequency of 7-7%.

**Immunofluorescence Patterns**

Previous studies in polyendocrine autoimmunity showed mostly prolactin-cell\(^5\) and more rarely growth-hormone (hGH)-cell antibodies\(^6\) with a granular cytoplasmic IFL confined to one cell type (fig. 2). When diabetic sera were similarly tested, most of them stained multiple cells. With some sera whole cords of pituitary cells appeared positive, some brighter than others (fig. 3). With the double IFL technique it was possible to show that only 3 of 24 positive sera from the young-onset patients and unaffected relatives reacted exclusively with prolactin cells: 10 showed staining of prolactin and hGH cells, whereas 11 had a “multiple cell” pattern.

**Other Characteristics of Pituitary Antibodies**

The titres were low in the young-onset patients, not exceeding 1:4. All sera contained IgG and IgM but no IgA antibodies. About half the pituitary-positive sera fixed complement when tested with C3 conjugates. The ongoing nature of the family study enabled us to test multiple samples in 9 relatives; the antibodies persisted in up to 10 samples tested over 3 years. Only 1 gave fluctuating results on pituitary, probably owing to the weakness of the reactions.

**Prevalence of Other Antibodies in Patients with Pituitary Antibodies**

The patients with polyendocrine diabetes all had the appropriate organ-specific antibodies. In the juvenile series and the susceptible relatives, 24 were positive on pituitary; and of these, 4 had thyroid microsomal antibodies, 1 had thyroglobulin antibodies, and 3 reacted with gastric parietal cells. 1 of the probands was positive on adrenal in the absence of Addison’s disease, but neither he nor his relatives showed any reactivity on pituitary. The prevalence of antinuclear, smooth-muscle, and mitochondrial antibodies was unremarkable.

**Correlation between Pituitary Antibodies and ICA in Diabetics and Relatives**

As expected, 51 of 61 patients with juvenile diabetes of recent onset had islet-cell antibodies, and all those who reacted on pituitary also had ICA. However, of the 48 patients with longstanding type-1 diabetes who were selected to contain 50% with persistent ICA, the only patient with pituitary antibodies had ICA-IgG 5 years after onset of his disease.

Only half the “unaffected” relatives were chosen for susceptibility to diabetes, yet pituitary antibodies were found almost entirely in association with ICA, irrespective of complement-fixing ability against islets. 4 of the 7 relatives in whom diabetes developed during the follow-up reacted with pituitary (table ii). These relatives all had at least one HLA haplotype in common with the proband, but the presence of pituitary antibodies appeared unrelated to this set of predisposing genes: of 9 pituitary-positive sibs, 1 was HLA identical, 5 had one common haplotype, and 3 sibs shared no haplotypes with the proband.
TABLE II. CORRELATION BETWEEN PRESENCE OF PITUITARY-CELL ANTIBODIES AND ONSET OF DIABETES IN 63 FIRST-DEGREE RELATIVES OF DIABETIC CHILDREN

<table>
<thead>
<tr>
<th></th>
<th>Pituitary positive</th>
<th>Pituitary negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Become diabetic during 3 yr follow-up:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICA negative</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ICA positive</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Healthy:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICA negative</td>
<td>1</td>
<td>29</td>
</tr>
<tr>
<td>ICA positive</td>
<td>8</td>
<td>18</td>
</tr>
</tbody>
</table>

Discussion

The pathogenesis of type 1 (insulin-dependent) diabetes involves an inherited HLA-linked genetic susceptibility and a probable environment-induced initiating event, in most cases leading to a relatively slow immune-mediated destruction of the islet insulin-secreting beta-cells. Both humoral and cellular mechanisms are involved in this latter process. Diabetes is not infrequently seen in association with other known endocrine disorders (e.g., Schindler's syndrome, candida-endocrinopathy syndrome, thyrogastric autoimmune disease). Conversely, a proportion of patients presenting with diabetes may have multiple endocrine-organ involvement at a subclinical level when their sera contain the appropriate organ-specific antibodies. There is also preliminary evidence that gastrin-inhibitory-peptide cells and secretin cells of the gut endocrine system may be engaged in the autoimmune response of diabetes. Hitherto, reports of antibodies reacting with the pituitary have been relatively sparse. Our first study showed that approximately 2% of patients with longstanding type 1 diabetes had pituitary antibodies, and this is now confirmed. The intriguing finding, however, is that these antibodies were not only present in 16% of patients with newly diagnosed juvenile diabetes but were also found in over a third of genetically predisposed first-degree relatives with ICA. In particular, they were observed in a number of siblings who subsequently developed classical type 1 diabetes. The pituitary-cell reactivity was found consistently in multiple samples tested throughout the 3-year follow-up. The other striking finding is the correlation with islet-cell antibodies and, in particular, complement-fixing ICA. Such temporary and simultaneous autoimmune responses to two glands occurring in very young subjects and showing no female sex bias may be compatible with a viral infection. The role of viruses in the causation of type 1 diabetes has been extensively studied in animals, both in vivo and by infection of islet-cell cultures. In isolated cases of virus-induced diabetes mellitus in man, these organisms have been extracted from the patient's pancreas and shown to induce insulins in mice. Some beta-cell cytotoxic reoviruses can also infect the anterior pituitary. After these infections the mice produced autoantibodies to insulin and to growth hormone. Perhaps the human pituitary is also capable of being infected with viruses, and this might then give rise to a transient autoimmunisation reflected by the appearance of pituitary-cell antibodies in the circulation. A challenging difference between the experimental models and human diabetes is the absence of organ-specific markers comparable to islet-cell and pituitary-cell antibodies in the animals. Conversely, patients with endocrine pancreatic autoimmunity rarely produce spontaneous antibodies to islet-cell or pituitary hormones. It must always be kept in mind that cytoplasmic autoantibodies detected on sections of endocrine glands are directed against the intracellular membranes and not against the hormone secreted by these cells. These antibodies are highly organ-specific, and in the case of pituitary they are probably also cell-specific. Pituitary antibodies were of lower titres in patients with early-onset type 1 diabetes than in those with polyendocrine autoimmunity, and they stained more cells in the glands. The pituitary antibodies of the diabetic children, unlike those of patients with polyendocrine autoimmunity, lacked IGA. Antibodies reacting with multiple pituitary-cell types have also been reported by Pouplard et al. in animal tissues. The striking resemblance with the ICA was the restriction of complement-fixing ability to about half the pituitary-positive cases and the disappearance of these antibodies with ICA after onset of overt diabetes.

The function of the serum markers we have found in the prediabetic state and their connection with autoimmune hypophysitis must for the present time remain speculative. The autoimmune nature of destructive lymphocytic hypophysitis has been suspected since 1962, but in-vivo proof of pituitary antibodies has been obtained in only 2 patients so far. Although pituitary fibrosis was observed in longstanding diabetes, it was attributed to vascular changes. Autoimmune hypophysitis is increasingly reported in connection with pregnancy, and pituitary antibodies may occur in upwards of 2% of women after delivery. In this context it may be of interest to look for pituitary antibodies in gestational diabetes, especially in mothers who have detectable islet-cell antibodies, since this association has been found so often in the prediabetic state. The findings reported in the present study do not suggest a strict analogy between autoimmune hypophysitis and childhood diabetes, since there is no evidence of impaired pituitary function and the autoimmunisation in the diabetic children is transient. The increased rate of somatic growth in early diabetes suggests hypersecretion by some pituitary cells. Could it be that the reactions detected with immunofluorescence serve as indicators of other antibodies of a stimulatory nature resembling those found in Graves' thyrotoxicosis? Functional bioassays with pituitary tissue may help to clarify this issue. The role of the pituitary in the compensatory hyperplasia of pancreatic islets observed in early diabetes is at present unknown. Stimulatory factors influencing islet regeneration may explain the long incubation period preceding the acute onset of symptoms. The rapidly expanding work on multiple endocrine responses in diabetes will strengthen the present efforts to elucidate the degree of heterogeneity in this multifactorial disorder.

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REFERENCES

References continued on next page
Summary

Colonic absorption of deoxycholate and the proportion of secondary bile-acids in duodenal bile were significantly higher in 11 patients with adenomatous polyps than in 11 controls matched for age, sex, and gut transit time. In the patients with adenomas the large bowel made a greater contribution to bile-acid conservation, and this was associated with a more rapid turnover of cholic acid, but not with the adenomas patients’ higher consumption of cholesterol. These data accord with the hypothesis that bacteria degraded bile-salts are involved in the adenoma-carcinoma sequence of the colon.

Introduction

ADENOMATOUS polyps, particularly large ones, are regarded as precancerous lesions. 1,2 Although environmental factors, especially diet, are believed to influence the incidence of large-bowel cancer, 3,4 there is still disagreement about which substances are involved in the development of adenomas and carcinomas of the colon. 5-9

High consumption of animal fat may be important because it increases bile-acid excretion. 10 Exposure of the large bowel to bacterially degraded bile-salts enhanced carcinogenesis in rats. 10 High dietary fibre intake may protect against malignancy by accelerating intestinal transit, diluting colonic contents, and lowering pH. 11-13

Faecal concentrations of deoxycholate (DCA), a major metabolite of cholic acid (CA), and faecal 7α-dehydroxylase activity, which is responsible for DCA formation, were higher in patients with colonic adenomas than in controls. 14-15

We view absorption of DCA from the colon as a measure of the exposure of large-bowel mucosa to bacterial bile-acid products. We have measured DCA absorption in patients with adenomatous polyps at high risk of colon cancer and have compared these results with those in controls. Patients and controls were matched for age and gut transit time, since these factors have been shown to influence DCA absorption. We assessed DCA absorption by measurement of the rate that DCA entered the bacterial bile-acid pool. We also collected data on the diet in both groups to determine whether this was related to bile-acid metabolism.

Patients and Methods

Experimental procedure.— Eleven patients with histologically proven adenomatous polyps were investigated. In all the risk of colon cancer was high because they had severe epiphielial dysplasia, large adenomas (mean diameter i±sD) 1.5 x 0.7 cm), or numerous adenomas. 12 Three patients had undergone cholecystectomy. None of the patients with adenomas were taking any medication or had any other illness of any note. Eleven healthy volunteers matched for age, sex, and gut transit time were studied as controls. Subjects were included in the trial only if they had guaro-negative stools, non-compromised intestinal hepatic, renal, and gallbladder function.