Autoimmune endocrinopathies 5
Autoimmune disease of the adrenal cortex, pituitary, parathyroid glands and gastric mucosa

F. A. KARLSSON, O. KÄMPE, O. WINQVIST & P. BURMAN
From the Section of Endocrinology and Diabetes Care, Department of Internal Medicine, University Hospital, Uppsala, Sweden

Introduction
Over the past several years, our laboratory has been interested in characterizing autoantigens in the adrenal cortex and the stomach. In this paper, we discuss in some detail the current knowledge in this field. We also review observations made by other investigators of autoimmune diseases of the pituitary and parathyroid glands. As can be seen, the presumed immunological target structures in these latter organs remain to be elucidated.

Adrenal autoimmunity
In his classic paper of 1855, Thomas Addison described 11 patients with a disease he termed hypoadrenalism, of which four had adrenal tuberculosis, four malignancies, and three an adrenal fibrosis of unknown cause [1]. Six years earlier in the London Medical Gazette, Dr Addison had reported [2] on the post-mortem examination of three anaemic patients which were found to have a diseased condition of the suprarenal capsule', which suggested to him a state of cause and effect. Nowadays, the anaemia might be regarded as pernicious anaemia due to autoimmune gastritis and the adrenal lesions as autoimmune adrenitis or idio- pathic 'Addison's disease'. In 1930, Swingle & Pflüger found that suprarenal cortical extracts could cure the hypoadrenic state in adrenalectomized cats [3], and 1 year later, Rowntree et al. employed this type of extract to treat humans [4]. Following the discovery of antibodies directed towards thyroglobulin in patients with Hashimoto's disease by Roitt, Doniach and co-workers in 1956 [5], the reaction of antibodies in the sera of two patients with Addison's disease with the adrenals was described by Anderson et al. (1957) who used a complement fixation technique [6]. Today, we know that the majority of patients presenting with primary adrenal insufficiency are the victims of an autoimmune disease. Most patients have adrenal antibodies, which can be readily demonstrated by indirect immunofluorescence.

Addison's disease in polyendocrine syndromes
In up to 40% of cases Addison's disease is associated with a dysfunction in another endocrine organ [7, 8]. Most often the thyroid gland, the insulin-producing cells in the islets of Langerhans or the ovaries (Table 1). In the rare type I autoimmune polyglandular syndrome (APS-I), the hypoadrenalism is typically associated with hypoparathyroidism and gonadal insufficiency and, in certain cases, with autoimmune diabetes mellitus, pernicious anaemia, malabsorption, chronic active hepatitis and/or hypothyroidism. In addition, mucocutaneous candidiasis is usually present and some patients also suffer from vitiligo, alopecia, keratopathy, and nail and enamel dystrophies [9, 10]. This syndrome, which has its onset in childhood, is found amongst siblings.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Endocrine disease found in association with Addison's disease*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid disease†</td>
<td>18-19%</td>
</tr>
<tr>
<td>Ovarian failure‡</td>
<td>9-17%</td>
</tr>
<tr>
<td>Insulin-dependent diabetes mellitus</td>
<td>8-15%</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>2-5%</td>
</tr>
<tr>
<td>Hypoparathyroidism§</td>
<td>4-7%</td>
</tr>
</tbody>
</table>

* The data have been calculated from the reports of 295 and 246 patients, respectively, described in 1978 and cited in ref. 8. On average, 40% of the Addisonian patients present with one associated disorder.
† Autoimmune thyroiditis is twice as frequent as Graves' disease.
‡ Ovarian failure is a part of the type I autoimmune polyglandular syndrome in about 40% of cases.
§ Hypoparathyroidism is part of the type I autoimmune polyglandular syndrome.
thought to be inherited as an autosomal recessive trait and is not associated with any particular major histocompatibility complex (MHC) haplotype. A different type of autoimmune polygalandular syndrome APS-II can be identified in adults [9]. This syndrome is more common, is often found in multiple generations within a family and involves several genes, including the MHC class II region. APS-II is characterized by different combinations of thyroid diseases, hypoadrenalism, autoimmune diabetes mellitus and pernicious anaemia. However, the most prevalent combinations do not include the adrenals but are associations of thyroid disease and parietal cell autoimmunity [11] and of thyroid disease and diabetes mellitus. When not present alone, hypoadrenalism with an adult onset is most often linked to hypothyroidism and may also develop in patients with diabetes mellitus, in particular in cases having a simultaneous thyroid autoimmunity. Therefore, it has been suggested that adrenal function should be monitored on a regular basis in thyroid antibody-positive diabetic patients [12]. It should also be remembered that patients with a combination of Addison’s disease and autoimmune thyroid disease, i.e. ‘Schmidts syndrome’, have a higher risk of developing pernicious anaemia. Likewise, the frequency of ovarian insufficiency is increased in such cases.

Autoantigens in steroid-producing cells in the adrenal cortex and ovaries

The adrenal cortex is stained by sera from patients with idiopathic Addison’s disease when indirect immunofluorescence is utilized [6, 9]. The zona glomerulosa and zona reticularis in particular express the autoantigen(s), whereas the middle layer, the zona fasciculata, stains to a variable extent. We have found that the major autoantigen in idiopathic Addison’s disease is 21-hydroxylase [13], a microsomal cytochrome P450 enzyme responsible for the conversions of 17-hydroxyprogesterone to 11-deoxycortisol and progesterone to deoxycorticosterone in the glucocorticoid and mineralocorticoid pathways, respectively. The identification of the enzyme as the autoantigen was based on experiments utilizing immunofluorescence. Western blot analyses of adrenal extracts (Fig. 1) and immunoprecipitations of $^3$S-methionine-labelled cell lysates from a human adrenal cell line. Subsequently, the finding that 21-hydroxylase was a main autoantigen in Addison’s disease has been confirmed [14]. In an earlier publication, Krohn et al. [15] described that the sera of patients suffering from hypoadrenalism as part of the autoimmune polygalandular syndrome type I interact with the microsomal enzyme 17α-hydroxylase, which, in contrast to 21-hydroxylase, is present in the steroid-producing cells of both the adrenals and the gonads. We have examined five patients with APS-I and found that the sera stains both types of tissues, whereas the majority of sera from patients with idiopathic Addison’s disease only stain the adrenals. However, in our experiments, the sera of APS-I patients did not bind to either of the microsomal enzymes, 21-hydroxylase or 17α-hydroxylase, but to a separate antigen of mitochondrial origin (unpublished data). In addition, Betterle et al. [16] have previously described that the autoantibodies of APS-I patients react with a mitochondrial antigen. Thus, it is clear that there exist multiple antigens in the steroid-producing cells and that further work is needed to clarify the full spectrum of autoantibody reactivity.

In general, the antibody reactivity of the sera taken from patients with a combined adrenocortical and ovarian failure can be absorbed with adrenal extracts [7, 17], but the reverse does not apply. The sera of such patients may sometimes recognize several antigens in the ovaries as a clumpy immunofluorescence in the corpora lutea is found with certain sera, others produce a confluent or patchy stain, and yet others react only with the theca interna cells [7]. Gonad-restricted antibodies, i.e. ones that stain the gonads but not the adrenals, appear to be very rare and have not been documented to any great extent [18–21]. In one study of sera from 10 patients with premature ovarian failure, three such sera were described, two of which reacted with the theca interna and granulosa cells and one with the theca interna cells only.

Our current knowledge about steroid-cell autoantibodies allows a categorization of patients with adrenal hypocortisolism into at least three groups. In idiopathic Addison’s disease, the major antigen is 21-hydroxylase. Secondly, it is most probable that patients with adrenal antibodies not binding 21-hydroxylase belong to the category known as autoimmune polygalandular syndromes type I. In these cases, the sera react with a different antigen(s) present in both the adrenals and the gonads. Thirdly, there are patients with combined adrenocortical and ovarian failure, not belonging to the APS-I category,
that have 21-hydroxylase antibodies as well as antibodies directed towards a separate antigen common to both the adrenal cortex and the ovaries. This antigen is possibly 17α-hydroxylase which, like 21-hydroxylase, is located in the endoplasmic reticulum, or may represent yet another enzyme or protein. Finally, in cases with isolated gonadal insufficiency, there might exist antibodies directed towards ovary-restricted antigens, which have yet to be characterized.

Cell-mediated immunity has been less extensively examined in patients with idiopathic Addison’s disease. In about half the patients studied, evidence of organ-specific hypersensitivity has been obtained in in-vitro studies using adrenal extracts and peripheral blood leukocytes in a migration inhibition assay (22) and also in in-vivo tests using intradermal injections of antigen (23).

Recently, heterogeneous T-cell responses were observed with blood cells and fractionated adrenal extracts (24). It is probable that the identification of the specific antigens involved in antibody formation will provide a better opportunity of studying cell-mediated immunity, as the cellular and humoral immune responses seem to be directed towards the same antigen in autoimmune disease. This is illustrated by myasthenia gravis, autoimmune thyroiditis and insulin-dependent diabetes mellitus, where cellular responses as well as autoantibodies directed towards the acetylcholine receptor (25), thyroid peroxidase (26) and glutamic acid decarboxylase (27), respectively, have been documented.

**Pituitary autoimmunity**

An autoimmune basis for idiopathic hypopituitarism has been claimed for a long time (28). This is mainly based on circumstantial evidence and several reports of lymphocytic infiltrates in the pituitaries of patients...
with varying clinical pictures [29, 30]. An autoimmune aetiology of isolated ACTH deficiency has been suggested in certain cases [31–33], some of which displayed serum antibodies directed towards the adrenals or thyroid glands. Furthermore, autoimmune hypophysitis was suggested as a cause of isolated gonadotropin deficiency acquired after puberty [34] in two men with an autoimmune polyglandular syndrome, although no pituitary antibodies could be detected. Enhanced lymphocytic sensitivity to extracts of human pituitary tissue, in the absence of circulating pituitary antibodies, has been described in a child presenting with alopecia, candidiasis and idiopathic hypopituitarism [35].

The first description of autoantibodies directed towards the pituitary was made by Bottazzo et al. [36] in 1975 who used the sera of patients with autoimmune polyglandular disease associated with hypoparathyroidism. However, there was no evidence of pituitary dysfunction in these cases. Antibodies against the GH-producing cells have been reported in a young girl with arrested growth and an attenuated GH response to stimulation tests [37]. In an investigation of 220 children with growth disorders, 10% of the sera reacted with isolated cells in the pituitary, the majority stained prolactin-producing cells, three sera reacted with GH cells and in a few sera, the reactivity was not conclusively identified [38]. About 15% of the sera of patients with insulin-dependent diabetes mellitus contain immunoglobulins directed towards pituitary cells [39]. Vercammen et al. [40] have also described pituitary reactive antibodies of the IgM and IgG types in the sera of patients with newly diagnosed insulin-dependent diabetes. The significance of these two reports remains obscure. By using rodent pituitary cell lines to detect antipituitary antibodies, the sera of more than 70% of patients with empty sellae were reported to produce positive results [41], whereas the sera of normal subjects did not. The nature of the observed reactivity has not yet been clarified. In an experimental system, immunization with rubella virus glycoproteins has been found to produce an organ-specific disease resulting in a lymphocytic infiltration of the pituitary and the production of antibodies directed towards pituitary cells [42].

Thus, there exist several reports of lymphocytic infiltration of the pituitary in material obtained at surgery or autopsy. Furthermore, there are reports of antibodies that react with distinct cells in the pituitary, notably the prolactin-producing cells. No conclusive data linking antibodies to an associated pituitary dysfunction have so far appeared and the nature of the antigens in the pituitary remains to be clarified.

**Parathyroid autoimmunity**

In the type I autoimmune polyglandular syndrome, hypoparathyroidism is a typical feature and the parathyroids are usually the first endocrine organs to be immunologically attacked. Blizzard and coworkers [43] studied the sera of patients belonging to this category and found evidence of parathyroid antibodies in 38% of the cases as opposed to 6% in the sera of normal individuals. Some years later, parathyroid extracts were found to produce positive migration inhibitory tests in seven out of ten investigated subjects with idiopathic hypoparathyroidism [44]. In a recent study [45], cytotoxic antibodies have been reported in the sera of hypoparathyroid patients. Isolated hypoparathyroidism in adulthood, not associated with the type I autoimmune polyglandular syndrome, is exceedingly rare. The reason for this behaviour is obscure, as is the nature of the parathyroid antigens.

**Parietal cell autoimmunity**

In 1849 Thomas Addison [2] reported the postmortem examinations of three anaemic patients with adrenal destruction and suggested that this observation had no relevance to the anaemia. The basis for the anaemia in these cases was not clarified, however, and later Dr Flint suggested that a degeneration of gastric tubular glands could be of importance [46]. The subsequent work of several investigators disclosed a relationship between atrophic gastritis and the development of pernicious anaemia (review. ref. 47). The autoimmune nature of the disease was demonstrated by the existence of antibodies directed towards the intrinsic factor [48] and antibodies that reacted with the parietal cells [49]. Today, the combination of Addison's disease and anaemia is rare, in literature, one finds only a few reports of such a combination [50, 51]. In several of these cases, the patients had a thyroid autoimmunity and vitiligo also seemed to be overrepresented [52]. Thus, in idiopathic Addison's disease an anaemia due to a simultaneous autoimmune gastritis appears to be a very rare occur-
ence and if anaemia is present, it has probably developed via other mechanisms.

There are several reports of an increased frequency of parietal cell antibodies in patients with Addison’s disease [7, 9, 53] although most of these studies were carried out before the present classification of the polyglandular syndromes was acknowledged. When carefully examined, one finds only a few descriptions in literature of pernicious anaemia in combination with only hypoadrenalism of the adult onset type. However, autoimmune gastritis in adulthood is often associated with thyroid disorders or insulin-dependent diabetes mellitus. Thus, about 10% of patients with hypothyroidism, 1–2% of patients with a history of Graves’ disease and about 5% of those with autoimmune diabetes mellitus also have pernicious anaemia (the ultimate end-stage of autoimmune gastritis). As both thyroid autoimmune disease and diabetes mellitus occur in a significant proportion of patients with Addison’s disease, the relatively lower frequency of autoimmune gastritis in Addisonian patients appears somewhat exceptional. One could speculate that cortisol therapy reduces the risk of a later development of severe autoimmune gastritis. Previous reports of a return of acid secretion, partial parietal cell regeneration and improvement in vitamin B₁₂ absorption after the administration of high doses of steroids [54, 55] could favour such an idea. In the type 1 autoimmune polyglandular syndrome, parietal cell dysfunction is reported in about 10% of the patients and is more common in patients over 40 years of age who often present with type 1 diabetes mellitus and seldom with hypoadrenalism [10], thus mimicking the APS-I profile. Since periodic intestinal malabsorption of an early onset is also a prominent feature in 20% of the probands, the defective uptake of vitamin B₁₂ cannot be solely attributed to intrinsic factor deficiency.

The autoantibodies in autoimmune gastritis are directed towards H⁺,K⁺-ATPase [56], the proton pump of the stomach wall. Some patients also have antibodies against intrinsic factor [47], a secretory product which binds vitamin B₁₂ to facilitate its absorption. The H⁺,K⁺-ATPase antibodies are found in most patients with pernicious anaemia and the levels fall only gradually over the years [57]. Amongst patients with autoimmune thyroid disorders, parietal cell antibodies are common and are sometimes accompanied by pernicious anaemia. Women with autoimmune thyroiditis, who in the postpartum period experience a flare-up of their disease, display an increased frequency of ‘post-partum gastritis’ with transiently enhanced levels of antibodies directed towards the H⁺,K⁺-ATPase [58]. As the antibodies inhibit the enzyme in vitro [59], they may also possibly contribute to parietal cell dysfunction in vivo.

The H⁺,K⁺-ATPase is a non-covalent heterodimer. The alpha subunit has a molecular weight of 114 000 and contains the catalytic site, whereas the beta subunit is a glycoprotein with an apparent molecular weight of 65 000–80 000 where the protein core accounts for 35 kDa. The patient antibodies are directed towards both the alpha and the beta subunits and, in experimental animal models of autoimmune gastritis, the antibodies produced have been found to be similarly directed towards both the subunits of H⁺,K⁺-ATPase [60], as well as a protein corresponding to intrinsic factor [61].

Intrinsic factor is protein with a molecular weight of 45 000, produced in the parietal cells and secreted into the gastric lumen. Vitamin B₁₂ is liberated by the degradation of food, binds to ‘R proteins’ and is transported to the duodenum where it attaches to intrinsic factor after the digestion of the R proteins by pancreatic proteases. This complex is taken up in the distal ileum by a receptor mechanism. Vitamin B₁₂ is liberated in the enterocyte, binds to transcobalamin II and is transported to the liver and the target cells. It is believed that antibodies against intrinsic factor inhibit the binding and/or the absorption of vitamin B₁₂ and in some patients may precipitate the onset of vitamin B₁₂ deficiency and the development of pernicious anaemia by neutralizing the remaining amount of intrinsic factor.

Parietal cell antibodies can be determined by several methods and in clinical practice the most frequent technique is an indirect immunofluorescence assay using rat gastric mucosa as a substrate. The results compare well with determinations of H⁺,K⁺-ATPase antibodies using an enzyme-linked immunosorbent assay with purified enzyme from porcine gastric mucosa [62]. Antibodies directed towards intrinsic factor are determined in radioassays using labelled vitamin B₁₂ or assays using iodinated intrinsic factor protein as a tracer [63]. As mentioned above, because up to 10% of patients with hypothyroidism due to autoimmune thyroiditis, 1–2% of patients with a history of Graves’ disease, and up to 5% of patients with autoimmune diabetes mellitus develop pernicious anaemia, screening for autoimmune gastritis is indicated in these groups.
Also, in the rare patients with the type I autoimmune polyglandular syndrome, the increased risk of pernicious anaemia motivates regular screening.

Perspectives

Autoantigens in human organ-specific autoimmune disease often seem to be membrane-bound proteins (e.g. H⁺,K⁺-ATPase, thyroid peroxidase, glutamic acid decarboxylase, 21-hydroxylase) and/or secretory products (intrinsic factor, thyroglobulin, insulin) of endocrine cells. Although the mechanism by which tolerance to these proteins is broken is not understood, studies of organ-specific autoimmune disease in experimental animals have provided interesting insights during recent years. Thus, it has been found that neonatal thymectomy promotes the development of disease in susceptible strains of animals, presumably via mechanisms linked to the appearance in peripheral blood of a population of autoreactive T cells, which are normally deleted in the thymus at the time of T-cell education [64]. In this context, the reported reduced incidence of autoimmune diabetes in BB rats given glucose and arginine neonatally to stimulate their immature beta cells [65] seems particularly interesting. Furthermore, animal studies demonstrate that the functional activity of an endocrine organ influences the intensity of disease. Thus, the stimulation of the ovaries by gonadotropins markedly enhances the development of experimental oophoritis [66], and in the BB rats, autoimmune diabetes develops following weaning whereas insulin administration reduces the incidence of the disease [67]. These observations are of great interest since they indicate possible ways of modulating autoimmune disease. Today, it is becoming increasingly apparent that ‘target cell rest’ may reduce the intensity of an autoimmune disease in humans and, in the future, the silencing of autoreactive antigen-specific T cells by the presentation of peripheral proteins at the time of thymic education could be of fundamental importance. We wish to speculate that ‘vaccination’ early in life with key autoantigens of pancreatic beta cells and of thyroid cells would provide a means of reducing the incidence of autoimmune diabetes mellitus and autoimmune thyroid disease in generations to come.

References

20 Cameron IT, O’Shea FC, Rolland JM, Hughes EG, De Kretser DM, Healy DL. Occult ovarian failure: a syndrome of infertility.


60 Jones CM, Collaghan J, Gleeson PA, Mori Y, Masuda T, Toh BH. The parietal cell autoantibodies recognized in neonatal thymectomy-induced murine gastritis are the α and β subunits of the gastric proton pump. Gastroenterology 1991; 101: 287–94.


Received 24 March 1993, accepted 7 May 1993.

Correspondence: Professor F. Anders Karlsson MD, Section of Endocrinology and Diabetes Care, Department of Internal Medicine, University Hospital, S-751 85 Uppsala, Sweden.