Autoimmunity in isolated Addison’s disease and in polyglandular autoimmune diseases type 1, 2 and 4

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INTRODUCTION

Primary adrenal insufficiency or Addison’s disease (AD), was first described by Thomas Addison in 1855 [2], when reporting cases with adrenal tuberculosis, metastatic tumors and also with an « idiopathic » adrenal atrophy. AD in general is a quite rare manifestation, being present in 40-110 cases per million inhabitants [30, 37, 49]. In the past, tuberculosis was the most frequent form of AD. At present, in developed countries, the most common form of AD is the autoimmune type causing up to 75-85 % of all cases, while tuberculosis is responsible for up to 10-20 % of the cases. Many rare syndromes are involved in the remaining 5 % of the patients [9, 29]. Over a period of 30 years we studied 300 cases of AD. The etiologies are summarized in Table I.

AUTOIMMUNE ADDISON’S DISEASE

In 1957 Witebsky proposed the criteria which define a disease as autoimmune [57]; these criteria were recently revised by Rose [42]. It was firstly discovered in 1957 that autoimmune AD had : a) circulating...
Autoantibodies directed against the adrenal cortex [4] and subsequently that the disease satisfied many of the above criteria such as: b) cell-mediated immunity against the adrenal cortex; c) steroidogenic enzymes as self-antigens; c) lymphocytic infiltrations of the adrenal cortex; d) association with genes of the HLA region; f) association with other autoimmune diseases [12, 35].

POLYGLANDULAR AUTOIMMUNE DISEASES (PGAD)

In 1980 Neufeld reorganized and classified PGAD into four main types summarized in Table III.

Autoimmune AD is one of the major components of the PGAD Type 1, 2 and 4 [38]. About 50-60 % of the patients with the autoimmune AD have, or will develop during their lives, a PGAD. In the remaining cases, the adrenal failure is not associated with the above syndromes [12]. In our patients a PGAD was found in 59 % of the cases (13 % Type 1, 40 % Type 2, 5 % Type 4) and 41 % had an isolated AD (Table IV).

EPIDEMIOLOGY

PGAD Type 1 is a very rare disorder world-wide, except in the Iranian Jewish community, in Finland and in Sardinia where the incidence, probably as a result of a founder gene effect, is about 1 : 9,000 and 1 : 25,000 inhabitants. The F/M ratio varies from 0.8 to 2.4, and the syndrome occurs almost exclusively in childhood [3, 11, 38]. In our population we observed 32 cases with this PGAD that represent 13 % of the cases (13 % Type 1, 40 % Type 2, 5 % Type 4) and 41 % had an isolated AD (Table IV).

Key words: Addison’s disease, polyglandular autoimmune diseases, adrenal cortex autoantibodies, steroid-producing cell autoantibodies.

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Isolated AD is the fourth clinical type of the disease. We observed 102 cases that represent 41% of the autoimmune AD in our population. The F/M ratio was 0.7, the mean age of onset was 30 years (Table IV).

### CLINICAL ASPECTS

PGAD Type 1, also called APECED (Autoimmune Poly-Endocrinopathy, Candidiasis, Ectodermal Dystrophy) manifests itself with chronic candidiasis, hypoparathyroidism and autoimmune AD (Table III). At least two of the three diseases need to be present in each patient and they are all present only in one-third of the observations [38]. The main diseases generally occur in a fairly precise chronological order [3, 11, 38]. Candidiasis is usually the first manifestation to appear in childhood. It affects the nails, the dermis and the mucous membranes, and it is considered the clinical expression of an immunological selective T cell deficiency to Candida Albicans [3, 11, 38]. On the contrary, the B cell response to Candida is normal and this prevents the development of a systemic candidiasis. Hypoparathyroidism is the second disease to appear and Addison’s disease is the third. The complete evolution of the PGAD takes place during the first 20 years of life [3, 11, 38] in most of the cases. Other minor autoimmune or immuno-mediated diseases may occur with a high frequency during the patients’ lives [3, 11, 38]. Table IV shows the frequencies and the main characteristics of diseases and (fig. 1) shows the age of onset of the fundamental diseases in this PGAD in our patients.

PGAD Type 2, also known as Schmidt’s syndrome [43], is characterized by the presence of autoimmune AD and either thyroid autoimmune diseases and/or Type 1 diabetes mellitus (DM) [10, 38, 39]. Type 1 DM, when present, develops before Addison’s disease, whereas thyroid autoimmune disease can develop before,
contemporary with or after the appearance of adrenal insufficiency [10, 39]. Out of our 100 patients with PGAD Type 2, only 11 had the complete triad and 89 had 2 fundamental diseases (AD and thyroid autoimmune disease or Type 1 DM). Thyroid autoimmune diseases include Graves’ disease, Hashimoto’s thyroiditis, primary myxoedema, symptomless thyroiditis and endocrine ophthalmopathy. In addition, other minor diseases may be present with a low frequency [10, 38, 39]. Table IV summarizes the main features of this PGAD and fig. 1 the age at onset of the main diseases in this Type of PGAD in our patients.

PGAD Type 4 manifests when autoimmune AD is associated with other autoimmune diseases which do not fall into Type 1 or 2. Table IV shows the main features of this Type of PGAD in our patients.

Isolated autoimmune AD is a condition not associated with other clinical autoimmune or immuno-mediated manifestations. Table IV summarizes the main features of this condition and fig. 1 the age at onset of the AD.

**PATHOLOGY**

The pathology of the parathyroid glands in patients with hypoparathyroidism in the context of the PGAD Type 1 reveals an atrophy with a lymphocytic infiltration [11]. The pathology of the adrenal cortex in patients with AD in the context of these PGADs and in isolated AD reveals a glandular atrophy, with the presence of a mononuclear cell infiltration constituted by lymphocytes, plasma cells and macrophages [34].

**GENETIC STUDIES**

PGAD Type 1 is a condition occurring sporadically or among siblings [3, 11]. The mode of presentation argues for an autosomal recessive inheritance. A Finnish family study has shown that the responsible gene is located on chromosome 21 [1]. This gene, defined as AIRE (autoimmune regulator), has recently been isolated and cloned [36, 51]. Five main mutations have been identified. The most common is R257X responsible for 82 % of the Finnish APECED alleles [41]. As for the mutation in this gene we examined a group of Italian patients and the results are summarized in Table V. Also in Italian patients R257X is the most frequent mutation [44].

PGAD Type 2 and 4 sometimes occur in many generations of the same family, and show a pattern of inheritance consistent with autosomal dominance and incomplete penetrance. HLA-DR3, was found to be increased in patients with PGAD Type 2 from USA [26, 33], Germany [15], UK [55] and Italy [10]. In particular, the subtype DR3 DQB1*0201 was increased in US patients [26] and DR4 DQB1*0302 was increased in patients with AD + Type 1 DM [26]. HLA-DR5 was increased in Italian patients with AD + thyroid autoimmunity [10]. Recently, an increased frequency of alleles of class I HLA (MIC-A5.1) was found in patients with autoimmune AD, excluding Type 1 PGAD. On the contrary MIC-A6 was significantly reduced. The frequency of MIC-A5.1 was significantly increased only in the presence of HLA-DR3 DQ2 [24].
We studied the HLA-status in 38 patients with PGAD Type 2 and a significant increased frequency of HLA-DR3 was found (p corrected = 0.05) (fig. 2 left). We also studied 11 patients affected by isolated autoimmune AD and HLA-DR3 was increased (fig. 2 right), but after correction P lost significance (p not corrected = 0.008, p corrected = 0.08) probably due to the limited number of cases.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Allele 1/Allele 2</th>
<th>Major Clinical Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. F.T.</td>
<td>R257X/R257X</td>
<td>HP + CC + AD</td>
</tr>
<tr>
<td>2. F.L.</td>
<td>R257X/R257X</td>
<td>HP + CC + AD</td>
</tr>
<tr>
<td>3. A.E.</td>
<td>R257X/R257X</td>
<td>HP + CC + AD</td>
</tr>
<tr>
<td>4. T.P.</td>
<td>del 13/del 13</td>
<td>HP + CC + AD</td>
</tr>
<tr>
<td>5. C.A.</td>
<td>R257X/R257X</td>
<td>HP + CC + AD</td>
</tr>
<tr>
<td>6. C.G.</td>
<td>R257X/R257X</td>
<td>HP + CC + AD</td>
</tr>
<tr>
<td>7. C.G.</td>
<td>del 13/R257X</td>
<td>HP + CC + AD</td>
</tr>
<tr>
<td>8. D.G.F.</td>
<td>del 13/del 13</td>
<td>HP + CC + AD</td>
</tr>
</tbody>
</table>

Table V
Haplotype analyses of PGAD Type 1 in 8 Italian patients.

We studied the adrenal cortex (ACA), steroid-producing cells (StCA), 21-hydroxylase (21-OHAb), 17α-hydroxylase (17α-OHAb) and P450 side-chain cleavage (P450scCcAb) in patients with autoimmune AD in the context of PGAD Type 1, the frequency of ACA, detected using the indirect immunofluorescence technique, varied according to the duration of the disease, from 100 % at diagnosis to 78 % in longstanding disease [9]. In addition, StCAs were demonstrated in 60-70 % of the ACA-positive patients [9]. StCAs were always associated with ACA and they were generally considered to be serological markers of clinical or potential hypergonadotropic hypogonadism [8, 9, 28, 48] (fig. 3).

Steroid 17α-OH was the first autoantigen reported to be recognized by sera of patients with PGAD Type 1 [31]. Subsequently, P450scCc was also identified [56], and finally, steroid 21-OH was included in the family of target autoantigens [20, 47, 52]. In 1994, Uibo [52] reported that sera from patients with PGAD Type 1 reacted with at least one of the three above-mentioned autoantigens, and this observation was supported by a later study [40]. In contrast, we [13, 19, 20] reported that in PGAD Type 1, ACA recognized 21-OH as a major autoantigen, whereas StCAs recognized the other autoantigens, such as 17α-OH and/or P450scCc (fig. 3).

**IMMUNOLOGICAL STUDY**

Antibodies to adrenal cortex (ACA), steroid-producing cells (StCA), 21-hydroxylase (21-OHAb), 17α-hydroxylase (17α-OHAb) and P450 side-chain cleavage (P450scCcAb) in patients with autoimmune AD in the context of PGAD Type 2 (left) (**p = 0.05**) and in the isolated form (right) (*p = 0.08* corrected, **p = 0.008** not corrected).
In patients with autoimmune AD in the context of PGAD Type 2 and 4, ACA can be present in 100% at the onset and in 54% of those with a disease duration of more than 2 years. The StCAs were positive in about 30% of the cases and the majority of these had or will have developed a hypergonadotropic hypogonadism [8, 9] (fig. 3). To date, there is a consensus that 21-OH is the major autoantigen and that StCA recognizes 17α-OH and/or P450scc also in PGAD Type 2 [13, 19, 20, 45, 46] (fig. 3).

In isolated autoimmune AD ACA are present in 77% of the patients with a disease’s duration of less than 2 years and in 47% of those with long-standing disease. The autoantigen is 21-OH [12, 13, 19, 20, 45]. StCAs are rarely positive and also in these cases they recognize 17α-OH or P450sc. These autoantibodies can be herald of hypergonadotropic hypogonadism [8]. Data about the frequency of the main autoantibodies to adrenal and ovarian antigens in our patients with PGAD Type 1, 2 and isolated AD are summarized in fig. 3. The relationships between these antibodies are shown in fig. 4. Studies on autoepitopes recognized by the sera of patients with autoimmune AD were performed by Western blot analysis, using 21-OH expressed in an in vitro transcription/translation system, in bacteria or yeast: 90% of the sera reacted with the central portion of the protein (residues 165-379) and the majority of them reacted with an epitope located between residues 281-379, where the proposed steroid binding site is located [5, 47, 54]. Furthermore, half of the sera reacted with the central and C-terminal portion (residues 380-494) of the molecule [53]. We performed a study using the sera of patients with PGAD Type 1, 2 or with isolated AD. We demonstrated that these sera recognize the central and the C-terminal portion, without any significant difference between the various types of AD [53].

The demonstration that IgGs from patients with autoimmune AD were able to inhibit 21-OH enzyme activity in vitro [23] supported the hypothesis of a pathogenetic role of these autoantibodies, but these data were not confirmed in vivo [16].

**Parathyroid autoantibodies**

Parathyroid autoantibodies, detected by indirect immunofluorescence technique, were identified in 11-38% of the patients with hypoparathyroidism [14, 27]. Other studies have been unable to confirm these results, but demonstrated that the sera of patients with PGAD Type 1 reacted with parathyroid oxyphilic cells rich in mitochondria [7, 50]. Cytotoxic autoantibodies reacting with cultured bovine parathyroid cells were also found in all sera of the patients with hypoparathyroidism [18], but they lost their reactivity after absorption by endothelial cells [22]. Autoantibodies to the extracellular domain of the calcium-sensing receptors, evaluated by immunoblot analysis, have been detected in the sera of 56% of the patients with hypoparathyroidism, most of whom had PGAD Type 1 [32].

**Other autoantibodies**

In patients with PGADs Type 1, 2 and 4 pleiades of other autoantibodies is present and, in general, these autoantibodies are correlated with the respective clinical autoimmune disease (Table VI). Frequently these antibodies can be present in patients without clinical manifestations and they can be heralds of the future clinical presentation (Table VI).

In patients with apparently isolated autoimmune AD, an autoantibody screening reveals the presence of many autoantibodies (see Table VII). When present these autoantibodies can be considered markers of latent PGAD, in fact a proportion of these cases will develop the respective clinical diseases [10]. Therefore, the true isolated autoimmune AD is a quite rare condition.
CONCLUSION

These data suggest that patients with autoimmune AD have a high frequency of other clinical or latent autoimmune manifestations. This frequency is exceptionally elevated in the context of PGAD Type 1 and that can be due to an impairment in immunotolerance as a consequence of the particular genetic status. The enzyme 21-OH is the main target of adrenal cortex autoantibodies in AD, independently from the clinical presentation. StCA can additionally be present, it is correlated with hypogonadism and recognizes one of the other steroidogenic enzymes (17α-OH/P450scc). In patients with apparently isolated autoimmune AD the screening of organ-specific autoantibodies can help to individuate patients with latent or potential PGAD.

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