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Radioimmunoassay for autoantibodies against interferon omega; its use in the diagnosis of autoimmune polyendocrine syndrome type I

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Abstract Patients with the autoimmune polyendocrine syndrome I (APS I) have high titers of neutralizing IgG autoantibodies against type I interferons (IFNs), in particular IFN-ω. Until now, the most specific assay has been the antiviral interferon neutralizing assay (AVINA), which has the drawbacks of requiring a cytolytic virus, being cumbersome and difficult to standardise. We have developed a fast and reliable immunoassay based on radiolabelled IFN-ω for quantifying anti-IFN-ω antibodies. Sera from 48 APS I patients were analysed together with those from 5 control groups. All sera from APS I patients were positive for anti-IFN-ω, while, except one serum, all sera from the controls were negative. This method has the advantage over bioassays that it is readily adapted to high throughput. It provides an alternative, sensitive and specific diagnostic test for APS I, and an ideal screening tool to precede mutational analyses of the *AIRE* gene in suspected APS I cases.

Introduction

Autoimmune polyendocrine syndrome type I (APS I) is an inherited autosomal recessive disease. The main components are chronic mucocutanous candidiasis, hypoparathyroidism

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Table 1 Manifestations and mutations in the autoimmune regulator (*AIRE*) gene in the Swedish and Finnish patients

Patients	Sex	Manifestations	Mutation
1 (Swedish)	F	H, AD, G, A, V, C	p.R257X/ R257X
2 (Swedish)	M	AD, A, C, CAH	p.R257X/ R257X
3 (Swedish)	M	AD, D, M, G, C, CAH, PA	n.d.
4 (Swedish)	M	H, AD, A, V, C	p.R257X/ R257X
5 (Swedish)	M	H, AD, M, G	p.R257X/ R257X
6 (Swedish)	F	H, A	n.d.
7 (Swedish)	F	H, AD, G, M, C, CAH, PA	p.M388fsX36/A21V
8 (Finnish)	F	C, HP, AD, G, K, T	p.R257X/ R257X
9 (Finnish)	F	C, HP, AD, D, G, A, V, K, T	p.R257X/X546C
10 (Finnish)	M	C, HP, AD, A, V, K	p.R257X/X546C
11 (Finnish)	M	C, HP, AD, PA, V, K, H	p.R257X/ R257X
12 (Finnish)	M	C, HP, G, V, K	p.R257X/ R257X
13 (Finnish)	F	C, HP, AD, G, A, K	p.R257X/ R257X
14 (American)	M	C, HP, D, A	p.Y85C
15 (American)	M	HP	Not found
16 (American)	F	HP	p.Y302C/not found

Manifestations: AD, Adrenal insufficiency; HP, hypoparathyroidism; C, mucocutaneous candidiasis; G, primary gonadal insufficiency; V, vitiligo; A, alopecia; D, diabetes type 1; PA, pernicious anemia; K, keratopathy; H, hepatitis; T, hypothyroidism; CAH, congenital adrenal hyperplasia; M, malabsorption.

and adrenal insufficiency (Addisons disease) [1]. Affected individuals may additionally suffer from various other organ-specific autoimmune manifestations such as type 1 diabetes, gonadal failure, autoimmune gastritis, autoimmune hepatitis and intestinal malabsorbtion. Moreover, a number of ectodermal manifestations are common, notably vitiligo, alopecia, keratitis and enamel dysplasia. The last two are quite specific for APS I [2,3].

The genetic cause of APS I was recognised in 1997 and has been attributed to inherited mutations in the autoimmune regulator (*AIRE*) gene [4,5]. More than 50 deleterious mutations in *AIRE* have been identified, the most common being frame shift and nonsense mutations resulting in truncated AIRE proteins [6]. Two large deletions have also been described [7,8]. Studies of AIRE-deficient mice and structural comparisons to transcription factors suggest that AIRE functions as a transcription factor. It appears to control autoimmunity by regulating expression of organ-specific transcripts in thymic medullary epithelial cells that induce self-tolerance in developing T cells [9–12]. APS I patients have reduced function of regulatory T cells that could contribute to recognised immune defects [13].

Patients frequently develop high titers of autoantibodies against molecular targets in their affected endocrine organs. Several autoantigens have been identified, among them 21-hydroxylase (21OH) [14–16], trypthophan hydroxylase (TPH), side-chain cleavage enzyme (SCC) [16,17], 17-hydroxylase (17OH) [16,18], aromatic L-amino acid decarboxylase (AADC) [19], the newly discovered tudor domain containing protein 6 (TDRD 6) [20] and the highly specific NACHT leucine-rich-repeat protein 5 (NALP5) [21]. Although discovery of these autoantigens has provided disease markers in APS I patients, autoantibodies directed against AADC, TPH and NALP5 are only found in about half of them. The organ-specific antibodies appear only within the immune attack against the glands and tissues, while the youngest patients may be seriously ill already earlier [2]. Furthermore, some autoantibodies, such as those against 21OH and SCC, are not specific but occur in the more common conditions of isolated autoimmune Addison's disease and autoimmune polyendocrine syndrome type II (APS II).

Recently, using specific antiviral interferon neutralisation assays (AVINA), high titers of circulating neutralizing IgG autoantibodies against type I interferons (IFNs), IFN-α and IFN-ω, were found in Nordic APS I patients [22]. In contrast to the other autoantibodies they were present in all patients, regardless of age, gender, precise *AIRE* genotype and APS 1 disease components. Subsequently, similar findings have

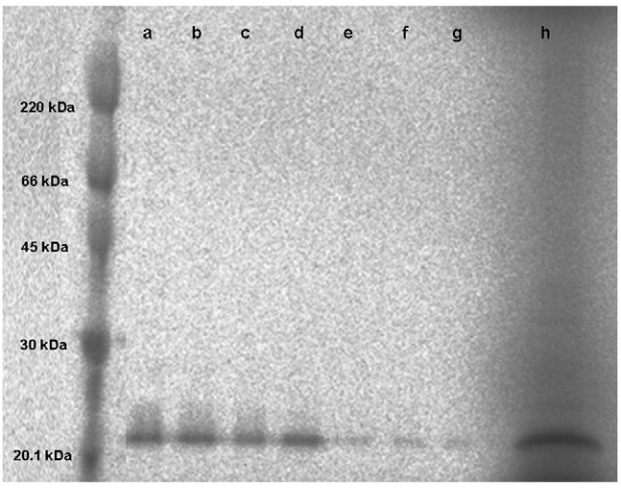

Figure 1 Immunoprecipitation of antibodies against interferon omega. SDS-PAGE of *in vitro* coupled transcribed and translated ^{35}S-labeled interferon omega (IFN-ω) migrated to the expected position of an apparent molecular weight of 28 kDa (lane h). Lanes a–c show the immunoprecipitation of anti-IFN-ω with sera from 3 different APS I patients, lane d shows the precipitation with a specific murine antibody against human IFNω and lanes e–g show three negative control sera.