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Aire deficient mice do not develop the same profile of tissue-specific autoantibodies as APECED patients

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Abstract

Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, or APS1), is a monogenic autoimmune disease caused by mutations in the *autoimmune regulator* (*AIRE*) gene. The three main components of APECED are chronic mucocuteaneous candidiasis, hypoparathyroidism and adrenocortical insufficiency. However, several additional endocrine or other autoimmune disease components, or ectodermal dystrophies form the individually variable clinical picture of APECED. An important feature of APECED is a spectrum of well-characterized circulating autoantibodies, reacting against tissue-specific autoantigens. Aire deficient mice develop some characteristics of APECED phenotype. In order to investigate whether the Aire deficient mice produce autoantibodies similar to human APECED, we studied the reactivity of Aire mouse sera against mouse homologues of 11 human APECED antigens. None of the APECED antigens indicated elevated reactivity in the Aire knock-out mouse sera, implying the absence of APECED associated autoantibodies in Aire deficient mice. These findings were supported by the failure of the autoantigens to activate mouse T-cells. Furthermore, Aire knock-out mice did not express increased levels of antinuclear antibodies compared to wt mice. This study indicates that spontaneous induction of tissue-specific autoantibodies similar to APECED does not occur in the rodent model suggesting differences in the immunopathogenic mechanisms between mice and men.

Keywords: APS1; Autoantigen; Autoimmune regulator; Knock-out mouse

1. Introduction

APECED (OMIM 240300) is a unique, organ specific monogenic autoimmune disease with severe disabilities for the patients. The first symptoms which typically appear during the early years of life are candidiasis and hypoparathyroidism. Until late adulthood, a myriad of subsequent symptoms can develop in APECED patients. The clinical picture can vary strongly between patients, even between siblings. The three main components are *Candida* infections, hypoparathyroidism, and adrenocortical insufficiency (Addison's disease). In addition, several ectodermal diseases such as alopecia, nail dystrophies and vitiligo, may appear. Endocrine autoimmune diseases such as gonadal atrophy, type 1 diabetes and hypothyroidism and other autoimmune diseases, such as autoimmune hepatitis as well as other, rare manifestations may also occur. Half of the patients demonstrate multiple endocrine manifestations [1,2].

APECED is caused by mutations in the *AIRE* gene [3,4]. The consequences of the mutations in the *AIRE* gene have

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been extensively characterized at molecular and cellular levels by us and others, revealing the functions of the different domains of the AIRE protein [5–7] and linking the function of AIRE to the regulation of transcription [5,8–10]. Recent functional data of mouse Aire emerges from studies of Aire knock-out mouse strains. These investigations have illustrated that Aire may function as a transcriptional regulator [11,12] and plays a role in the process of negative selection of developing T cells in the thymus [13,14]. Although it first appeared that Aire activates the expression of ectopic genes in medullary thymic epithelial cells (mTECs), recent data indicates that Aire may also down regulate a large number of genes of different functions [14–17]. Despite this progress, the function of Aire in the periphery remains incompletely defined, stressing further that the functions of Aire at a molecular level are still unclear.

Studies on Aire deficient rodent models have shown consistently that the phenotype of the Aire deficient mice is surprisingly mild. The three reported Aire deficient mouse models share a rather similar phenotype which is considerably less severe than that of APECED patients [12,18–20]. Ramsey et al. [19] constructed and characterised the first Aire deficient mice (C57/BL6 strain). The genetic defect in these mice mimics the $Finn_{Major}$ mutation (p.R257X), which is the most common mutation among APECED patients worldwide. These mice have several features suggesting pathogenesis: infertility, lymphocyte infiltrates in several organs and circulating autoantibodies against liver and sperm. The T cell receptor Vβ-chain (TCR-Vβ) repertoire of peripheral T cells was altered and the T cells from the $Aire^{-/-}$ mice hyperproliferated when immunized with hen egg lysozyme (HEL) together with Freund's complete adjuvant. The two other Aire deficient mouse models (C57/BL6) [12,18] provided similar findings. The lymphocytic infiltrates were demonstrated to increase in an age-dependent manner. Furthermore, the number of mTECs and activated memory T cells ($CD44^{hi}CD62L^{lo}$) in peripheral lymphoid organs was increased, in agreement with the findings showing that autoreactive T cells escape the thymus in Aire deficient mice [13]. The most recent findings using Aire deficient mouse models show that the strains used for construction of the models have a strong influence on the immune symptoms found in these mice [20]. However, it is noteworthy that $Aire^{-/-}$ mice have not shown any endocrinopathies, typical for APECED patients. By indirect immunofluorescence technique autoantibodies targeted to endocrine organs have been described in Aire deficient mice [18,19], suggesting that autoimmune processes against these tissues might be present although no clinical disease was observed.

These discrepancies between the symptoms of Aire deficient mice and patients with APECED prompted us to carry out a careful study of the autoantibody profile in these mice in order to characterize similarities and differences in the immunological phenotype of Aire deficient mice compared to APECED patients. In Aire deficient mice, we measured the presence of antibodies against the human APECED antigen homologues (Table 1). This knowledge is important not only for evaluating the relevance of Aire deficient mice as a model of APECED and autoimmunity in general, but also for comparison of the mechanisms of autoimmunity between humans and mice. Our study reveals that despite their well characterized defect in the central tolerance the Aire knock-out mice fail to develop autoimmunity against organs typically affected in the patients with APECED.

2. Methods

2.1. Mice

Aire knock-out mice (C57/BL6) were constructed using a targeted disruption of the mouse *AIRE* gene and preliminarily characterized by Ramsey et al. [19]. Aire deficient and wt congenic mice were kept in a barrier at specific pathogen free conditions at the National Public Health Institute (NPHI) in Helsinki, Finland and non-congenic mice were kept at the animal facilities at Salmisaari and Biomedicum, Helsinki. The mice were bred as heterozygotes. Serum samples were taken from both congenic and non-congenic mice, females and males between ages 2.5 months to 6 months.

2.2. Genotyping

Genotyping took place after weaning and was performed as described in Ramsey et al. [19] with slight modifications: DNA was extracted from a small punch of ear obtained in connection with earmarking of the mice. The QIAGEN DNeasy kit was used for DNA isolation, followed by PCR using Expand long template enzyme (Roche) or Dynazyme PCR. Primers: F 5′ TGA GAC AGT TCC TCT GTG TAG CTT TGG CTG TCC TGG 3′, R 5′ TCT TGG GAC TTA CCT GGT TAA CCT GGG GCT CAC TG 3′ The wt allele yielded a 748 bp and the mutant allele a 2024 bp DNA-fragment., which were analyzed by agarose gels and detected by EtBr-staining.

2.3. Cloning

2.3.1. PCR/ RT-PCR

The human antigens were produced as described in Söderbergh et al. [31], except for the 21-OH plasmid, which was obtained from A. Falorni, GAD-65 from S. Baekkeskov and 1A-2 from E. Bonifacio.

The mouse complementary DNA (cDNA) clone coding for each protein listed in Table 1 was isolated. RT-PCR with the Advantage RT-for-PCR kit (Clontech, BD Biosciences) or PCR (Platinum *Taq* High Fidelity, Invitrogen, Carlsbad, CA, USA) with the Platinum Taq Polymerase High Fidelity enzyme (Invitrogen) were carried out. Some of the templates were amplified using a commercial cDNA tissue panel (Mouse Multiple Tissue cDNA (MTC) Panel I, BD Biosciences). For cloning of some of the antigens, RNA was extracted from mouse tissues (C57BL/6) using TRIzol®-reagent (Invitrogen) and the RNeasy® Midi Kit (Qiagen) according to the protocol of the manufacturer, followed by RT-PCR. Primer sequences are available from the author upon request.