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Defect internalization and tyrosine kinase activation in Aire deficient antigen presenting cells exposed to *Candida albicans* antigens

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Abstract Patients with Autoimmune polyendocrine syndrome type I (APS I) present with multiple endocrine failures due to organ-specific autoimmune disease, thought to be T-cell-mediated. Paradoxically, APS I patients suffer from chronic mucocutaneous candidiasis. The mutated gene has been identified as the Autoimmune regulator (AIRE). Aire is expressed in medullary epithelial cells of the thymus and in antigen presenting cells in the periphery. T cells from Aire deficient mice and men displayed an enhanced proliferative response against *Candida* antigen *in vitro*, suggesting that Aire deficient T cells are competent in recognizing *Candida albicans*. In contrast, monocytes from APS I patients displayed a decreased and delayed internalization of zymosan. Furthermore, *Candida* antigen activated monocytes from APS I patients show decreased and altered phoshotyrosine kinase activation. In conclusion, Aire deficient APCs have a defect receptor mediated internalization of *Candida* which affects kinase activation, likely altering the innate *Candida* immune response.

Introduction

Autoimmune polyendocrine syndrome type I (APS I, APECED, OMIM 240300), is a rare inherited monogenic autosomal recessive disease [1,2] with an increased

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prevalence in isolated populations in Finland, Sardinia and among Iranian Jews [3,4,5]. The APS I patients develop a progressive loss of tolerance against self which leads to multiple organ failures due to the immunological destruction of adrenals, parathyroid glands, β cells of islet of Langerhans [6], stomach and small intestine [7]. Many of the auto-antigens have been identified and characterized [8,9]. The gene causing APS I was identified by positional cloning [10] and named the *A*uto*i*mmune *Re*gulator (AIRE) [11,12]. Functional studies of AIRE suggest a role as a transcription factor and *in vitro* transactivating studies support this idea [13,14,15,16]. The mouse Aire orthologue shares 71% amino acid homology with the human counterpart [17]. Aire is mainly expressed in a subset of the medullary thymic epithelial cells (mTEC) as well as in DCs in the spleen and lymph nodes [18,19,20,21]. To characterize the role of Aire in immunological tolerance, we previously engineered an Aire deficient mouse targeting the most common APS I mutation [22]. Aire deficient mice develop multiple organ-specific autoantibodies and lymphocytic infiltrates, therefore mimicking the human APS I disease manifestations [22]. Since Aire is expressed in medullary epithelial cells of thymus, studies have focused on central tolerance and have addressed the role of Aire in negative selection [23,24]. In a recent study [25], we found that Aire has role in the peripheral DC regulation of T cell activation suggesting that Aire takes part in the peripheral tolerance.

In the classical triad of APS I, in addition to autoimmunity, the patients suffer from chronic mucocutaneous candidiasis [7,26]. Chronic mucocutaneous candidiasis is a feature present in all patients with APS I starting out in early childhood, with the exception of Iranian Jews [5]. Chronic mucocutaneous candidiasis may occur as an isolated phenomenon, or as a part of APS I. Protection against *Candida albicans* infections involves innate, adaptive and humoral immunity [27]. The antibody response against *Candida* in chronic mucocutaneous candidiasis patients with or without endocrinopathy is found to be intact suggesting a normal B cell response [27,28,29].

In chronic mucocutaneous candidiasis, the fungal protection normally appears to depend on cell-mediated immunity, especially by the generation and activation of a dominant Ag-specific Th_1-cell response [30,31,32]. The strong *Candida*-specific Th_1-type immune response seen in almost all healthy humans safely allows the fungus to be present as a commensal in various mucocutaneous tracts [33,34]. In addition to the protection by Th1 immune responses, some Th2 cytokines have been shown to be required to maintain the antifungal protection [35]. The importance of T helper cell responses against *C. albicans* is further substantiated with the fact that patients with HIV and a state of T helper cell depletion frequently suffer from *C. albicans* infections [36,37]. Therefore, most work on *Candida* infections has focused on T cells.

Patients with chronic mucocutaneous candidiasis have been reported to display an impaired or low lymphocyte proliferation to *Candida* antigens [38]. In chronic mucocutaneous candidiasis without endocrinopathy, the protection seems to depend on the cell-mediated immunity, especially by the generation of a dominant Th_1-cell response [30,31,32]. However, in an early study of three patients with APS I and chronic mucocutaneous candidiasis, one patient displayed an elevated T-cell response whereas two patients were found to respond adequately [39].

In this study, we have examined the role of AIRE for the defense against *C. albicans* by investigating proliferative response, T-cell activation and the ability of antigen presenting cells to internalize and become activated against *Candida* antigens.

Materials and methods

Mice

Breeding pairs of $Aire^{+/-}$ C57Bl/6 congenic mice were raised under non-pathogen-free (non-sterile shoebox without filter top) and pathogen-free conditions (sterile shoebox with controlled/closed HEPA filter air supply). Female and male mice 8—45 weeks old, congenic and non-congenic were used. For the non-congenic mice (C57Bl/6 Sv129), wild type ($AIRE^{+/+}$) controls were littermates. Congenic mice were designed as described previously [22,40]. Ovalbumin T-cell receptor transgenic mice (OT II) [41] were a gift from Dr. Carbone. All animal experiments were conducted with approval from the local ethical committee.

Patients

Peripheral blood from three APS I patients suffering from mucocutaneous candidiasis. All APS I patients displayed severe mucocutaneous *Candida* growth needing frequent ketoconazole treatment. Samples were drawn in treatment free intervals at least 1 week after last therapy. Peripheral blood from sex and age matched healthy controls were used with their informed consent and the approval of the local ethical committee.

Preparation of mice single cell suspension and peritoneal macrophages

Spleens and lymphatic nodes were collected and the spleens were treated with 1 mg/ml collagenase (Sigma) for 1 h at 37°C in a humidified incubator supplemented with 5% CO_2. Single cell suspensions were obtained by using a glass homogenizer. Spleen single cell suspensions were treated with Red Blood cell lysing buffer for 5 min. The samples were then washed and resuspended in RPMI (Invitrogen) supplemented with 5% NCTC 109 (Invitrogen), 10% cosmic calf serum (Hyclone), 1% Pest/Glutamine (Sigma) and 0.05 mM 2-ME (Sigma). Peritoneal macrophages were collected through injection of 5 ml ice-cold PBS (Invitrogen) to the peritoneum and massaging during 2 min. The cells were then washed and resuspended in the above medium without 2-ME.

PBMC purification

Peripheral blood was collected in heparinized tubes (Vaccutainer, BD). PBMC were enriched with Ficoll-Hypaque (Amersham) gradient and washed 3 times with HBSS (Invitrogen) and finally resuspended in RPMI supplemented