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Modulation of Aire regulates the expression of tissue-restricted antigens

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Abstract

Intrathymic expression of tissue-restricted antigens (TRAs) has been viewed as the key element in the induction of central tolerance and recently, a central role for the autoimmune regulator (*Aire*) has been suggested in this process. The aim of this study was to establish whether down or up-regulation of Aire leads to alterations in TRA expression and whether this is limited to thymic epithelial cells. This study also characterized whether TRAs follow Aire expression during normal development, and whether thymic microenvironment plays a role in the expression of Aire and TRAs. We did several in vivo and in vitro experiments to manipulate Aire expression and measured expression of four TRAs (Trefoil factor-3, Insulin-2, Major urinary protein-1 and Salivary protein-1) by real-time RT-PCR. Aire had an allele dose-dependent effect on TRA expression in the thymuses of mice from two strains, C57BL/6J and Balb/c, but had no effect on TRA expression in the lymph nodes. In the thymus, Aire and TRAs were both localized in the medulla and were co-expressed during normal development and involution. In the primary stromal cells as well as thymic epithelial cell line, the adenoviral over-expression of Aire resulted in an increase in TRA expression. By manipulating in vitro organ-cultures we showed that thymic microenvironment plays a dominant role in Aire expression whereas TRAs follow the same pattern. The data underline a direct role for Aire in TRA expression and suggest that modulation of Aire has a potential to control central tolerance and autoimmunity.

Keywords: Autoimmune regulator; Thymus; Tissue-restricted antigens; Autoimmunity

1. Introduction

Thymus has an essential role in establishing immune tolerance. Previous studies have demonstrated that tissue-restricted antigens (TRAs) are expressed in thymus and that this expression is needed for the deletion of self-reactive T cells (Kyewski and Klein, 2006). A central feature in this process is the promiscuous expression of TRAs by epithelial cells in thymic medullary region, where the TRAs are presented and encountered by the thymocytes, leading to the induction of tolerance either by clonal deletion or functional inactivation (Derbinski et al., 2001). In this context, medullary thymic epithelial cells (mTEC) highly express MHC molecules with costimulatory signals and act as professional antigen presenting cells (APCs) in thymus. A detailed study of the gene expression pattern in mTEC revealed that many of the TRAs and, in particular, almost all putative autoantigen targets of experimental animal models and human diseases are expressed by mTEC (Derbinski et al., 2001; Gotter and Kyewski, 2004). Altogether, the pool of promiscuously expressed genes in thymus appears to be highly diverse including tissue and sex-specific genes and genes specifically involved in development (Kyewski and Klein, 2006).

An important molecule in regulation of TRA expression in mTEC is autoimmune regulator (Aire) (Nagamine et al., 1997). The Aire protein has several features such as SAND and PHD finger domains that are characteristic to proteins involved in transcriptional control and has been reported to bind directly to DNA (Kumar et al., 2001) and to a common transcriptional regulator and histone acetyltransferase, CREB binding protein (CBP) (Pitkanen et al., 2000). In the thymus and cell lines, the Aire protein is subcellularly located to the nuclear bodies (Bjorses et al., 1999; Heino et al., 1999), which have been associated with several functions, includ-

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ing modulation of chromatin structure, transcriptional control, DNA repair and antiviral response (Everett and Chelbi-Alix, 2007). Initial studies have shown the Aire protein to be predominantly expressed in mTECs and suggest it has a role in regulation of immune tolerance (Blechschmidt et al., 1999; Heino et al., 1999). In humans, mutations in AIRE cause autoimmune-polyendocrinopathy-candidiasis ectodermal dystrophy (APECED), a syndrome characterized by the presence of autoantibodies to multiple self antigens and lymphocytic infiltration of endocrine glands, leading to autoimmune endocrine disorders (Perheentupa, 2006; Peterson and Peltonen, 2005). In agreement with the human disease, the Aire deficient mice have autoantibodies and tissue infiltration, although the full development of autoimmune disease appears to depend on the genetic background of the mouse (Anderson et al., 2002; Kuroda et al., 2005; Ramsey et al., 2002). The Aire deficiency affects negative selection since there is a complete failure to delete the organ-specific thymocytes in this mouse model (Liston et al., 2003). More importantly, the microarray analysis of mTEC population shows a decreased or abolished expression of multiple tissue specific genes in the Aire deficient mouse suggesting thus that Aire plays a role in modulating TRAs in the mTEC (Anderson et al., 2002; Derbinski et al., 2005; Jiang et al., 2005).

This study aims to further clarify whether Aire can directly regulate the TRA expression by analyzing the expression of four antigens in several experimental settings where Aire's expression has been modulated. The study aims to establish whether there is a dose-dependent correlation between the number of Aire allele copies and TRA expression level in thymic epithelial cells, and whether TRAs are co-expressed with Aire during thymic development and involution. We also studied whether the over-expression of Aire as a sole factor is sufficient to induce TRA expression and whether thymic microenvironment plays a role in the expression of Aire and TRAs.

2. Material and methods

2.1. Mice and cell cultures

Aire deficient mice (C57BL/6J and Balb/c background) were generated at The Walter and Eliza Hall Institute (Melbourne, Australia). The inserted targeting construct containing LacZ gene replaced mouse Aire exon 8. For genotyping, the genomic DNA was extracted using JetQuick Tissue DNA Spin Kit (Genomed), and wild-type (WT) and knockout (KO) alleles were amplified using primers: 1042 5′-cagaagaacgaggat-3′, 1045 5′-cagactgccttggga-3′ or 1043 5′-ctgtcttctgtgaaggcttctagg-3′. As shown in Fig. 1A, primers pair 1042/1043 and 1043/1045 detect WT and KO alleles, respectively. Thymuses from 4- to 6-week-old WT, Aire HET (heterozygote) and Aire KO mice were used. Embryonic (E13.5, E15.5 and E17.5), newborn, neonatal D11 and adult (6 weeks, 6 months and 12 months) mouse tissues were used in developmental dynamics analysis. Mice were maintained at the mouse facility of the Institute of Molecular and Cell Biology, Tartu University. TEC 1C6 cell line (Mizuochi et al., 1992) was kindly provided by G. Holländer (University of Basel, Switzerland). Human embryonic kidney (HEK293) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS), 100 U/ml penicillin, 100 μg/ml streptomycin and 0.25 μg/ml amphotericin B (Gibco BRL).

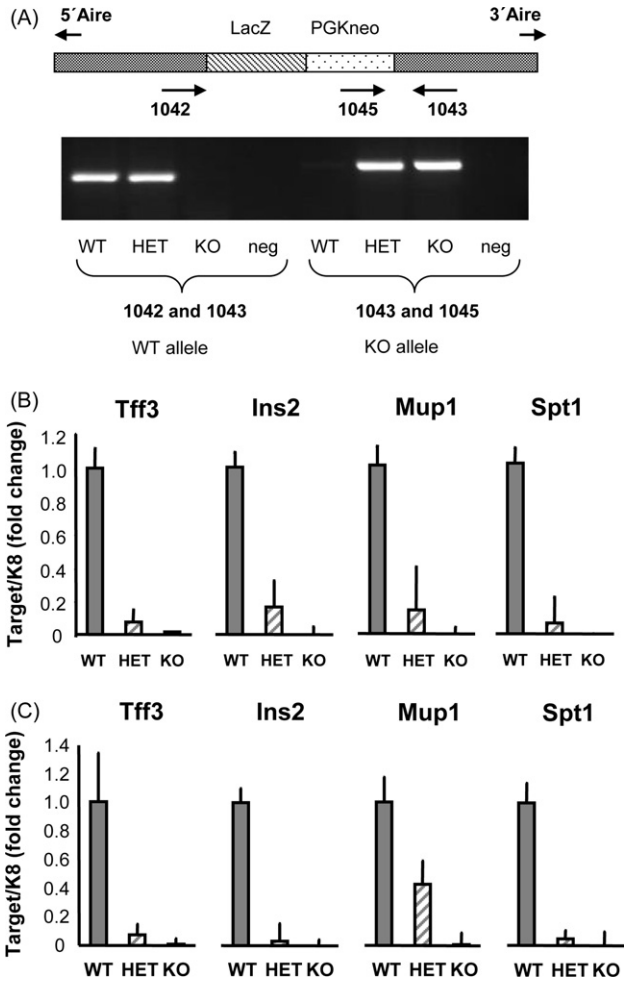

Fig. 1. Dose-dependent effect of Aire on TRA expression in C57Bl/6 and Balb/c mice. Four to six weeks old C57Bl/6 or Balb/c mice were genotyped by PCR (A) and whole thymuses from WT, Aire HET and Aire KO mice were analyzed for TRA gene expression by real-time PCR. TRA expression followed the expression of Aire in a dose-dependent manner in C57Bl/6 (B) as well as Balb/c (C) mice. Data are mean with S.E.M. of triplicate measurements of one out of two representative experiments.

2.2. EGFP and Aire adenovirus construction and infection

The pAdTrack-CMV (Stratagene) vector expressing enhanced green fluorescence protein (EGFP) gene was used as pAd-GFP plasmid. The mouse *Aire* gene was amplified from pcAire vector (Heino et al., 2000) using the primers: mAire-5-SalI 5′-tttgtcgac agatggcaggtggggatggaatg-3′ and mAire-3-NotI_stop 5′-tttgcggccgctcaggaagagaagggtggtgtc-3′ and cloned into SalI and NotI sites of pAdTrack-CMV resulting in AdAire-GFP. HEK293 cells (Invitrogen), which constitutively express AdEasy deleted E1 genes *in-trans*, were used for expression analysis of adenoviral vectors and for virus growth. To make recombinant adenoviruses, pAd-GFP and