REVIEW

Models to explore the molecular function and regulation of AIRE

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Abstract  Mutations in the Autoimmune Regulator (AIRE) gene are responsible for Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED). Within the thymic medulla, AIRE regulates the expression of a large number of tissue-specific self-antigens (TSAs) and the recognition of these TSAs by auto-reactive T-cells is a prerequisite step for thymic negative selection. APECED patients will therefore develop multi-organ autoimmune disease because of the defective role of AIRE in thymic negative selection. Aire-deficient mice also develop multi-organ autoimmune disease and in this review we will focus on how both animal and cellular models have been used to dissect biochemical function of AIRE/Aire which is an essential step toward the understanding disease pathogenesis.

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1. Introduction

Autoimmune Polyendocrinopathy Candidiasis Ectodermal Dystrophy (APECED) (also known as Autoimmune Polyglandular syndrome 1 – APS1) is an autosomal syndrome, diagnosed by the occurrence of two components of the clinical triad of chronic mucocutaneous candidiasis, hypoparathyroidism and adrenal insufficiency (Addison’s disease) [1–3], and was first reported in 1946 [4]. A wide variety of secondary disorders have been identified in APECED patients, but as these manifest with lower incidences, they are not required for diagnosis of APECED. These other clinical diseases include a variety of autoimmune endocrinopathies, ectodermal dystrophies and gastrointestinal diseases (Table 1), as well as Sjögren’s syndrome and keratoconjunctivitis. APECED patients also have a high incidence of squamous-cell carcinoma of the oral mucosa [1,2,5–10].

Studies reporting in 1997 had identified mutations in the Autoimmune Regulator (AIRE) gene as the causative factors of APECED [11,12]. Since then, a wide variety of different models systems have been used to investigate both human AIRE and murine Aire, varying from Aire knockout mice, what we have learned from these, and discuss the relevant merits and limitations of each system.

1.1. Tissue and cellular distribution of AIRE/Aire

The knowledge of the tissues and cells displaying AIRE/Aire expression (summarised in Table 2) can be particularly useful when making decisions upon which model system to use for further functional studies into AIRE/Aire. If using mice, these decisions can include which tissues to investigate in the study, or if using cell lines, whether to choose a cell line derived from a tissue that expressed AIRE/Aire natively, or to use a cell line identified to have some degree of AIRE/Aire expression.

Thymic expression of AIRE/Aire at both the RNA and protein level has been confirmed in both human and mice and identified in several other tissues across many studies investigating AIRE/Aire expression. However, it must be noted that the majority of studies investigating AIRE/Aire expression used mice, predominately the C57BL/6 strain, although Aire expression in the BALB/c, CD1, NIMR (Naval Medical Research Institute), NOD (Non-Obese Diabetic) and TRAMP (TRansgenic Adenocarcinoma of the Mouse Prostate) mice has been explored. Additionally, approximately half of the studies investigating extra-thymic AIRE expression in humans only examined PBMCs (Peripheral Blood Mononuclear Cells).

The reported extra-thymic expression of AIRE/Aire has proven to be much more contentious than that of thymic expression, as there has been an element of disparity between the different studies, with a recent review discussing these extra-thymic AIRE/Aire expression studies in more depth [13].

A major contributory factor to these differences between reports is the sensitivity of the assay used to detect AIRE/Aire expression. This is most clearly seen when certain studies using whole tissue homogenates could not detect AIRE/Aire in certain tissues, yet techniques that were able to detect AIRE/Aire at the cellular level, identified AIRE/Aire expression in specific cell sub-types of these tissues, typically either epithelial cells or cells of the myeloid lineage. Additionally, AIRE/Aire expres-

<table>
<thead>
<tr>
<th>Type of disease</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autoimmune endocrinopathies</td>
<td>IDDM, thyroid diseases, pituitary defects and hypergonadotrophic hypogonadism</td>
</tr>
<tr>
<td>Ectodermal dystrophies</td>
<td>Alopecia areata, vitiligo, keratopathy, nail and dental enamel dystrophy</td>
</tr>
<tr>
<td>Gastro-intestinal diseases</td>
<td>Pernicious anaemia, malabsorption, cholelithiasis, hepatitis and chronic atrophic gastritis</td>
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</table>
sion findings at the protein level did not always tally with those at the RNA level, with inadequate anti-AIRE/Aire antibodies suggested as a likely reason for this [13].

This knowledge of the tissues and cells displaying AIRE/Aire expression (summarised in table 2) can be used to decide upon which model system to use for further functional studies.

<table>
<thead>
<tr>
<th>Organ/tissue</th>
<th>Cell types/regions showing Aire expression</th>
<th>Protein detected</th>
<th>RNA detected [14,36,41,65–82]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>Detected in tissue. Also reported in medulla and as a scattered distribution amongst epithelial cells of the medulla (MECs); Dendritic cells; high levels found in CD80int and CD80hi MECs</td>
<td>Detected in tissue. Also reported in medulla and MECs; occasional medullary and cortical non-epithelial cells; (margins of) Hassal’s corpuscles. Found in CD80+MHC IIhi cells; ly51lowMHCIIhi cells; Cld3,4+ cells; CD45– cells; CD4–CD8+ thymocytes</td>
<td></td>
</tr>
<tr>
<td>Spleen</td>
<td>Detected in tissue. Also reported in border of red and white pulp; CD11c– cells; Dendritic cells</td>
<td>Detected in T-cell areas, T-cells and B-cells of white pulp; lymphocytes, some neutrophil granulocytes and macrophages of red pulp; MHC II+ cells</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Detected in tissue. Also reported in medulla and para cortical region; CD45+ cells; gp38–CD31– cell; very low levels found in gp38+CD31+ fibroblastic reticular cells, gp38+CD31+ blood endothelial cells and gp38–CD31+ lymphatic endothelial cells</td>
<td>Detected in medulla; also lymphocytes and some reticular cells of medulla; T-cell areas; light zone and some of dark zone of germinal centres; MHC II+ cells</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>Detected in tissue</td>
<td>Detected in epithelial cells of tubules; podocytes of glomerulus; transitional epithelium of bladder</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>Detected in tissue</td>
<td>Epithelial cells of large airways; alveolar macrophages</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>Detected in adult tissue and in foetal liver</td>
<td>Hepatocytes; Kupffer cells</td>
<td></td>
</tr>
<tr>
<td>Ovary, fallopian tubes and uterus</td>
<td>Detected in ovary and granulosa cells</td>
<td>Detected in ovary, also in epithelial cells of fallopian tube; follicular cells; oocytes</td>
<td></td>
</tr>
<tr>
<td>Testis and prostate</td>
<td>Detected in testes</td>
<td>Uterus – epithelial cells of mucosa and secretory glands; myometrium smooth muscle</td>
<td></td>
</tr>
<tr>
<td>Brain/nervous system</td>
<td>Detected in tissue</td>
<td>Detected in epithelial cells of lymph node.</td>
<td></td>
</tr>
<tr>
<td>Pituitary</td>
<td>Detected at low levels in tissue</td>
<td>Anterior and intermediate lobes</td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td>Detected at low levels in tissue</td>
<td>Parafollicular cells; epithelial cells of follicles</td>
<td></td>
</tr>
<tr>
<td>Adrenal gland</td>
<td>Detected at low levels in tissue</td>
<td>Zona glomerulosa; medullar chromaffin cells</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>Detected at low levels in tissue</td>
<td>Exocrine and endocrine cells</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Detected in tissue</td>
<td>Some large megacaryocytes; lymphoblasts; and myeloblasts</td>
<td></td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Detected in tissue</td>
<td>CD11c+ (Dendritic) cells; lymphocytes; monocytes; polymorphonuclear leukocytes; CD4– CD8+ cells (B-cells); and granulocytes</td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>CD14+ Monocytes; Dendritic cells (including monocyte derived, plasmacytoid and lymphoid); CD4+ and (weakly) in B-cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>Detected (at low levels) in tissue</td>
<td>Detected in secretory glands (accini and secretory duct secretory cells); Alimentary canal (Epithelial and glandular cells); goblet cells; GALT of large &amp; small bowel and appendix</td>
<td></td>
</tr>
<tr>
<td>Gut/Alimentary tract</td>
<td>Detected (at low levels) in tissue. Also found in gut associated lymphoid tissues (GALT)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Appendix</td>
<td>Low levels in homogenate</td>
<td>Bulb of hair follicles and outer root sheath</td>
<td></td>
</tr>
<tr>
<td>Skin and hair</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell lines</td>
<td>Jurkat; ATN-1; OTC-4; TW-EBV-LCL; MDK3; THP-1; HaCaT</td>
<td>MDK3; IL-2T; THP-1; HaCaT</td>
<td></td>
</tr>
</tbody>
</table>

* See Poliani et al. [68] for a comprehensive list of cell surface markers found on AIRE+ cells in lymph node.
into AIRE/Aire, with certain cell lines also observed to express AIRE/Aire to some degree.

1.2. Subcellular localisation of AIRE/Aire

Whilst the subcellular distribution of AIRE from immunohistochemical analysis of tissue sections has also been reported [14], the majority of studies investigating its subcellular localisation have used transfected cell-lines expressing recombinant variants of AIRE/Aire. These have included wild type AIRE/Aire, variants containing reported APECED mutations, and those with specific regions deleted in order to discern the function of specific domains in subcellular localisation [14–22].

Despite recombinant copies of both human AIRE and murine Aire being artificially expressed in differing species and tissue origins, consistent patterns of subcellular localisation was observed. AIRE was predominantly found in the nucleus where it was often described as a “speckled pattern” forming distinct nuclear bodies/dots (NBs), although it can also be found in the cytoplasm, where it has a more filamentous distribution [14,16,18,20,21]. Cytoplasmic AIRE was found on fibres that stretched from the nuclear envelope to the plasma membrane and co-localised with microtubules and with vimentin, a component of intermediate filaments [14,18,20,21]. A GFP-AIRE expression vector transfected into HeLa cells identified that whilst AIRE did form NBs, this was dependent on cell cycle and NBs would form after cell division but disappear before mitosis [16].

2. Studies using mouse models

2.1. Pathways affecting Aire expression

Mouse models have been used extensively in studies involving Aire, with a variety of transgenic species used to great effect to trace the development of Aire expressing cells, particularly of those in the thymus. Lta−/− and Ltbr−/− mice were found to have a reduction in thymic Aire expression, suggesting the lymphotoxin pathway regulated Aire expression [23]. It was subsequently determined that the lymphotoxin pathway appears to be involved in the organisation and/or development of the thymus, and that Aire expressing cells differentiate from a sub-set of the cells regulated by this pathway. In a related study, NFxB2 (Nuclear Factor κ B2) was found to be an important part of this development pathway, through the use of NFxB2−/− mice [24].

Studies involving RANK−/− (Receptor Activator of Nuclear Factor κ B) and TRAF6−/− (TNF Receptor Associated Factor 6) mice identified that RANK/RANKL (RANK Ligand) regulates Aire expression amongst mTEC precursor cells during development via TRAF6, and that RANK signalling is important for maintenance and turnover of Aire+ mTECs in adults [25–27]. It was also discerned using transgenic mice that Aire+ thymic cells were typically derived from Claudin-3+, Claudin-4+ (Cld3,4+) cells, with the minority of Cld3,4− Aire− cells being dendritic cell origin [28].

2.2. Aire knockout mice

Within a relatively short period after the identification of AIRE as the causative gene in APECED, Aire knockout mouse strains were developed as model organisms for the study of APECED. These have subsequently been used in studies investigating the molecular function of Aire and the pathways it is involved in.

The original Aire knockout mice were generated using the C57BL/6 mouse strain, and observed to produce auto-antibodies directed towards a variety of organs, as well as displaying lymphocytic infiltration in a number of organs too [29,30]. Comparisons between Aire knockout mice generated on different genetic backgrounds (C57BL/6, BALB/c, NOD) identified that there was variation in the targets of autoimmune attack between the different strains, indicating that the genetic background can modulate autoimmune symptoms [31]. These knockout mice also identified that Aire regulated the promiscuous expression of tissue specific antigens (TSAs) in the thymus, and that this function has a role in the negative selection of thymocytes in the thymus. Whilst Aire typically up-regulated gene expression, up to one third of the genes found to be differentially expressed were down-regulated in Aire+/+ mice compared to the Aire−/− mice [32–34].

The gene expression profile of Aire knockout mice compared against their reciprocal wild type mice have since been used in a number of studies to elucidate the repertoire of Aire regulated genes [29,32–39]. Studies investigating the targets of Aire regulated gene expression in medullary thymic epithelial cells (mTECs) identified that the differentially expressed genes tended to co-localise into chromosomal clusters (of approx. 200kb in size), but did not indicate a particular chromosomal preference for Aire [32]. The level of expression of Aire regulated genes has been shown to vary between not only between different mice of the same genetic strain, but has a similar degree of variation between the mTECs taken from the two thymic lobes of the same mouse. Nevertheless, comparisons made between the repertoires of Aire regulated genes in the mTECs generated from mice of different genetic backgrounds (C57BL/6, BALB/c and NOD) [34], showed elements of conservation as well as divergence between the repertoires from these different genetic backgrounds. Aire regulated gene expression has also been compared between the mTECs and pancreatic islet beta-cells of mice on the NOD genetic background, finding quite a large divergence between the repertoires of differentially expressed genes, although approx 1/3 of these genes were down regulated as would be expected. Despite alternate methods used to determine these repertoires, wild type vs Aire−/− for the mTECs and Rat Insulin Promoter driven expression of recombinant Aire (RIP-Aire) vs wild type for pancreatic beta cells, the level of divergence between these two repertoires suggests that the repertoire of Aire regulated genes is strongly influenced by tissue type [33].

The results from a number of studies using a range of transgenic mice that are either wild type (Aire+/+), heterozygote knockout (Aire+/−) or homozygote knockout (Aire−/−), have suggested that Aire functions in a dose dependent fashion. Specific peripheral tissue antigens (Tfif3, Ins2, Mup1, Sp1) were found to be expressed at lower levels in Aire+/− mice than in wild type mice, and at even lower levels in Aire−/− mice [40,41].

3. Studies using cell line models

Cell line models have mainly been used in two different ways in the study of AIRE/Aire function. The first is as a tool to ex-
press recombinant proteins and study the molecular interactions of these specific recombinant proteins, typically in the identification of proteins that AIRE/Aire is able to bind to, and the critical domains for this interaction. The second is as a cellular model with which to discern either the factors that affect AIRE/Aire expression, or the cellular effects of AIRE/Aire.

3.1. Interaction of AIRE/Aire with other macro-molecules

One of the earliest complexes identified to be formed by AIRE/Aire, was that of a homodimer and homotetramer, with this ability first determined using in vitro translated protein, and verified using hybrid assays in yeast and mammalian cells (HUH-7 and Cos-1) [42]. This oligomerisation was further corroborated in later studies using recombinant protein expressed in E. coli [43], and mammalian 2-hybrid assays using COS-1 cells [44].

In addition to its homo-multimerisation, AIRE has been found not only to associate with the nuclear matrix during pro-cells [44], but also bind to DNA [43,47] and found not only to associate with the nuclear matrix during pro-cells [44]. This oligomerisation was further corroborated using hybrid assays in yeast and mammalian cells [42].

One of the earliest complexes identified to be formed by AIRE/Aire is a homodimer and homotetramer, with this ability first determined using in vitro translated protein, and verified using hybrid assays in yeast and mammalian cells (HUH-7 and Cos-1) [42]. This oligomerisation was further corroborated in later studies using recombinant protein expressed in E. coli [43], and mammalian 2-hybrid assays using COS-1 cells [44].

Roles in repression of transcription, DNA binding, and histone modification studies of AIRE/Aire expression, and the cellular effects of AIRE/Aire.

### Table 3

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Cell line used</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBP (CREB Binding Protein)</td>
<td>Transcription co-factor</td>
<td>HEK293</td>
<td>AIRE binds to CBP via CH1 and CH3 domain on CBP [42]. AIRE and CBP act synergistically to activate IFNβ (interferon β) expression [35], and affects expression of other genes [59]</td>
</tr>
<tr>
<td>DAXX</td>
<td>Role in repression of transcription</td>
<td>Cos-1 and HeLa</td>
<td>N-terminal region of AIRE interacts with SPT domain of DAXX in Cos-1 cells. DAXX suppressed AIRE mediated activation of Insulin promoter, and suggests that this is via histone deacetylation [53]</td>
</tr>
<tr>
<td>DNA-PK complex(DNA Dependant Protein Kinase)</td>
<td>Protein kinase</td>
<td>THP-1 and HEK293</td>
<td>AIRE PHD1 domain interacted with Ku70, Ku80 and DNA-PKcs in THP-1 cells. In HEK293 cells, full length AIRE interacts with DNA-PKcs, and AIRE is phosphorylated by the DNA-PK complex in the N-terminus at residues T68 and S156 [46]</td>
</tr>
<tr>
<td>p63</td>
<td>Transcription factor</td>
<td>HEK293 and P1.4 (human thymic epithelial cells)</td>
<td>SAND domain of AIRE interacts with TA domain of p63, and is prevented by G228W-AIRE mutation. HLA class II expression is reduced by p63 alone, but increased with co-expression of p63 and AIRE [85]</td>
</tr>
<tr>
<td>PIAS-1(Protein Inhibitor of Activated STAT)</td>
<td>E3 SUMO ligase</td>
<td>Cos-1 and Caco2 (human colon epithelial cells)</td>
<td>CARD domain of AIRE interacts with c-terminal domain of PIAS-1. AIRE and PIAS-1 activate expression from Insulin promoter concurrently, whereas AIRE represses CSTB (Cystatin B) promoter, which PIAS-1 activates. AIRE does not undergo sumoylation [46]</td>
</tr>
<tr>
<td>pTEF-b(Positive Transcription Elongation Factor b)</td>
<td>Prepares RNAPII for transcription elongation</td>
<td>HeLa-MAGI and 1C6 (mouse mTECs)</td>
<td>AIRE promotes transcription elongation. AIRE interacts with cyclin T1 and cyclin dependent kinase9 components of P-TEFb complex, and recruits this complex to mouse Ins2 (Insulin 2) and Sptl (salivary protein 1) promoters [54]</td>
</tr>
</tbody>
</table>

The studies exploring the interaction of AIRE/Aire with other proteins, often used screening with yeast 2-hybrids [46,53] or pull-down assays/co-immunoprecipitation [52,54,55] to initially identify proteins that interacted with AIRE/Aire (Table 3). However, Pitkänen et al. selected CBP (CREB Binding Protein) for a series of in vitro assays, as a potential co-factor that interacted with AIRE as it was “the most common coactivator” [42]. After a target protein/complex had been identified, cell lines were typically then used either to corroborate in vitro findings, or help discern particular protein domains involved in the interaction, or to identify cellular effects of this interaction. A broad screen of proteins co-immunoprecipitating with recombinant Aire-FLAG fusion protein in HEK293 and 1C6 cells identified a range of proteins that interact with Aire, and tested the effect of these identified proteins on recombinant Aire mediated expression of KRT14 (Keratin 14) in HEK293 cells by knocking down their expression with shRNAs. The identified proteins were grouped into four broad functional classes of chromatin binding/structure, nuclear transport, pre-mRNA processing and transcription, and Aire’s involvement in these biochemical processes further tested. Aire was found to elevate
the levels of spliced (mature) mRNA, but not unspliced (pre-) mRNA in HEK293 cells, and in the human 4D6 (thymic epithelial) cell line, knockdown of the NPC-associated proteins NUP93, XPO1, KNPB1 and RanBP2, caused cytosolic retention of Aire, suggesting an indirect effect on Aire mediated regulation of expression [52].

3.2. Regulation of AIRE/Aire expression

A number of studies have used cell lines to either identify mechanisms that induce AIRE expression, or factors that are involved in the regulation of AIRE expression.

Investigations by the Pärt Peterson research group have used COS-7 cells, HeLa cells, the THP-1 and U937 human mononcytid cell lines and the TEC 1A3 human thymic cell line to study the regulation of AIRE transcription. They determined the minimal AIRE promoter, consisting of the first 350bp upstream of the ATG start codon (223bp upstream of the transcription start site). Additionally, they identified and validated transcription factor binding sites in this region for Ets-1, AP-1, Sp-1 (GC box), NF-Y (an inverted CCAAT box) and a TATA box motif [56,57]. They also discerned that regulation of AIRE expression is mediated by methylation of the AIRE promoter, and were able to up-regulate AIRE expression by treating cells with the DNA methyltransferase inhibitor 5-aza-2’-deoxycytidine, which was further increased upon addition of trichostatin A, a histone deactylase inhibitor, to the treatment regimen [56]. Nagafuchi et al. [58] were able to induce AIRE expression in the OTC-4 myelomonocytic leukaemia cell line for a limited time (less than 72 h from the commencement of treatment) by treating the cells with granulo-monocyte colony stimulating factor (GM-CSF), triggering the cell line to differentiate from monoblast-like cells into monocytes. They were able to determine that this expression was mediated through the p38 MAPK pathway, through the identification of phosphorylated proteins in the treated cells, and co-treatment with specific inhibitors alongside GM-CSF.

3.3. AIRE/Aire regulated gene expression

One of the earliest studies to identify targets of AIRE/Aire regulated gene expression in cell lines used ribonuclease protection assays to determine differential expression of a limited selection of cytokine and chemokine genes in the murine Raw 264.7 macrophage cell line [59]. Comparisons were made between the unmodified cell line and a variant carrying a stable transfectant of a recombinant Aire expression vector. IL-1ra, whose expression is normally regulated in a CBP dependent manner, was one of the few genes indentified to have a significant change in expression as a result of recombinant Aire expression. It was hypothesised that Aire dependent down-regulation of expression of this, and other genes, might be mediated by Aire sequestering CBP and quenching its normal function.

A report into AIRE regulated gene expression in the U937 cell line was released shortly after this study was published, and used a custom cDNA chip to investigate the effect of stable expression of recombinant AIRE on the cells transcriptome [60]. Similar to findings in mice, AIRE was found to both up- and down-regulate gene expression, with approx 1/3 of the genes affected being down-regulated (48 genes up-regulated and 24 down regulated). Later studies using HEK293 cells found that recombinant AIRE up-regulated the expression of Insulin, Involucrin and S100A8 by QPCR [49], and using Illumina beadchip arrays, identified significant up- and down-regulation of 140 and 154 genes respectively [61]. The genes identified in the beadchip array were also found to be more clustered than would be expected at random, and that AIRE down-regulated genes often had high levels of initial expression, and up-regulated genes typically had low levels of initial expression and lacked H3K4me3 and ACh3 markers on histones near to their promoters. Recombinant Aire expression in HEK293 cells was found to generate a 2-fold or greater up-regulation of expression in ~900 genes and similar down-regulation of expression for ~300 genes [52].

4. Choice of model system

There are many good reasons why mice have been used so extensively out of all the studies investigating AIRE/Aire function. Firstly, they provide a reproducible and modifiable model system that can have cells which have prolonged native high level expression of Aire. Secondly, they are the only genetically manipulatable system which can be used in investigations into the systemic effects of AIRE/Aire and its role in the development of central tolerance and autoimmune pathogenesis. Additionally, the variety of different genetic strains of mice enabled the identification that genetic background had a significant effect on Aire function. However, this can be both a curse and a benefit. On one hand this could complicate the interpretation of results, for example, whether certain downstream effects of Aire have been modulated due to the genetic background. Whereas on the other hand, experiments using a wide variety of different mouse strains could help identify the full range of the downstream effects of Aire, and comparisons of related experiments using different strains of mice can identify conserved Aire functionality.

A particular disadvantage using mice to determine the biochemical targets and downstream pathways of Aire is that there is likely to be increased heterogeneity between the cells extracted and tested. Aire has been found to be expressed in only specific cells of certain tissues, and thus there is the potential for non-relevant cells to modulate observed results. In addition, intra-tissue variation has already been reported, and thus this increased background noise may serve to reduce the sensitivity to detect all Aire dependent effects. Finally, whilst one must always be conscious of the potential for variation in a gene’s effects when using mice (or another organism) and their corresponding gene homolog as a model for the study of a human disorder and/or gene, particular consideration must be taken for the study of AIRE and APECED. There has been published debate over the relevance of Aire knockout mice as an appropriate model for APECED [62–64], particularly as these mice have not displayed the full range of symptoms of APECED. However, as pointed out, genetic background can modify disease symptoms observed in these knockout mice, thus the lack of a wide range of genetically diverse Aire knockout mice, compared to the genetic diversity amongst APECED patients, may in part explain this absence of a full range of symptoms.

Cell line models can overcome some of the problems that could affect mouse models, although they too have certain drawbacks in their use as model systems. One of the major benefits of using cell lines, is that it is possible to test the bio-
chemical interactions of human AIRE within a human cell line, or those of murine Aire without the need of whole mice. This is coupled with the fact that a single mouse will have a limited number of Aire+ cells from a particular tissue, whereas cell culture can generate many more AIRE+ or Aire+ cells, and thus provide sufficient cell numbers from a single source for use in experiments, as has previously reported [52]. Additionally it may be possible to obtain cell lines that have been derived from appropriate tissues, such as the thymus, which as human tissue samples could be harder to resource. Unfortunately, there is still a limitation to the range of the different sub-populations that make up a tissue that are available as a cell line. Thus, as AIRE/Aire is only expressed in certain cell types within a specific tissue, there may need to be compromise in the design of experiments, as to how similar the cell line of a tissue is to the native cellular environment in the tissue where AIRE/Aire is natively expressed. This is obviously less of an issue if the study is focusing on the interaction between recombinant AIRE/Aire or modified variant with another recombinant protein, rather than AIRE/Aire’s interaction with macro-molecules native to the cellular.

In addition to this, due to different cell lines being derived from different donor sources, when comparing results from two different cell lines, there is likely to be an element of genetic heterogeneity. These genetic differences could provide additional “background noise” that might modulate the observed results, and thus may affect the sensitivity to determine effects of the other differences between the cell lines, such as species or tissue type the cell line was derived from.

One major advantage to cell lines is that they are relatively simple to modify, whether to express recombinant proteins, or to knockdown gene expression by RNAi, or to perform different treatment regimens with various additives to growth media. Unfortunately, this is coupled with the drawback of the relative dearth of cell lines that display native AIRE or Aire expression. Whilst, AIRE expression has been induced in several cell lines after treatment [56,58], the resultant cell lines either expressed AIRE for only a relatively short period of time [58], or the treatments to demethylate DNA and deacetylate histones would have altered the cellular biochemistry to make it an inadequate model for AIRE function [56]. Consequently, many of the cell lines used to examine AIRE or Aire, needed to introduce an artificial system to express recombinant AIRE/Aire or another construct, such as a fusion protein, or protein variants containing mutations or deletions or specific domains, although there may be side-effects as a result of this. For example, artificial expression of recombinant AIRE/Aire, if driven by the CMV promoter (which is commonly used in expression vectors) will generate higher levels of expression than native AIRE/Aire expression, and thus the observed functional effects may differ than what would be observed under more native expression levels. However, this will be tempered if different experimental variants are required, whether using different variants of AIRE/Aire, or the expression of additional recombinant proteins.

In short, all studies need to carefully decide whether the cellular environment, whether murine or human tissue sample, or tissue derivation of cell line, is appropriate for the focus of the investigation. Mice can provide a model where the effects of natively expressed Aire can be easily investigated, and can identify Aire’s systemic effects on other cells and tissues, in addition to the cellular environment. Whereas cell lines can be used to model AIRE as well as Aire and provide larger numbers of a more homogenous sample of cells in a system that can be more easily modified, although the choice of cell lines natively expressing AIRE or Aire is limited.

Conflict of interest

The authors declare that there is no conflict of interest.

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