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STUDIES ON PERIPHERAL TOLERANCE IN AIRE DEFICIENT MICE

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ABSTRACT

Autoimmune diseases such as diabetes mellitus and multiple sclerosis are increasing today, but the mechanism behind these diseases remains largely unknown. Autoimmunity arise when the immune system of an individual start to attack its own organs and tissues. Immune cells go through a selection in central and peripheral organs where they are taught to be non-reactive to self structures, a process referred to as tolerance. In this thesis we have investigated the function of the autoimmune regulator (AIRE), a gene that is important for the establishment of tolerance. The autoimmune polyendocrine syndrome type 1 (APS I) is a rare severe autoimmune disease caused by a single mutation in the *AIRE* gene. These patients suffer from a range of endocrine and non-endocrine manifestations such as hypoparathyroidism and chronic mucocutaneous candida infections. Aire deficient mice have been created in order to enable the investigation on how tolerance is lost in APS I patients. These mice have revealed that AIRE is involved in the deletion of autoreactive T-lymphocytes in the thymus. AIRE is thought to induce the expression of tissue specific antigens (TSAs) that are presented to T-lymphocytes in the thymus. However, *Aire* deficient mice display a phenotype suggesting that additional tolerance mechanisms are affected.

In the first paper presented in this thesis we show that AIRE is involved in the regulation of T-cell independent B cell responses and that *Aire*$^{-/-}$ mice display an increased activation of B cells. This increased activation was demonstrated to be due to increased serum levels of the B cell activating factor of the TNF-family (BAFF) in *Aire*$^{-/-}$ mice and APS I patients. The increased levels of BAFF were in turn found to be due to uninhibited signaling of IFN-γ through the STAT1-pathway in absence of AIRE. In the second paper it was demonstrated that AIRE is expressed by a specific dendritic cell in the marginal zone of the spleen. These dendritic cells were found to regulate the activation of T lymphocytes in germinal center reactions and displayed a phenotype suggesting their involvement in tolerance mechanisms. In the third project we demonstrated that the expression of *Aire* in the marginal zone dendritic cells was regulated by IFN-γ. Upon IFN-γ stimulation both the expression of *Aire* and the TSA insulin was quickly down-regulated while the expression of inflammatory cytokines was up-regulated. These data suggest that the marginal zone dendritic cells are able to participate in tolerance induction during steady state and switch to an immunogenic state during an immune response. In the last project we investigated the development of the marginal zone dendritic cell in the bone marrow and found that *Aire* is expressed in a precursor cell to the dendritic cell in the spleen. Further, it was found that in absence of AIRE the regulation of transcription factors important for the development of this particular dendritic cell subset was impaired.

The findings from this thesis suggest that AIRE play an important function in the periphery and adds to the view that AIRE regulates both central and peripheral tolerance.

LIST OF PUBLICATIONS

I. Lindh E., Lind S., Lindmark E., Hässler S., Perheentupa J., Peltonen L., Winqvist O. and Karlsson M.C.I. (2008).
AIRE regulates T cell independent B cell responses through BAFF
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AIRE regulates genes important for development of dendritic cells in the bone marrow
In manuscript.

CONTENTS

LIST OF ABBREVIATIONS

AADC	Aromatic L-amino acid decarboxylase
AID	Activation-induced cytidine deaminase
AIRE	Autoimmune regulator protein
AIRE	Human autoimmune regulator gene
Aire	Murine autoimmune regulator gene
ALPS	Autoimmune lymphoproliferative syndrome
APECED	Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy
APS	Autoimmune polyendocrine syndrome
BAFF	B cell activating factor of the TNF family
BCR	B cell receptor
BMDC	Bone marrow-derived dendritic cell
CARD	Caspase recruitment domain
CBP	CREB-binding protein
CMC	Chronic mucocutaneous candidiasis
cTEC	Cortical thymic epithelial cell
CTLA-4	Cytotoxic T lymphocyte antigen 4
DC	Dendritic cell
DN	Double negative
DNA-PK	DNA protein kinase
DP	Double positive
FOB	Follicular B cell
GAD	Glutamic acid decarboxylase
GC	Germinal center
GCB	Germinal center B cells
HEL	Hen egg lysozyme
HLA	Human leukocyte antigen
HSR	Homogenously staining region
IA-2	Insulinoma associated tyrosine phosphatase like protein
ICOS	Inducible co-stimulator
IFN	Interferon
IDDM	Insulin dependent diabetes mellitus
ILT	Inhibitory immunoglobulin like receptor
iNKT	Invariant natural killer T cell
IPEX	Immune-dysregulation polyendocrinopathy enteropathy X-linked
IRBP	Interphotoreceptor retinoid-binding protein
LC	Langerhans cell
LTβ	Lymphotoxin β
MBP	Myelin basic protein
MHC	Major histocompatibility complex
MS	Multiple sclerosis
mTEC	Medullary thymic epithelial cell
MZB	Marginal zone B cell
NIK	NF-κB inducing kinase
NK	Natural killer cell

NOD	Non-obese diabetic
NTS	Nuclear target sequence
PD-1	Programmed cell death 1
pDC	Plasmacytoid DC
PHD	Plant homeodomain
PIAS	Protein inhibitor of activated STAT
P-TEFb	Positive transcription elongation factor b
PV	Pemphigus vulgaris
RA	Rheumatoid arthritis
RAG	Recombination activating gene
RIP	Rat insulin promoter
SCC	Side chain cleavage enzyme
SLE	Systemic lupus erythematosus
SP	Single positive
STAT	Signal transducers and activator of transcription
TCR	T cell receptor
Tfh	T follicular helper cell
TH	Thyrosine hydroxylase
TNF	Tumor necrosis factor
TPH	Tryptophan hydroxylase
TRAF6	TNF receptor-associated factor 6
Treg	T regulatory cell
TSA	Tissue-specific antigen
TSLP	Thymic stromal lymphopoietin
VCAM-1	Vascular cell adhesion molecule-1
21-OH	21-hydroxylase
17α-OH	17α-hydroxylase

1 INTRODUCTION

Our immune system is made up of a dynamic network of cells, organs and tissues that work together to protect our body from pathogens. The term immunity derives from the Latin word immunis, meaning "exempt", aiming at the phenomenon that an individual that has recovered from a disease is later on protected against the same disease. Observations of immunity were taken into practice by Edward Jenner and Louis Pasteur during the 18th and 19th century where they demonstrated that immunity could be deliberately achieved by the administration of a weakened pathogen, e.g. vaccination. However, immune responses can also lead to pathological conditions. Early in the 20th century Paul Ehrlich acknowledged that the immune system could go awry and direct its responses against the hosts own organs and tissues. He termed these events, which were later rephrased to autoimmunity, "Horror autotoxicus" (Kindt et al., 2007).

Today, autoimmune disorders affect 3-5% of a given population in western countries that may contribute to both psychosocial and economic burden for individuals as well as for the society (Jacobson et al., 1997). There is no treatment for autoimmune disease as one, instead the efforts lie on mending the isolated disease components.

In order to prevent autoimmunity the immune system has evolved an educational system referred to as tolerance. During this education, immune cells go through several checkpoints in order to behave properly when encountering both friends and foes. The complex mechanisms of autoimmunity and tolerance can be better understood by studying patients with primary immunodeficiency where tolerance is affected, Autoimmune polyendocrine syndrome type I (APS I). In APS I the tolerance machinery is flawed by a single mutation in the autoimmune regulator gene (AIRE), leading to severe organ destruction. In order to study the function of the *AIRE* gene, mice that lack *Aire* have been engineered and revealed parts of the mechanisms behind AIREs function in tolerance.

The aim of this thesis was to study the role of AIRE in peripheral tolerance mechanisms in order to investigate how immunity is maintained or broken.

1.1 OVERVIEW OF THE IMMUNE SYSTEM

The state of balanced immunity is maintained by two interconnected systems termed innate immunity and adaptive immunity. Innate immunity comprises the first line of defense and reacts rapidly but non-specifically to invading pathogens and the adaptive immunity makes up the specific and long term defense (Kindt et al., 2007).

The innate immunity consists of physical barriers such as skin and mucosal membranes as well as a range of different cells. The phagocytic cells make up a very important part of the innate immune system by their ability to engulf pathogens and particles and thereby neutralize danger or envisage it to other components of the immune system. These cells include macrophages, neutrophils and dendritic cells (DCs) (Tauber, 2003). The innate immune system is further backed up by mast cells, basophils, eosinophils, natural killer (NK) cells and γδ T cells. The mast cells, basophils and eosinophils fight off pathogens, deal with wound healing and allergic reactions and asthma. The NK cells have cytotoxic activity and the ability to recognize a wide range of tumor cells and certain viruses and are, thus, very important in the

defense against these intruders. The γδ T cells, have a distinct feature from αβ T cells in the adaptive immunity, one of them is their ability to function as a phagocytic cell (Wu et al., 2009). Also, a variety of soluble factors contribute to the innate immunity such as lysozyme in mucous membranes that destroy the cell wall of bacteria, interferons and cytokines that are produced by infected cells in order to warn other cells, and the complement system that is a group of proteins that can destroy pathogens or mark them for destruction by other cells (Kindt et al., 2007).

Due to their ability to phagocytose and present parts of pathogens on their cell surface, the dendritic cell is an important bridge between innate and adaptive immunity. DCs are a heterogeneous collection of migratory cells whose main function is to present antigens to T cells. The DCs will be reviewed in greater detail in section 1.2.3. The adaptive immune system is made up of lymphocytes that mediate the specificity during an immune response and also contribute to the diversity and memory of the immune system. The lymphocytes are equipped with distinct antigen binding receptors and thus divide into two distinct categories, B lymphocytes and T-lymphocytes (Kindt et al., 2007).

The B lymphocytes, or B cells, bear an antigen receptor, or B cell receptor (BCR), that is a membrane bound antibody molecule that binds antigens (the active part of a molecule that induces an immune response). When a B cell encounter an antigen it responds by rapid division and is further maturated into plasma cells and memory B cells. The plasma cells produce antibodies as the one encountered by the antigen, but in a soluble form that can be secreted and engage the rest of the immune components. The memory B cells are long lived cells that will react quickly when encountering the same antigen a second time. In addition there are specialized B cell subsets residing in different organs, like the B-1 cells in the peritoneal cavities and the marginal zone B cells (MZBs), follicular B cells (FOBs). Activated B cells that have come in contact with T-cells in spleen and lymph nodes will enter germinal centers (GCs) to enhance their antigen specificity and are then referred to as germinal center B cells (GCBs). The B cell subsets are best characterized in mice, but the knowledge of these cells is emerging in humans (Allman and Pillai, 2008; Martin and Kearney, 2002; Shlomchik and Weisel, 2012).

Conventional T cells, or αβ T cells, bear a T cell receptor (TCR) that typically only recognizes antigens that are bound to major histocompatibility complex (MHC) molecules, for example MHC II expressed on dendritic cells. There are two major subpopulations of these T cells, T helper cells that express CD4 on their cell surface and cytotoxic T cells that express CD8. The T helper cells assist other immune cells in different immunological functions as well as activating the cytotoxic T cells which main function is to destroy infected cells and tumor cells (Kindt et al., 2007). When activated, the T helper cells can divide into one of several different subtypes that secrete different sets of cytokines depending on the type and location of activation. The Th1 cells secrete IFN-γ and IL-2 and activate cytotoxic T cells. Th2 cells secrete, among others, IL-4 and IL-10 and mainly aids in the activation of B cells (Murphy and Reiner, 2002). Th17 cells secrete IL-17, important in inflammation and tissue injury (Harrington et al., 2005). Finally, T follicular helper (Tfh) cells are activated T cells that have come in contact with a B cell and make up a crucial part of the germinal center reactions in the spleen and lymph nodes together with GCBs (Crotty, 2011). Apart from the cytotoxic T cells and the T helper cells there are also T regulatory cells (Tregs) that are important for immunological tolerance, two subsets of long lived

memory T cells and NKT cells that recognize antigens presented by the molecule CD1d instead of MHC that, as the innate NK cells, can kill target cells (Rossjohn et al., 2012; Sakaguchi et al., 2008; Sallusto et al., 1999).

1.2 TOLERANCE AND AUTOIMMUNITY

As reviewed above, our immune system has evolved with the purpose of fighting off invading pathogens. However, equally important is the ability to distinguish self from non-self, an important mechanism referred to as tolerance. The breakdown, or loss of tolerance to self, results in responses against self-components that may lead to autoimmune disease. During their development, B and T lymphocytes undergo educational processes in primary lymphoid organs where they are taught to distinguish between self and non-self. These mechanisms are together referred to as central tolerance. Although the central tolerance machinery makes an excellent work of keeping the lymphocytes in check, nobody's perfect, and the immune system has therefore developed an artillery of safety mechanisms outside of the primary lymphoid organs, termed peripheral tolerance.

1.2.1 T-lymphocytes

T cell precursors originate in the bone marrow, but the development into the different T cell subsets, $CD4^+$ T cells and $CD8^+$ T cells, occur in specialized structures in the thymus that is populated by antigen presenting cells; cortical thymic epithelial cells (cTECs), medullary thymic epithelial cells (mTECs) and DCs (Capalbo et al., 2012; Holländer et al., 1995). The T cell progenitors, thymocytes, enter the cortex of the thymus as CD4/CD8 double negative (DN) cells where they make a commitment to the αβ T-cell lineage. During this process the DN thymocytes first rearrange their TCR β chain, through a series of steps involving induction of recombination activating genes (RAG) and the expression of a pre-TCR α chain. Once a functional TCR β chain complex is arranged, the RAG, pre-TCR α chain, and CD25 is downregulated, resulting in a burst of cell proliferation and subsequent upregulation of CD8 and CD4. The double positive cells (DP) then finally rearrange their TCR α chain and display a fully assembled TCR complex ready for positive selection (Anderson et al., 2006; von Boehmer and Fehling, 1997).

During positive selection the DP thymocytes are presented with self-MHC molecules on the surface of cTECs. In order to increase the chances of binding to a presented MHC complex, the DP thymocytes can extrapolate the rearrangement of their TCR α chain during a process referred to as receptor editing (Capalbo et al., 2012). Those DP thymocytes bearing TCRs that are able to bind to MHC with adequate affinity will receive rescue signals through the TCR and differentiate into $CD4^+CD8^-$ and $CD4^-CD8^+$ single positive (SP) thymocytes. The preference of the thymocytes to bind to either MHC I or MHC II molecules will determine whether it will downregulate the CD4 or CD8 co-receptor , respectively. DP thymocytes with TCRs unable to bind to self-MHC molecules will not receive a rescue signal are programmed to undergo apoptosis. Approximately 95% of all DP thymocytes are eliminated at this stage (Jameson et al., 1995; Kisielow et al., 1988; Zinkernagel et al., 1978).

Thymocytes that have gone through the process of positive selection will be directed to the medulla of the thymus to undergo negative selection. During this selection, thymocytes are checked for unresponsiveness against self-peptides so that potentially autoreactive T cells can be cleared off before entering the circulation (Palmer, 2003). This process is dependent on the display of a wide range of tissue-specific antigens (TSAs) that the cells may encounter in the periphery. These TSAs are expressed by mTECs along with more than 1000 other promiscuous genes (Derbinski et al., 2005). However, only 1-2% of the mTECs express a particular antigen and one mTEC only expresses a few antigens. Thus, the mTECs are aided by DCs that have the ability to scan a huge amount of mTECs for a short period of time. The DCs are also thought to take up self-peptides through apoptotic mTECs or picking them up in the periphery before migrating to the thymus (Derbinski et al., 2008; Koble and Kyewski, 2009). Thymocytes that bind with high affinity to self-antigens presented by mTECs or DCs will be subjected for clonal deletion by apoptosis (Punt et al., 1994). Thus, both the mTECs and the DCs are equipped with a range of co-stimulatory molecules, such as CD40, CD80 and CD86, that can mediate activation of the Fas signaling pathway leading to apoptosis (Capalbo et al., 2012; Foy et al., 1995). Some of the thymocytes that binds to self-antigen complexes with high affinity are thought to develop into $CD4^+CD25^+$ regulatory T cells (Treg) expressing FOXP3 (Schubert et al., 2001). The Tregs have been shown to play an important role in maintenance of peripheral tolerance, but how these cells escape negative selection is more elusive. However, the induction of Treg differentiation is thought to be mediated by DCs activated by thymic stromal lymphopoietin (TSLP) in the Hassal's corpuscles (Watanabe et al., 2005).

Thymic deletion of autoreactive T-cells is efficient, although, T-cells with low affinity for self-antigens are usually found in the periphery. (Bouneaud et al., 2000). Thus, several mechanisms exist for preventing self-reactive T-cells to become activated in peripheral lymphoid organs. These include ignorance, induction of anergy, deletion by apoptosis, and the suppressive actions of Tregs (Abbas et al., 2004). Ignorance is the simplest of all mechanisms, which means that T cells in the periphery ignore self-antigens either because of to low levels of antigen or no accessibility of the antigen (Walker and Abbas, 2002). Anergy is induced when T-lymphocytes are activated via their TCR, or perhaps also through other surface receptors, by a certain set of co-stimulatory signals that results in functionally unresponsive lymphocytes. Two of these co-stimulatory signals are thought to be the cytotoxic T-lymphocyte associated antigen 4 (CTLA-4) and programmed cell death 1 (PD-1) (Freeman et al., 2000; Perez et al., 1997). Tregs exert their function as suppressors of autoreactive T cells by the release of inhibitory cytokines such as IL-10 and TGFβ (Groux et al., 1997; Shevach, 2009). Tregs have been shown to be indispensable for the maintenance of peripheral tolerance, demonstrated by the fact that absence of Tregs in both mice and humans leads to severe autoimmune disease. Further, transfer of Tregs from healthy mice does prevent disease in autoimmune mice models (Vignali et al., 2008).

1.2.2 B-lymphocytes

The mechanisms of tolerance during B cell development in the bone marrow resemble those for the T cells in the thymus. One major difference, though, is the fact that B cells are not yet fully mature lymphocytes when entering the periphery. This is possibly a consequence of a less promiscuous antigen expression in the bone marrow compared to

the thymus (Wardemann and Nussenzweig, 2007). B cell tolerance mechanisms seem to be similar in mice and humans (Ghia et al., 1998).

B cells develop from hematopoietic stem cells and go through three sequential recombination steps to become immature B cells with a functional BCR. At the pro-B cell stage expression of RAG genes induce rearrangement of immunoglobulin (Ig) heavy chain gene segments (Pieper et al., 2013). At the second step, the pre-B cells stage, a surrogate light chain is rearranged, resulting in a pre-BCR complex (Melchers, 2005). The pre-BCR has two important tasks; the first is to shutdown the RAG genes in order to stop heavy chain rearrangement, referred to as allelic exclusion, and the second is to initiate light chain rearrangement (ten Boekel et al., 1998). At the last step, assembly of the heavy and light chains results in the expression of an IgM on the cell surface of an immature B cell. The rearrangement of the heavy and light chains of the BCR creates a diverse repertoire of antibodies capable of recognizing more than 5×10^{13} different antigens (Pieper et al., 2013). However, the advantages of recognizing such a diverse range of pathogens might be overshadowed by the fact that some of these randomly generated antibodies may react to self-antigens. To avoid this, the developing B cells are checked for autoreactivity at two different check points (Meffre et al., 2004; Wardemann et al., 2003). One of these checkpoints occurs at the early immature B cell stage in the bone marrow (before appearance of surface IgM) where the BCR plays a crucial role. B cells that receive a strong signal from the newly formed BCR will be subjected to deletion by apoptosis. B cells that receive an intermediate signal through their BCR will be given a second chance to display a non-self reactive BCR through the process of receptor editing. During this process, the B cell will further rearrange the light chain genes until a non-self-reactive BCR is displayed. B cells that fail to do this will be deleted (Gay et al., 1993). Also, self reactive B cells can be rendered non-responsive and leave the bone marrow in a state of anergy (Goodnow et al., 1988). However, anergy may only account for the removal of a few autoreactive B cell clones, whereas receptor editing applies to 20-50% of all developing B cells (Casellas et al., 2001). The importance of the BCR in the prevention of autoreactive B cells has been demonstrated in mice and humans. For example, a polymorphism in a specific allele in humans, PTPN22, leads to decreased BCR signaling that is thought to be the reason for the entering of autoreactive immature B cells in the periphery in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) (Meffre, 2011).

IgM expressing immature B cells leaving the bone marrow are regulated by a second tolerance checkpoint in the periphery. The mechanism of this checkpoint is less understood compared to the bone marrow checkpoint, however, the immature B cells are thought to go through two transitional stages on the way to a mature naïve B cell (Wardemann and Nussenzweig, 2007). The survival of the transitional B cells is dependent on the expression of a functional BCR and signals from the B cell activating factor of the TNF family (BAFF), a cytokine secreted by DCs and stromal cells (Schneider et al., 1999). BAFF promotes the survival of transitional and naïve B cells through a BAFF receptor and two additional receptors depending on the subtype of naïve B cell (Gross et al., 2000). Thus, a defect in the expression or secretion of BAFF affects the regulation of all B cells. For example, BAFF deficient mice display a severe reduction in B cell numbers and halted development at the second transitional stage (Batten et al., 2000). Conversely, overexpression of BAFF alters the development of transitional B cells leading to increased survival of autoreactive B cells (Mackay et al.,

1999). In humans, increased levels of serum BAFF is usually seen in autoimmune diseases as Sjögren's syndrome and SLE (Tangye et al., 2006). The importance of Tregs in the peripheral tolerance checkpoint has recently been demonstrated in patients lacking this celltype, which display a severe phenotype including autoreactive B cells (Kinnunen et al., 2013).

The naïve mature B cells are further differentiated into one of three different subtypes depending on its homing site. In mice, marginal zone B cells (MZB) are non-circulating cells in the marginal zone of the spleen that participate in the early immune response against blood-borne antigens in a T cell-independent manner (Lopes-Carvalho and Kearney, 2004). Human MZB cells are recirculating and express IgM carrying somatic hypermutations (SHMs) that is thought to develop independently of clonal expansion in germinal centers. Apart from this, human and mouse MZB cells seem to share similar functions (Pieper et al., 2013). MZB cells need low levels of antigen for activation, and the findings that autoreactive MZB cells are spontaneously activated without T cell help implicate that these cells are involved in autoimmune responses (Pillai et al., 2005, Mandik-Nayak et al., 2006). Furthermore, MZB cells also participate in T-cell dependent responses by capturing antigens and presenting them to follicular B cell (FOB). FOBs migrate through the blood, lymph and the follicles of the spleen where they take part in T-cell dependent responses (Allman and Pillai, 2008). T-cell dependent responses results in the differentiation of plasma cells and memory B cells. Some plasma cells can differentiate in the extrafollicular areas of the spleen without further T cell help and quickly secrete antibodies (MacLennan et al., 2003). Memory B cells, as well as plasma cells, are generated in germinal centers (GCs) where activated B cells, GCB cells, go through SHMs to further diversify their antibody repertoire. The GCB cells are presented with cognate antigen by follicular dendritic cells in the light zone of the GC and migrate to the dark zone where activation induced cytidine deaminase (AID) initiate SHM. The SHMs will either result in an increased BCR affinity leading to the formation of memory B cells or plasma cells, removal of non-functional BCRs by apoptosis or a change in the antibody specificity of the BCR, leading to an autoreactive B cell (MacLennan, 1994; Wardemann and Nussenzweig, 2007). Competition for antigen together with stable interactions with Tfh cells are thought to favor the assembly of high affinity BCRs (Allen et al., 2007). Tfh cells are equipped with several cytokines and several co-stimulatory molecules such as IL-21, PD-1 and the inducible co-stimulator (ICOS) respectively, making them important for the activation and survival of GCB cells. Conversely the GCB cells are important for Tfh cells and together they facilitate the survival of one another (Crotty, 2011). The choice between extrafollicular antibody responses and GC selection seems to be influenced by the signaling from the BCR upon initial antigen crosslinking. BCRs with high affinity are more frequently found in the extrafollicular areas while low affinity BCRs go into germinal centers (Wardemann and Nussenzweig, 2007). Apart from proper BCR signaling, BAFF secretion, Tregs and AID, MHC II expression and CD40-CD40L interactions also seems to play a role for the prevention of autoreactive B cells in the periphery (Meffre, 2011).

1.2.3 Dendritic cells

Dendritic cells do not express antigen specific receptors such as the TCR and BCR and therefore, do not go through the self/non self education as the T- and B-lymphocytes

do. However, the dendritic cells play a major role in governing both the education of lymphocytes and their action during an autoimmune response. Since the DCs are professional antigen presenters, and the main producers of cytokines in the immune system, they are responsible for the initiation, direction and strength of an immune response. Although the DCs were discovered 40 years ago the research on DCs in tolerance and autoimmune diseases has been standing in the shadow of the lymphocytes (Steinman and Cohn, 1973). However, emerging data in the recent years have shown the importance of DCs in the immune response and appreciation of the role of different subtypes.

One obstacle in DC research has been the fact that they are a much more heterogeneous group of cells then earlier thought and the identifying markers differ greatly between mice and human DCs. Common for both mice and human DCs are, though, the division between non-lymphoid tissue migratory and lymphoid tissue resident DCs and plasmacytoid DCs (pDCs) (Merad and Manz, 2009). The main function of the resident lymphoid DCs is to maintain self-tolerance and induction of specific immune responses, whereas the pDCs are important producers of cytokines in response to viral infections (Banchereau and Steinman, 1998; Liu, 2005; Steinman et al., 2003).

In mice, the non-lymphoid tissue DCs includes those that express CD103 (e-cadherin ligand), those that do not express CD103 and the langerin expressing DCs referred to as Langerhans cells (Merad and Manz, 2009). The lymphoid tissue resident DCs are made up of two major subpopulations both of them mainly situated in the spleen. One population is located to the T cell zone of the spleen and expresses CD8a and the C-type lectin DEC205 and the other population resides in the marginal zone and red pulp and expresses the surface marker 33D1 and CD4 (Dudziak et al., 2007). The $CD8a^{+}DEC205^{+}$ DCs have been shown to be specialized for MHC I restricted cross presentation (den Haan et al., 2000), whereas the $33D1^{+}CD4^{+}$ DCs primarily elicit MHC II restricted T cell responses (Dudziak et al., 2007). There is also the presence of a double negative DCs population ($CD8a^{-}CD4^{-}$ DCs) in the spleen, but it is usually more prominent in mesenteric lymph nodes. The $CD8a^{+}DEC205^{+}$ is the only DC population of these three to be found in the thymus (Shortman and Liu, 2002). Mouse pDCs express low levels of CD11c and high levels of PDCA-1 and the lectin siglec-H. The pDCs are widely distributed throughout the body, but are more frequently accumulated in the liver (Merad and Manz, 2009). An overview of the murine subtypes of DCs in primary and secondary lymphoid organs, blood and skin is depicted in Figure 1.

Accessibility to mouse lymphoid organs and tissues compared to humans has meant fewer studies on human DC subsets. Human DCs have therefore been mainly studied in blood and it has been difficult to assess whether differences in the expression of surface markers reflect subpopulations of DCs or simply differences in maturation (Ueno et al., 2011). However, there are three distinct DC populations in human blood. The major population of the $lin^{-}HLA^{-}DR^{+}CD11c^{+}$ DCs expresses the molecule CD1c (BDCA-1) and express a variety of Toll-like receptors (TLRs). A second, smaller population, expresses CD141 (BDCA-3) and the c-type lectin CLEC9A. The $CD141^{+}$ DCs have been given much attention lately since they have been suggested to correspond to the mouse $CD8a^{+}$ DC population (Jongbloed et al., 2010; Poulin et al., 2010). The human DCs do not express CD8α, the correlation has instead been made through the shared expression of CLEC9A, NECL2 and XCR1

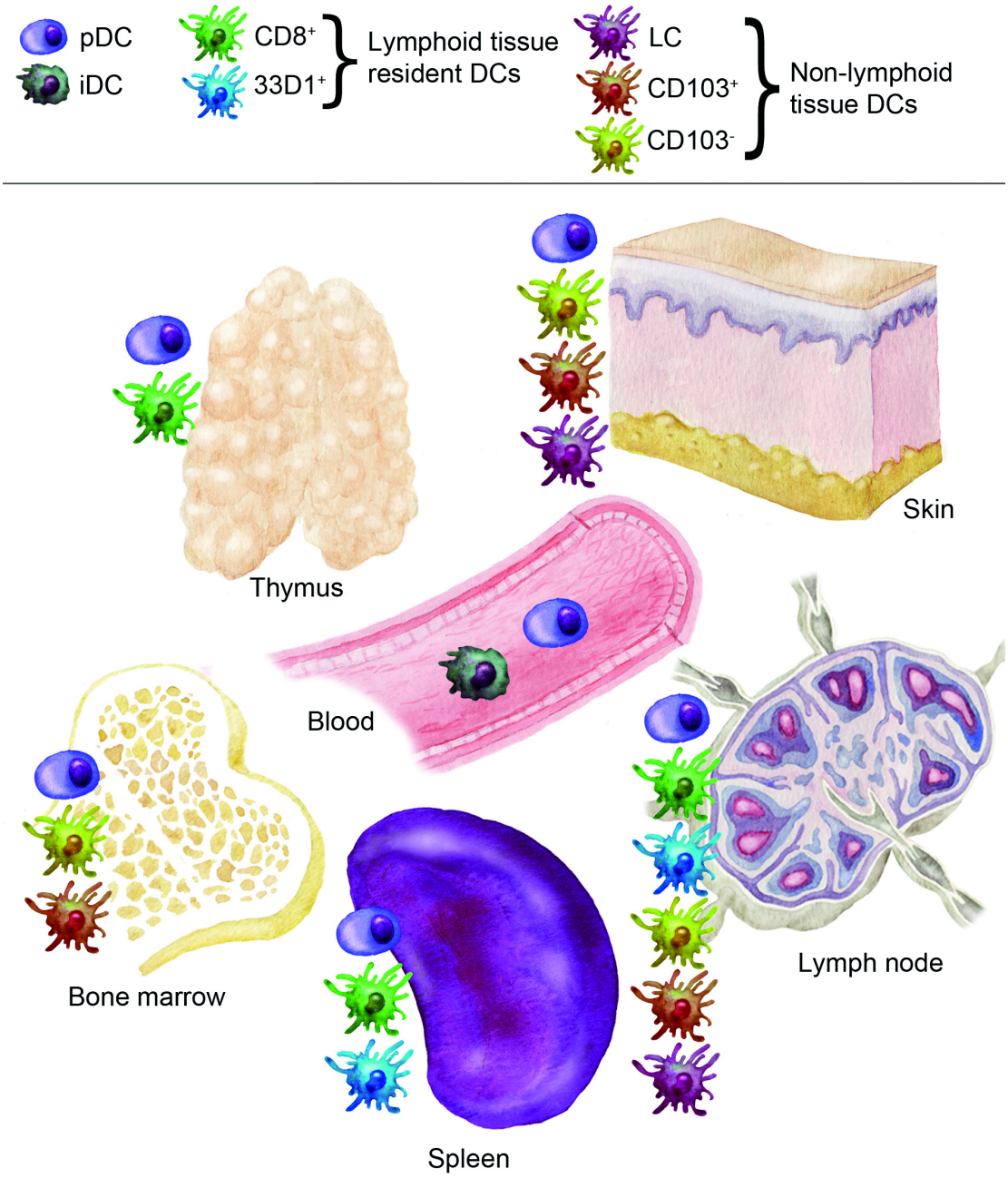

Figure 1. A schematic overview of the different murine subtypes of dendritic cells in primary lymphoid organs, secondary lymphoid organs, skin and blood during steady state. Illustration courtesy of Dr. M Winerdal.

(Bachem et al., 2010; Crozat et al., 2010). The third human DC population in blood is the pDCs that is distinguished by the expression of BDCA-2 and ILT7 and is thought to have the same function as the mouse counterpart.

Interestingly, there are two subtypes of pDCs in humans compared to mice who only have one. These subtypes are distinguished by the differential expression of CD2 and represent distinct transcription profiles. The $CD2^{high}$ subset is thought to induce a more potent allogeneic T cell proliferation than the $CD2^{low}$ subset (Matsui et al., 2009). Another site of DC characterization in humans has been the skin and here one can also find three distinct subtypes of DCs. In the epidermis of the skin reside only the Langerhans cells (LCs) expressing langerin and DCIR while the dermis

contains two subsets of DCs. The dermal DCs are $CD1a^+$ DCs and $CD14^+$ DCs. The $CD14^+$ DCs have been shown to be able to induce naïve $CD4^+$ T cells to differentiate into T follicular helper- like cells (Ueno et al., 2011; Valladeau and Saeland, 2005).

Taken together, all these reports demonstrate that dendritic cells are a heterogeneous population of migrating cells, which have strong influences on tolerance induction and activation of regulatory cells depending on the location, activation status and type of DC. DCs that maintain tolerance in the immune system are collectively named tolerogenic DCs and any imbalance in the homeostasis of these cells could consequently lead to the development of autoimmune disease. An example where altered migration and maturation of DCs leads to autoimmune disease have been demonstrated in multiple sclerosis patients where increased percentage of DCs express co-stimulatory molecules such as CD80 and CD40 at the same time as another DC subset, expressing inhibitory molecule PD-L1, is reduced (Karni et al., 2006). Another example is seen in rheumatoid arthritis where an increased and selective recruitment of DC precursors expressing inflammatory chemokines is observed in the joints of patients (Santiago-Schwarz, 2004). Several research groups have linked the hyperactivation of T cells typically seen in patients with SLE to alterations in DCs, for example a down-regulation of the differentiation marker CD83 compared to healthy controls (Ding et al., 2006).

Furthermore, it has become increasingly apparent that the production of cytokines from DCs is an important step in either mediating tolerance or causing autoimmunity. The most prominent example of cytokine-mediated induction of tolerance by DCs is the induction of Tregs. Although natural Tregs are thought to be induced through antigen presentation by DCs without activation signals, the induction of adaptive Tregs in the periphery is thought to be dependent on IL-10 and TGF-β from DCs (Wakkach et al., 2003; Yamazaki et al., 2008). Interestingly, activation of Tregs by DCs has shown to induce the production of IL-10 also by the Treg itself, thus indicating the presence of a Treg-DC homeostasis mechanism (Maldonado and von Andrian, 2010). Examples of DCs causing autoimmunity are the overproduction of TNFα in psoriasis and RA, and increased levels of type I interferons in Sjögrens syndrome and SLE thought to originate from pDCs (Steinman and Banchereau, 2007). Furthermore, IL-12 produced mainly by activated cDCs play a crucial role in the differentiation of Th1 cells and blocking of this cytokine has been found to ameliorate disease in Crohn´s patients (Mannon et al., 2004).

It has long been suggested that only immature DCs are involved in tolerance induction and that mature DCs instead have immunogenic properties (Shortman and Liu, 2002). Recent reports do, however, point at a mechanism where both immature, semi mature or mature quiescent DCs may induce tolerance by presenting self-antigens, killing T cells, or inducing Tregs. If these DCs then are subjected to an activation triggering signal, like a viral infection, they will shift to an immunogenic state providing T cells with MHC and co-stimulatory molecules leading to their activation (Blanco et al., 2008; Torres-Aguilar et al., 2010). Specific markers for tolerogenic DCs have not yet been identified, however, one candidate is the inhibitory immunoglobulin like transcription (ILT) receptors found on some tolerazing DCs (Manavalan et al., 2003). The potential capacity of tolerogenic DCs to prevent and ameliorate autoimmune disease have led to emerging studies of inducing tolerogenic DCs for the use in clinical practice both as *ex vivo* generated DC vaccines and DC targeting. Some of these studies have been successful in generating immune tolerance

in both animal models and human patients. However, due to the diversity and the plastic nature of DCs there are still a lot of obstacles to overcome before DC-therapy can be used as a safe and validated treatment. Routes and time points of administration, the origin and subtype of DC and the type of antigen chosen to be presented are examples of factors that need to be carefully considered (Gross and Wiendl, 2013; Ueno et al., 2011).

1.3 AUTOIMMUNE DISEASE

Despite the machinery of preventive mechanisms against auto reactivity in both primary and secondary organs, individuals still suffer from autoimmune disease. It is estimated that approximately 5% of the population in western countries suffers from autoimmune disease, where autoimmunity in thyroid organs, the bowel and stomach accounts for the highest prevalence (Jacobson et al., 1997; Marrack et al., 2001). The definition of autoimmune disease was re-postulated by Rose and Bona in 1993 to include 3 categories: 1) direct evidence that a disease can be reproduced in a healthy human or animal by the transfer of autoantibodies or antigen specific T cells, 2) indirect evidence that an autoimmune disease can be induced by immunization with target autoantigen or by administration of isolated autoantibodies from an autoimmune animal model or 3) circumstantial indirect evidence of disease where autoantibodies are isolated from the major target tissue of the individuals affected by disease (Rose and Bona, 1993; Witebsky et al., 1957).

Clinically, autoimmune diseases are classically divided into two major categories: organ specific and non-organ specific or systemic autoimmune disease. Organ specific disease is either caused by direct cellular damage or by stimulating or blocking autoantibodies (Kindt et al., 2007). An example of autoimmune disease caused by cellular damage is insulin dependent diabetes mellitus (IDDM) where an autoimmune attack directed against the β-cells of the pancreas leads to decreased levels of insulin and consequently to increased blood glucose levels (Lernmark and Larsson, 2013). In Myasthenia gravis, on the other hand, the autoimmune disease is caused by antibodies that block the acetylcholine receptors on muscles, hindering the binding of acetylcholine consequently leading to weakened skeletal muscles. Examples of systemic autoimmune disease are SLE and RA where the autoimmune response is affecting a wide range of organs and typically several target antigens and most commonly is a result of hyperactive B and T cells (Kindt et al., 2007).

1.3.1 Etiology of autoimmune disease

The understanding of autoimmune diseases has emerged over the past century. However, the underlying mechanism resulting in immune destruction still confuses researchers today. Several mechanisms for the initiation and maintenance of autoimmune disease have been proposed and thus, the appreciation that several factors may lead to disease progression (Davidson and Diamond, 2001; Marrack et al., 2001).

Multiple genetic factors accounts for the susceptibility in a majority of autoimmune diseases. Typically, clustering of several diseases and co-association of autoimmune disease in relatives is often seen. In addition, the concordance rate of autoimmune disease is higher in monozygotic twins than in dizygotic twins (Klein and Sato, 2000). Most autoimmune diseases are polygenic i.e. several susceptibility genes contribute synergistically to the development of disease. The majority of autoimmune

diseases are linked to the human leukocyte antigens (HLA). The strongest association is seen with the major histocompatibility complex class II (MHC II) and certain autoimmune diseases where a specific self-antigen is attacked, as is seen in RA and IDDM. Interestingly, it is also known that some HLA alleles can protect from disease even if other susceptibility genes are present (Davidson and Diamond, 2001). There are also susceptibility genes that act on immune cells directly and thus, control the overall immune response of an individual. An example of this is seen in a polymorphism of the CTLA-4 gene, which is important for down regulation of T cell activation, often associated with IDDM and celiac disease (Holopainen et al., 1999; Marrack et al., 2001).

A few autoimmune diseases are, however, caused by mutations in one single gene, nevertheless, the disease etiology can still be complex. The best characterized monogenic autoimmune diseases are immune-dysregulation polyendocrinopathy enteropathy X-linked (IPEX), autoimmune lymphoproliferative syndrome (ALPS) and autoimmune polyendocrine syndrome type I (APS I) (Davidson and Diamond, 2001). IPEX is caused by mutations in the FOXP3 gene, which is responsible for the development of regulatory T cells. Patients with IPEX develop severe and lethal symptoms very short after birth, most typically diabetes mellitus, severe diarrhea and hemolytic anemia (Bennett et al., 2001). ALPS is a autosomal dominant inherited disease where defects in the Fas protein or its receptor causes hyperproliferation of lymphocytes due to inability to induce apoptosis (Rieux-Laucat et al., 1995). APS I is an autosomal recessive disorder due to mutations in the AIRE gene which will be discussed in more detail in chapter 1.4.

It is apparent that also in these monogenic autoimmune diseases patients with the same disease display differences in disease spectrum and severity. For example it is not uncommon that siblings carrying the same mutation have different clinical features (Davidson and Diamond, 2001; Marrack et al., 2001). Also, the autoimmune disease concordance rate between monozygotic twins is far from fulfilled (Salvetti et al., 2000). Thus, other genes and non-genetic factors may also affect the etiology of autoimmune diseases.

The initiation of several autoimmune diseases has been connected to the presence of infectious agents such as bacterial and viral products. This may occur through several different mechanisms such as molecular mimicry and the release of sequestered antigens. Molecular mimicry describes the situation where an epitope of a microorganism resembles that of an antigen in a host leading to cross-reactivity. For example, cross-reactivity between streptococcal and cardiac proteins causes rheumatic fever and cross-reactivity between a peptide from the autoantigen glutamic acid decarboxylase 65 (GAD65) and the protein 2C of coxsackie B virus has been suggested as the mechanism resulting in the development of IDDM (Guilherme et al., 1995; Kukreja and Maclaren, 2000). Antigens that are normally protected from immune recognition at immune privileged locations such as the eye can be released during organ destruction and encounter a T cell for the first time leading to re-stimulation and a further exaggerated autoimmune response. This spreading of sequestered antigens may account for the cause of autoimmunity in some diseases (Kindt et al., 2007).

Theories that chance plays a role in the induction of autoimmune diseases have been proposed. Thus, stochastic events during T and B cell receptor recombination, somatic mutations and the degree of cell death may account for the differences of disease spectrum seen between monozygotic twins with the same

autoimmune defect (Germain, 2001; Mackay, 2005). Other non-genetic factors that may lead to autoimmunity are diseases or stress leading to lymphopenia, meaning that the number of lymphocytes in an individual is reduced. In order to balance this, the immune system turns on a homeostatic proliferation of cells which can lead to a skewed lymphocyte repertoire, for example favoring the development of autoreactive T cells (Ernst et al., 1999; Surh and Sprent, 2002).

Disease susceptibility between sexes differs greatly in several autoimmune diseases. The incidence of the rheumatic systemic diseases RA and SLE are for example about 70-90% higher in women than in men. Both genetically and physiological differences are thought to account for this predisposition. For example, in SLE, several X-linked genes such as CD40L and (IRAK1) are thought to be over-expressed in women due to incomplete X inactivation (Tiniakou et al., 2013). Also, female hormones may influence disease susceptibility, since estrogen support the survival of auto reactive T cell clones (Pennell et al., 2012).

Apart from describing the concept of tolerance as the immune systems recognition of self versus non-self, another model of unresponsiveness is the danger model. This model proposes that antigen presenting cells are activated only by danger signals from injured cells and thus need to be able to distinguish what is harmful from what is not (Matzinger, 1994; Matzinger, 2002). The danger model would subsequently better explain some of the mechanisms of the autoimmune disease etiologies reviewed in this chapter.

1.3.2 Autoantigens and autoantibodies

The autoantigens recognized by immune cells leading to the destruction of tissues and organs are only known for a small fraction of all autoimmune diseases. One reason for this is suggested to be due to changes in T cell and B cell responses as disease progresses in such a way that the response is focused on another autoantigen than the initial target autoantigen. This phenomenon is referred to as epitope spreading (Davidson and Diamond, 2001). Also, the autoimmune attack does not always occur at all of the sites where the autoantigen is found and it is now evident that both lymphocytes and autoantibodies contribute to devastation (Lernmark A, 2001).

Among the autoimmune diseases where the autoantigen have been identified and correlated with disease are those at the neuro-muscular junction, for example the voltage-gated calcium channels in acquired neuromyotonia or Isaac's disease. Desmoglein I have been identified in pemphigus vulgaris (PV) and the TSH receptor have been found in Graves' disease. In models of these diseases, the antibodies to these autoantigens will cause disease when transferred to an animal model (Steinman, 1995). Other well characterized autoantigens with a strong correlation to disease is the glutamic acid decarboxylase GAD in IDDM, myelin basic protein (MBP) in multiple sclerosis (MS) and fibrinogen in RA (Hayter and Cook, 2012; Lernmark A, 2001).

Autoantibodies are the immunoglobulins produced in response to a given autoantigen. Despite the fact that autoantibodies are directed against self, they class switch from IgM to IgG, show high specificity and undergo affinity maturation just like normal antibodies (Plotz, 2003). Both activated autoreactive T cells and the autoantibodies themselves contribute to tissue destruction during an autoimmune attack. For example autoantibodies in Grave's disease interfere with the physiology of

thyroid cells and the autoantibodies against desmoglein I t in PV induce release of proteases leading to the blister formation that is characteristic for the disease (Davidson and Diamond, 2001).

Despite the difficulties of pinpointing the right autoantibodies to the right autoantigen, and the corresponding disease, autoantibodies have proved to be a valuable tool for the characterization and prediction of some autoimmune diseases. Autoantibodies against organ specific diseases such as IDDM and thyroiditis can, for example, be detected long before the actual tissue destruction has started. Also, there are some diseases, such as athropic gastritis, which can have several causes but the detection of autoantibodies against H/K ATPase determine the cause as autoimmune gastritis (Lernmark and Larsson, 2013; Leslie et al., 2001).

When it comes to treatment or prevention of autoimmunity a lot of focus in the recent years has been on the use of autoantigens. Most of the efforts have been directed at Non-obese diabetic (NOD) mice and patients with IDDM where autoantigens are administered with the purpose of redirecting the immune response into a protective one and thus maintain β-cell function (Culina et al., 2013; Skyler, 2013). Although, these treatments have not had much success so far, perhaps a deeper knowledge of the actual pathogenesis of autoantigens and autoantibodies are still needed in order to be able to reinstate tolerance.

1.3.3 Autoimmune polyendocrine syndromes

Autoimmune polyendocrine syndromes are a collection of rare autoimmune diseases with mainly endocrine manifestations. The syndromes are divided into four different groups (APS I-IV) that tend to associate with each other and sometimes also with other autoimmune diseases (Eisenbarth, 2004; Neufeld et al., 1981).

APS I is the only monogenic disorder of the four and is characterized by the presence of hypoparathyroidism, adrenocortical insufficiency (Addison's disease) and chronic mucocutaneous candidiasis (CMC). APS I will be described further in chapter 1.4.

APS II, also called Schmidt's syndrome, is more common than the other syndromes and probably the most diverse. APS II is a complex genetic disorder showing strong association with HLA, in particular to a heterozygous HLA-DR3/HLA-DR4 locus. The same locus is also strongly associated with Addison's disease which is the most frequent disease component in APS II (Husebye and Anderson, 2010). Apart from Addison's disease APS II is typically characterized by the presence of autoimmune thyroid disease and/or IDDM, but also minor autoimmune diseases such as vitiligo and alopecia may be present. APS II is typically more frequent in women and usually has an insidious onset, where manifestations typically present in adult life (Eisenbarth, 2004).

APS III is characterized by the presence of autoimmune thyroiditis and at least one other autoimmune disease without the presence of Addison's disease. The inheritance of APS III is currently unknown. Individuals with a combination of autoimmune diseases, apart from the ones found in APS I-III, belong to the very rare group, APS IV. The combinations can for example be the presence of IDDM together with hypogonidism, chronic gastritis and coeliac disease (Betterle and Zanchetta, 2003).

Several mouse models of autoimmune disorders have been studied with regards to endocrine disorders. The most common ones are the NOD mice in which type I diabetes and sialitis develops spontaneously, and mice that have undergone neonatal thymectomy where massive infiltrates of lymphocytes in several organs lead to thyroiditis and gastritis (Eisenbarth and Gottlieb, 2004).

1.4 AUTOIMMUNE POLYENDOCRINE SYNDROME TYPE I

Autoimmune polyendocrine syndrome type I (APS I) also called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is an autoimmune primary immunodeficiency caused by mutations in the AIRE gene, leading to multiple organ destruction (Ahonen et al., 1990; Nagamine et al., 1997). APS I was first described in 1929 in a study of the association between hypoparathyroidism and chronic candidiasis (Thorpe and Handley, 1929). Since then APS I have been widely studied throughout the years in relation to both genetics and autoimmunity because of its monogenic recessive mode of inheritance, thus making it a very valuable model for autoimmune diseases in general.

1.4.1 Prevalence and genetics

APS I is a rare autosomal recessive inherited disease more prevalent in certain historically isolated populations; Iranian Jewish (1:9000), Sardinian (1:14500) and Finnish (1:25000) populations (Björses et al., 1998; Rosatelli et al., 1998; Zlotogora and Shapiro, 1992). The disease is also relatively common in Slovenia (1:43000), Norway (1:80000) and Poland (1:29000) compared to the rest of the world where, only isolated cases are seen (Myhre et al., 2001; Podkrajsek et al., 2005; Stolarski et al., 2006).

Individuals with APS I display a high variability in their clinical features both among the patient group and also between family members and, thus, hint that despite the monogenic inheritance of the disease other genetic and non-genetic factors influence its clinical expression (Gallo et al., 2013). A prominent example of an intrafamilial variability was recently seen in two siblings carrying the same mutation but with strikingly different disease manifestations. The younger sister had a very mild disease, while the older brother displayed a progressive and severe disease affecting nearly every organ leading to life-threatening encephalopathy (Capalbo et al., 2012). An HLA association is not found in APS I. However specific HLA haplotypes are associated with alopecia and Addison's disease in APS I patients, although HLA does not have any influence on the autoantibody formation characteristically seen in APS I (Gallo et al., 2013).

Compared to most autoimmune diseases there are no major gender differences in the prevalence of APS I (Perheentupa, 2006). However, it has been reported that female APS I patients have a slightly increased predisposition for hypoparathyroidism compared to males (Gylling et al., 2003).

The localization of the disease causing gene in APS I patients, AIRE, lead to the identification of a nonsense mutation, R257X, which is a C to T transition found in 85% of the Finnish APS 1 patients (Björses et al., 1998). This mutation was later found also in the majority of APS I patients in Italy, Norway, UK, and USA (Heino et al., 1999b; Pearce et al., 1998; Peterson et al., 2004). In contrast, an Y85C mutation is the major mutation among Iranian Jews a mutation absent in other countries. This

mutation is thought to induce a dimerization defect instead of resulting in a truncated protein as is seen in the common Finnish mutation R257X. In addition, the Y85C mutation is thought to be the reason for the milder form of APS I among Iranian Jews, typically without the presence of candida infections and fewer disease manifestations, as compared to Finnish APS I patients (Björses et al., 2000). However, the genotype correlation to disease manifestation in APS I is still very unclear, where the only correlation is seen in the presence of candida infections. Apart from the Y85C mutation, one more mutations seem to give a much milder candida infection (Kisand et al., 2010). To date over 60 APS I causing mutations in the AIRE gene have been found, most of them being nonsense or frameshift mutations leading to truncated polypeptides (Kisand and Peterson, 2011).

1.4.2 Clinical phenotype

As indicated above, patients with APS I suffer from a wide range of clinical manifestations with varying severity and disease components. The manifestations include both endocrine and non-endocrine disorders and can be up to 10 different disease manifestations in one single patient. However, the order in which these manifestations appear in the patients is very consistent (Ahonen et al., 1990; Husebye et al., 2009; Perheentupa, 2006). The first clinical symptoms of the disease are usually the infections of *C. albicans* leading to chronic mucocutaneous candidiasis (CMC) which is seen in 75-93% of the patients (Kisand and Peterson, 2011). The candida infections are typically followed by hypoparathyroidism and adrenocortical failure (Addison's disease) at age four to five (Perheentupa, 2006). The presence of at least two of these three above mentioned diseases are used for the diagnosis of APS I that is further verified by genetic testing and serological screening of autoantibodies (Blizzard and Kyle, 1963; Buzi et al., 2003). As the disease progresses, the patients usually continue to acquire several additional syndromes, such as chronic diarrhea, keratitis, autoimmune hepatitis, rashes with fever, alopecia and vitiligo (Husebye et al., 2009). It is common that by the age of 30 many APS I patients have up to 10 different disease components. The most common endocrine manifestations apart from Addison's disease and hypoparathyroidism are IDDM, hypogonadism, autoimmune thyroid disease and ovarian and testicular failure. Except for the already mentioned disease components alopecia and vitiligo, also enamel hypoplasia, keratoconjunctivitis and nail dystrophy belongs to the non-endocrine manifestations often seen in APS I (Perheentupa, 2006).

The most prevalent and most peculiar of all manifestations in APS I seems to be the CMC infections. CMC is a selective immunodeficiency that usually affects the oral mucosa, but also the esophageal and vaginal mucosa and the intestine. The yeast sometimes also spread from the mouth to the hands and nails. The CMC infections in APS I patients are superficial and rarely develop into systemic infections, but if left untreated the chronic inflammation may develop into squamous cell carcinoma (Rautemaa et al., 2007). Recently, it has been shown that APS I patients lack the anti-fungal peptide cystatin SA1, due to destruction of the submandibular glands (Lindh et al., 2012). An overview of the most common manifestations and their prevalence in APS I patients in Finland, Norway and Italy are summarized in Table 1.

To date, the treatments for APS I consist of hormonal replacement for the endocrine manifestations and aggressive antifungal treatment of CMC. Immunosuppressive treatment for some of the non-endocrine disorders has been tried in

small series of patients (Akirav et al., 2011; Husebye et al., 2009). The efforts are usually directed at ameliorating the different disease components as separate diseases, therefore, disease monitoring of all of the manifestations in a patient with APS I is utterly important. A number of manifestations other than CMC can develop into fatal disease such as chronic hepatitis, autoimmune gastritis and Addisons's disease (Husebye et al., 2009; Perheentupa, 2006).

Except for adequate clinical monitoring of APS I patients, the psychological burden of carrying such a severe disease with the stress of constant appearing and recurrent manifestations is also important to monitor. Support from family or a support counselor may account for a better life quality for APS I patients (Husebye et al., 2009).

Table 1. Frequency (%) of disease manifestations in APS I patients in Finland (Perheentupa, 2006), Norway (Myhre et al., 2001) and Italy (Betterle et al., 1998).

Disease manifestation	Finland	Norway	Italy
Diagnostic triad			
CMC	100	85	83
Hypoparathyroidism	87	85	93
Addison's disease	81	80	73
Other endocrine manifestations			
IDDM	23	0	2
Hypogonadism	69[f]28[m]	31	43
Hypothyroidism	21	10	10
Malabsorption	18	10	15
Autoimmune hepatitis	18	5	20
Pernicious anemia	13	-	15
Other non-endocrine manifestations			
Enamel hypoplasia	77	40	-
Alopecia	39	40	37
Vitiligo	31	25	15
Keratoconjunctivitis	22	10	12
Nail dystrophy	52	10	-

[f](females), [m](males).

1.4.3 Autoantibodies in APS I

In association with the disease manifestations in APS I, circulating tissue-specific autoantibodies are the hallmark of the disease (Eisenbarth and Gottlieb, 2004). The presence of autoantibodies in APS I was first discovered in 1963 by detection of autoantibodies against the adrenals and thyroid in a patient with Addison's disease (Blizzard and Kyle, 1963). Since then, several of the APS I associated autoantibodies and there corresponding autoantigens have been identified. The autoantibodies are typically directed against a specific antigen of a damaged organ or tissue and mainly of the IgG subclass. The autoantibodies are often present before the clinical manifestations

are visible and can therefore be used for prediction of APS I (Eisenbarth and Gottlieb, 2004).

Several of the identified autoantigens in APS I are key enzymes in hormone synthesis. Among the best characterized are a group of cytochrome P450 steroidogenic enzymes such as 21 hydroxylase (21-OH) in the adrenal cortex and side-chain cleavage enzyme (SCC) and 17α-hydroxylase (17α-OH) in the adrenal cortex and in the gonads and also aromatic L-amino-acid decarboxylase (AADC) and P450c1A2 in the liver (Gebre-Medhin et al., 1997; Krohn et al., 1992; Uibo et al., 1994; Winqvist et al., 1993; Winqvist et al., 1992). Another group of well characterized autoantigens are enzymes involved in neurotransmitter synthesis such as glutamic acid decarboxylase 65 (GAD65) in the pancreas, tryptophan hydroxylase (TPH) in the intestine and the tyrosine hydroxylase (TH) in hair follicles (Ekwall et al., 1998; Hedstrand et al., 2000; Velloso et al., 1994). Many of the autoantigens in APS I are also found in the isolated autoimmune diseases, i.e. 21-OH in Addison's disease and GAD65 in IDDM whereas others autoantigens are specific for APS I, such as TPH and SCC (Ekwall et al., 1998; Winqvist et al., 1993; Winqvist et al., 1992). An overview of the different autoantigens in APS I and their respective organ or tissue expression is depicted in table 2.

Table 2. Autoantigens in tissues and organs of APS I patients.

Tissue/organ	Disease manifestation	Autoantigen
Adrenal cortex	Addison's disease	21-OH, SCC, 17α-OH
Thyroid gland	Hypothyroidism	Thyroid peroxidase, thyroglobulin
Gonads	Hypogonadism	SCC,17α-OH
Pancreas	IDDM	GAD65, AADC, IA-2, insulin
Liver	Autoimmune hepatitis	P450c1A2, P4502A6, AADC
Parietal cells	Gastritis	H/K ATPase
Intestine	Intestinal dysfunction	Tryptophan hydroxylase, histidine decarboxylase
Hair follicle	Alopecia	Tyrosine decarboxylase
Melanocytes	Vitiligo	SOX9, SOX10

The prevalence of specific autoantibodies varies greatly between APS I patients, probably reflecting the variations in disease manifestations. Also, some individuals with APS I display tissue specific autoantibodies without any signs of clinical disease. Some autoantigens are, though, more frequently occurring than others. In a study of 90 Finnish, Swedish and Norwegian APS I patients it was revealed that the presence of autoantibodies against 21-OH, SCC and AADC was seen in 89% of the patients (Söderbergh et al., 1996). 7% of the patients displayed autoantibodies against insulinoma associated tyrosine phosphatase like protein (IA-2) and 37% had autoantibodies against GAD65, although IA-2 is a prevalent autoantibody in isolated IDDM (Söderbergh, 2004). Surprisingly, autoantibodies against interferon (IFN), in particular IFN-α and IFN-ω were found in all Finnish and Norwegian APS I patients (Meager et al., 2006b). These antibodies were later found to neutralize the action of IFN *in vitro*, but a functional role in mediating disease pathology for these autoantibodies was questioned (Akirav et al., 2011). Furthermore, the presence of

autoantibodies against Th17 related cytokines as IL-22 and IL-17 have been reported in APS I patients. Interestingly, these autoantibodies have been associated with CMC infections (Kisand et al., 2010; Puel et al., 2010).

1.5 THE AUTOIMMUNE REGULATOR

Mutations in the autoimmune regulator (*AIRE*) gene were found to be the causative agent of APS I approximately 15 years ago, when two groups independently cloned the *AIRE* gene and localized it to chromosome 21q22.3 (Consortium, 1997; Nagamine et al., 1997). Two years later the mouse Aire homologue was cloned and mapped to chromosome 10. The mouse *Aire* gene was shown to share 73% homology with human *AIRE* (Blechschmidt et al., 1999; Wang et al., 1999).

1.5.1 Molecular biology

Currently, three different splice variants of the human AIRE protein and eleven different variants of the mouse Aire protein have been identified by alternative splicing, where one of the variants are found in both species (Ruan et al., 1999). However, the human and mouse AIRE protein share striking similarities in both genetic sequence and structural organization (Gallo et al., 2013).

The AIRE protein is mainly found in the cell nucleus in specific nuclear bodies that forms a distinct speckled pattern, characteristic for transcription factor and thus AIRE is suggested to be involved in regulation of gene transcription (Björses et al., 2000). AIRE also contains several conserved structural domains that further support AIREs role as a transcriptional regulator. Three of the identified domains are also found in proteins of the Sp100 family that are known to interact with DNA-binding proteins. These domains include a SAND domain thought to be important for the transactivating capacity of AIRE and its subcellular localization (Ilmarinen et al., 2005). At the N-terminal region resides a caspase recruitment domain (CARD), formerly identified as a homologous staining region (HSR), at the N-terminal region important for dimerization (Ferguson et al., 2008). Finally, at the C-terminal domain of AIRE two separate plant homeodomains (PHD) with zinc finger motifs are found, thought to be DNA-binding (Consortium, 1997). Moreover, AIRE contains four interspersed LXXLL domains found in many transcriptional co-activators and a nuclear targeting signal (NTS) thought to be able to localize AIRE to the nucleus (Consortium, 1997; Savkur and Burris, 2004). The functional domains of the AIRE protein is illustrated in figure 2.

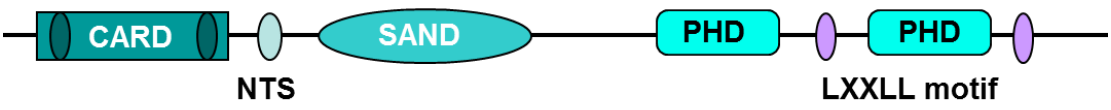

Figure 2. Schematic of the functional domains of the AIRE protein.

Two independent studies have indicated that AIRE may take part in the elongation step of transcription, but the exact molecular mechanisms for Aire's function in transcriptional regulation remains to be determined (Org et al., 2008; Oven

et al., 2007). Perhaps the interaction of AIRE with other proteins may reveal parts of the story. The first identified protein to interact with AIRE was the CREB-binding protein (CBP), a histone and nonhistone acetylase that binds to the LXXLL domain of AIRE and activates transcription (Pitkänen et al., 2000). AIRE has also been found to bind to DNA protein kinase (DNA-PK) and deficiency of DNA-PK has been found to lead to decreased levels of tissue specific antigens in mTECs in the thymus (Liiv et al., 2008). A functional interaction for AIRE and the protein inhibitor of activated STAT1 (PIAS1) in transcriptional regulation of signal transducers and activator of transcription 1 (STAT1) targeted genes have been identified (Ilmarinen et al., 2008). Furthermore, AIRE has been found to bind to and recruit complexes of the positive transcription elongation factor b (P-TEFb) to RNA polymerase II (Oven et al., 2007). In 2009, a large study on AIRE's interaction with an array of different proteins was performed that identified about 20 different proteins that directly or indirectly interacted with AIRE (Abramson et al., 2010).

1.5.2 Expression of AIRE/*AIRE*

Since the cloning of the *AIRE* gene, its expression in different organs and cells has been widely studied. Hence, expression of AIRE in humans and mice have been found in different myeloid and epithelial cells in various tissues involved in autoimmune reactions such as in the thymus, spleen, lymph nodes and in the bone marrow. The expression of human and mouse AIRE are found at similar locations, with a few exceptions, given that the accessibility of organs and tissues are greater in mice than in humans. However, the greater challenge with the mapping of AIRE expression are the contradictory results from different investigations, both when it comes to the transcript and protein expression of AIRE.

When it comes to primary lymphoid organs, it is well established that AIRE expression is found in the thymus, but expression has also been found in the bone marrow (Anderson et al., 2002; Halonen et al., 2001). In peripheral organs AIRE expression has been localized to lymph nodes, spleen and fetal liver (Adamson et al., 2004; Gardner et al., 2008; Halonen et al., 2001; Heino et al., 1999a). In addition, *Aire* transcript has been found in the heart, kidney, skeletal muscle, lung, ovary and testis (Adamson et al., 2004; Heino et al., 2000). AIRE expression has not been found in any of the target organs in APS I (Björses et al., 1999; Heino et al., 1999a).

The highest level of AIRE expression is found in mTECs in the thymus and some groups claim that this is the only location for AIRE expression (Hubert et al., 2008; Mathis and Benoist, 2009). However, *AIRE*/*Aire* expression has also been found in several peripheral cells, such as subsets of stromal cells and DCs in spleen and lymph nodes (Fletcher et al., 2010; Gardner et al., 2008; Hubert et al., 2008; Pöntynen et al., 2008). Among these cell types AIRE protein has so far only been found in pDCs in the spleen and extrathymic stromal cells, eTACs, in lymph nodes and spleen (Gardner et al., 2008; Grupillo et al., 2012). AIRE protein has also been found in B cells and monocytes in peripheral blood, in particular in $CD14^{+}$ monocytes (Kogawa et al., 2002; Suzuki et al., 2008). To date, the only AIRE expressing cell that has been proven to have a mechanistic role in regulating tolerance through AIRE is the mTEC in the thymus (Liston et al., 2003; Zuklys et al., 2000).

One aspect of *AIRE/Aire* expression that has not been as intensively studied is the actual induction or regulation of the gene or protein. However, a few studies have been made on the induction of *Aire* in mTECs. Several conflicting reports have been published on *Aire* induction and lymphotoxins. One group demonstrated that signaling through the lymphotoxin β (LTβ) receptor regulated *Aire* expression and PTA gene transcription, without changing the structure of the thymus epithelium (Chin et al., 2003; Zhu et al., 2006). Another group showed that LTβ receptor signaling was required for accurate mTEC differentiation but showed no impact on *Aire* expression (Boehm et al., 2003). The latter was supported by groups showing that LTβ may induce PTA expression in *Aire* negative mTECs (Seach et al., 2008; Venanzi et al., 2007). Also, the RANK/RANK ligand pair and its downstream signal transducers TNF receptor-associated factor 6 (TRAF6) and NF-κB inducing kinase (NIK) have been found to have an effect of the generation of AIRE positive mTECs. Both the soluble RANK ligand and surface bound CD40 ligand were needed for the generation of AIRE, but it has not been shown whether RANK signaling induce *Aire* transcription independently of mTEC differentiation (Akiyama et al., 2008; Hikosaka et al., 2008). Further, some of the transcription factors belonging to the Ets family have been shown to positively activate *Aire* transcription (Meager et al., 2006a).

1.5.3 *Aire* deficient mice

Before the cloning of the murine *Aire* gene, several animal models were used to study the phenotype of APS I. White leghorn chicken, mice infected with cytomegalovirus, BioBreeding rats and NOD mice were among those models that developed some of the manifestations seen in APS I (Ikegami, 2002). However, the generation of *Aire* deficient mice made the research on APS I take leaps forward.

The two first *Aire* deficient mice were made in 2002 by two independent groups and were of a mixed 129/Sv and C57BL/6 background (Anderson et al., 2002; Ramsey et al., 2002). The mouse made by Ramsey et al., was engineered to mimic the most common human Finnish mutation, R257X (Björses et al., 1998). The *Aire* gene was knocked out with the insertion of a neomycin-cassette (termination codon) in the beginning of exon 6. The phenotype of this mouse was relatively mild with no signs of clinical endocrine disease, but infiltrates of lymphocytes in several organs, atrophy of thymus and adrenals, infertility and organ specific circulating autoantibodies were observed. In addition, the mice displayed increased T cell proliferation after immunization with hen egg lysozyme (HEL) antigen and a change in the distribution of the TCRVβ chain families (Ramsey et al., 2002). This mouse was later crossed onto several double transgenic mouse models. The first model expressed HEL as an organ specific antigen under the control of the rat insulin promoter (RIP) and T cells expressing a TCR specific for HEL. Studies on this mouse model confirmed the role of *Aire* in tolerance mechanisms by demonstrating that *Aire* is needed for deletion of autoreactive T cells in the thymus (Liston et al., 2003). Second, the *Aire* deficient mice were crossed to a similar system, where the HEL antigen was instead expressed under the control of the thyroglobulin promoter. These mice displayed a decreased thymic expression of endogenous insulin and an increased presence of islet specific-T cells in the spleen (Liston et al., 2004). This mouse was later backcrossed to a congenic C57BL/6 background that displayed normal endocrinology, but infiltrates of B cells in

the liver and MZB cell lymphomas developed when reaching above 15 months of age (Hässler et al., 2006).

The second *Aire* deficient mouse was created by Anderson et al., using conditionally targeted disruption of exon 2 and parts of the surrounding introns (Anderson et al., 2002). This induced knock-out displayed some differences in phenotype with the Ramsey model. Lymphocytic infiltrates of salivary glands, ovarian follicles and the retina of the eye, and autoantibodies against these targets were found. Gene array analysis of mTECs from these mice displayed a reduction of some tissue specific antigens in the absence of *Aire*. Among the reduced antigens were insulin, salivary protein 1 and fatty acid binding protein. Furthermore, bone marrow transfer experiments and thymic transplants in these mice revealed that *Aire* is important for the prevention of autoimmunity (Anderson et al., 2002). Later, a similar model of the HEL transgene, though with ovalbumin as an antigen, was crossed to this *Aire* deficient mouse. This model showed a defect in the regulation of negative selection of OT-II T cells (Anderson et al., 2005). Further, the original Anderson mouse have also been backcrossed onto several different strains including C57BL/6, BALB/c, NOD and SLJ. These mice displayed some differences in phenotypes that may reflect the disease varieties between APS I patients. For example, the C57BL/6 and BALB/c mice displayed a mild phenotype compared to the one seen on the mixed 129/sv and C57BL/6 background, while the *Aire* deficient mice on the NOD and SLJ background had a more severe phenotype with a higher magnitude of lymphocytic infiltrates and the occurrence of pancreatitis and thyroiditis. Surprisingly, autoreactivity in these mice was seen against the exocrine cells of the pancreas and not against the β-cells (Jiang et al., 2005).

In 2005, a third *Aire* deficient mouse was generated by targeting of exon 5 to 12 and backcrossed onto the C57BL/6 and BALB/c background. Similarly to the two previous *Aire* deficient models, this mouse also displayed reduced fertility and infiltrates of lymphocytes. However, these mice also displayed a reduction in tear fluid and autoreactivity against α-fodrin, an antigen found in Sjögren's syndrome. In addition, the *Aire* deficient mice on the BALB/c background showed infiltrates of lymphocytes in the gastric mucosa and autoantibodies against this tissue (Kuroda et al., 2005). A year later this *Aire* deficient model was backcrossed onto the NOD background. These mice exhibited reduced body weight and lymphocytic infiltrates of several organs. In addition, the autoreactivity against the pancreas was directed at exocrine cells, the acinar cells surrounding the β-cells. The presence of diabetes that is found in normal NOD mice did not develop in the *Aire* deficient NOD mice and might, thus, be explained by the non-reactivity against insulin and the β-cells (Niki et al., 2006).

A fourth *Aire* deficient mice was created to mimic the second most common human mutation in APS I patients, a 13 base pair deletion that disrupts the PHD domain in exon 8. In addition to a similar mild phenotype as the previously described Aire deficient mice on a B6 background, these mice also displayed an increased frequency of activated MHC class II expressing mTECs in the thymus and a reduction in the immature MECs (Hubert et al., 2009).

One double *Aire* knock-out mice has been described. Scurfy mice, deficient in FoxP3, on the C57BL/6 background were crossed with *Aire* deficient mice with the exon 2 deletion on a C57BL/6 or NOD background. These mice exhibited severe lymphocytic infiltrates of the liver and the lungs, approximately 14 days after

birth, resulting in death or early termination at 28 days of birth. Surprisingly, other tissues remained healthy and no presence of autoantibodies was found. Not surprisingly, though, the double-knockout mice on the NOD background developed an even more severe manifestation of disease in the liver and lungs than the C57BL/6 (Chen et al., 2005).

In addition, a knock-in mouse giving rise to a dominant negative *Aire* mutation, G228, has been constructed on the C57BL/6 and the NOD background. These mice displayed a global suppression of tissue specific antigens and developed a novel phenotype with autoimmune thyroiditis and peripheral neuropathy (Su et al., 2008). A summary of the different *Aire* deficient mice is given in table 3.

Table 3. Aire deficient mouse models.

Targeted exon	Background	Major findings
Exon 6 (Ramsey et al., 2002)	Mixed C57BL/6 /129	Liver infiltrates of lymphocytes, autoantibodies, increased response against HEL, altered TCRβ repertoire.
Exon 6 (Hässler et al., 2006)	C57BL/6	MZB cell lymphoma, liver infiltrates of B cells, normal endocrinology.
Exon 2 (Anderson et al., 2002)	Mixed C57BL/6/129	Infiltrates of lymphocytes in salivary glands, ovaries, stomach and eye, differentialy regulated genes in mTECs.
Exon 5-12 (Kuroda et al., 2005)	C57BL/6 and BALB/c	Autoreactivity against α-fodrin, infiltrates of lymphocytes in gastric mucosa, reduction in tear fluid.
Exon 2 (Jiang et al., 2005)	C57BL/6, BALB/c, NOD, SJL	Organ infiltrates depending on background, exocrine pancreatitis in NOD and SJL.
Exon 2 (Chen et al., 2005)	FoxP3$^{-/-}$ C57BL/6	Death at 28 days of age, severe lymphocytic infiltrates of lung and liver, no autoantibodies.
Exon 5-12 (Niki et al., 2006)	NOD	Exocrine pancreatitis, protection against diabetes.
Exon 8 (Hubert et al., 2009)	C57BL/6	Increased levels of activated mTECs in thymus, reduction of immature mTECs

Several of the findings in all of these *Aire* deficient mice suggest differences in the immunopathogenic mechanisms between the mice models and the human individuals with APS-1. One of them is the fact that the *Aire* deficient mice have a much milder phenotype than the human patients, with the NOD *Aire* deficient mice being the only one to develop autoimmune disease, suggesting that *Aire* deficient mice are lacking an environmental trigger or additional disease susceptibility genes (Hubert et al., 2009; Kuroda et al., 2005). It has been suggested that the captivity of

Aire deficient mice under specific pathogen free conditions account for the mild phenotypes and would for example explain the absence of mucocutaneous candida infections in the *Aire* deficient mice (Mathis and Benoist, 2009). Furthermore, it has been shown that *Aire* deficient mice do not develop the same type of autoantibodies as APS I patients, since the autoantibodies produced are not directed towards the most common organs destroyed in APS I (Pöntynen et al., 2006). For example the liver is the most common target organ in *Aire* deficient mice which is very rarely affected in APS1 patients (Ramsey et al., 2002; Söderbergh et al., 2004).

1.5.4 Aire function in mice and men

The findings that mTECs expressed high levels of AIRE along with a wide range of TSAs raised the speculations that AIRE could be involved in the regulation of central tolerance in the thymus (Blechschmidt et al., 1999; Derbinski et al., 2001). Also, the AIRE protein was found to have regulatory elements resembling that of transcription factors (Björses et al., 1999). Hence, AIREs role in central tolerance was established when absence of AIRE in transgenic mouse models lead to impaired deletion of tissue specific antigens in the thymus. This was demonstrated by several groups using TCR transgenic *Aire* deficient mice (Anderson et al., 2005; Liston et al., 2003). The deletion of autoreactive T cells in the thymus has also been shown to be *Aire* dose-dependent, since deletions induced by antigens under control of the thyroglobulin promoter in homozygous *Aire* mice was less efficient than in *Aire* heterozygous mice (Liston et al., 2004). The fact that *Aire* deficient mice do not develop antibodies against insulin or any of the autoantigens seen in APS I patients raise the question that AIRE in mice and humans may not have the same function (Pöntynen et al., 2006). Also, *Aire* deficient mice on the NOD background led to the development of exocrine pancreatitis and not diabetes (Jiang et al., 2005). In contrast, reduced expression of the stomach antigen mucin 6 and the eye antigen interphotoreceptor retinoid-binding protein (IRBP) in the thymus was shown to also appear as autoantigens in the periphery, displaying a direct cause and effect function for *Aire* in mice (DeVoss et al., 2006; Gavanescu et al., 2007). However, the *Aire* deficient mice do also show autoreactivity to autoantigens that are expressed at normal levels in the thymus as alpha-fodrin and a pancreas specific protein disulphide isomerase (Kuroda et al., 2005; Niki et al., 2006).

Functions of AIRE in the thymus other than regulating negativeselection through expression of TSAs have been proposed. For example, AIRE has been implicated to play a role in the differentiation of mTECs (Derbinski et al., 2005). On the other hand, AIRE expression seems to be dependent on a proper thymic composition, since mice with disrupted thymic epithelium and medulla lack mTECs and AIRE expression, as observed for example in RelB deficient mice (Heino et al., 2000). Furthermore, absence of AIRE and or mTECs leads to a block in the development of $CD4^+$ T cells in the thymus (Li et al., 2007). Also, *Aire* has been shown to regulate the expression of co-stimulatory molecules important for both maturation and migration of thymocytes. For example, *Aire* deficient mice have reduced expression of CCL22 in the thymus and thus implies that *Aire* is needed for the migration of DP T-cells to the medulla (Anderson et al., 2005). In contrast, CCL19 and CXCL10, expressed at the cortico-medullary junctions luring SP T cells to migrate to the blood, are increased in *Aire* deficient mTECs (Annunziato et al., 2001).

Aire deficiency has also been found to affect other cell types that develop in the thymus. It has been shown that TSA expression in the thymus is able to positively select Tregs, which raised the question whether this is an AIRE dependent event. At first, several Aire deficient mouse models showed normal Treg function and number (Anderson et al., 2002; Kuroda et al., 2005). However, a later study showed that AIRE positive mTECs targeted with a particular antigen were able to induce Tregs with the same antigen specificity (Aschenbrenner et al., 2007). Several recent reports have been able to further establish Aires role in clonal deletion and differentiation of Tregs (Lei et al., 2011; Malchow et al., 2013). In addition, Treg function in APS I patients were found to be impaired (Kekäläinen et al., 2007).

Furthermore, it has been shown that the development of invariant NKT (*i*NKT) cells is dependent of *Aire*. A recent study showed that Aire deficient mice displayed a reduction of *i*NKT cells in both thymus and peripheral organs a finding confirmed also in APS I patients who displayed a reduction of *i*NKT cells in peripheral blood (Lindh et al., 2010).

Apart from the expression of AIRE in mTECs AIRE is also expressed in the periphery and thus, AIRE is implicated to play a role in peripheral tolerance. In the periphery AIRE expression is mainly found in subsets of DCs in lymphoid organs and monocytes in peripheral blood (Grupillo et al., 2012; Kogawa et al., 2002). Thus, both APS I patients and *Aire* deficient mice have shown increased antigen presenting cell mediated T cell activation in spleen and lymph nodes. Further, microarray experiments on stimulated DCs from *Aire* deficient mice has pointed out several differentially regulated genes that could be involved in the activation of T and B cells. One of these are the vascular cell adhesion molecule-1 (VCAM-1) expressed on endothelial cells and lymphoid DCs (Ramsey et al., 2006). Also, immunization of *Aire* deficient mice with HEL antigen leads to a hyperproliferative T cells response (Ramsey et al., 2002).

Furthermore, the presence of marginal zone lymphomas has been reported in aged *Aire* deficient mice which imply an increased activation of MZB cells in the absence of *Aire* (Hässler et al., 2006). It has also been shown that depletion of B cells with anti-CD20 monoclonal antibody ameliorate the autoimmune pathology of *Aire* deficient mice (Gavanescu et al., 2008).

Murine AIRE expression has also been found in stromal cells in lymph nodes. These AIRE expressing stromal cells were suggested to be involved in TSA presentation to $CD8^+$ T cells, however some of the key TSAs, such as insulin were not expressed by these cells (Lee et al., 2007). However, in a second report AIRE expressing stromal cells were found at the T and B cell border in lymph nodes and spleen. These extrathymic stromal cells, were found to mediate deletion of autoreactive T cells and found to express some of the TSAs expressed by mTECs in the thymus (Gardner et al., 2008).

In summary, AIRE has been established to play an important role in mediating central tolerance in the thymus. Emerging evidence also point at a role for AIRE in the periphery and hopefully future research will aid in the understanding of Aires mechanism in peripheral organs.

2 PRESENT STUDY

The aim of this study was to investigate the role of AIRE in peripheral tolerance mechanisms by the use of *Aire* deficient mice in order to improve our understanding on how immunity to self is maintained or lost.

2.1 METHODOLOGY

A detailed description of the materials and methods used in the following studies are given in the original papers, I-IV.

Mice (Paper I-IV)
The generation of *Aire*$^{-/-}$ mice (B6.129s4-Aire^{tm1P1tn}) has been described previously (Ramsey et al., 2002). In addition, congenic *Aire*$^{+/-}$ C57BL/6 mice crossed with OTII TCR transgenic C57BL/6 mice, or OTII TCR transgenic C57BL/6 mice alone were used with the permission of Dr F. Carbone (Paper I and II). All Aire$^{-/-}$ mice used were age- and sex matched with wild type littermates from heterozygotic breeding as controls. Athymic BALB/c nude mice were from Charles River Laboratories. The mice were kept at the animal facility at the Department of Microbiology, Tumour and Cell Biology, Karolinska Institutet.

Patients (Paper I-IV)
Blood was collected from APS I patients and used with their informed consent (Paper I). PBMCs were collected from healthy blood donors by automated leukapheresis and used with their informed consent (Paper II-IV). Blood and bone marrow were collected from patients undergoing total hip arthroplasty surgery and used with their informed consent (Paper IV).

Immunizations (Paper I-IV)
Mice were injected subcutaneously with Flt3L transfected tumor cells 14 days before sacrifice (Paper II-IV). Mice were immunized i.p. with TNP-ficoll (Paper I) and NP(23)-CGG in alum (Paper II), serum was collected before and after immunization.

Bone marrow transfer experiment (Paper I)
Bone marrow cells were obtained from flushed mouse femurs. Bone marrow transfer was performed by intravenous injection of donor-derived bone marrow cells into recipient mice irradiated with 900 rad 4 hours prior reconstitution (Paper I).

Proliferation assay (Paper II)
CFSE labeled CD4^{+} splenic OTII transgenic T cells were injected into mice intravenously 12 hours prior to intra peritoneal injection of DC-targeted antibody coupled to ovalbumin. Mice were sacrificed three days after i.p. injection.

Flow cytometry (Paper I-IV)
Single cell suspensions were prepared from murine spleen and bone marrow. Cells were stained with fluorochrome-conjugated antibodies and analyzed by flow cytometry, either on a FACSAria or a FACSCanto using FACS diva (Becton Dickinson) or FlowJo (TreeStar, Inc).

Cell Sorting (Paper II, IV)
Single cell suspensions were prepared from murine spleen and bone marrow. Cells were stained with bead-conjugated antibodies and sorted with magnetic beads either manually or with AutoMACS (Miltenyi Biotech). Magnetically sorted cells were

stained and sorted on a FACSAria (Becton Dickinson) or a MoFlo XDP (Beckman Coulter).

ELISA and ELISPOT (Paper I-III)
Levels of antigen specific antibodies were measured in serum from mice using specific ELISA kits according to manufacturer's instructions. An ELISPOT assay was used to measure the numbers of IgM-secreting B cells (Paper I).

Quantitative RT-PCR amplification (Paper I-IV)
Total RNA was extracted with Trizol® reagent (Invitrogen) or Rneasy mini spin kit (Qiagen) and cDNA was synthesized by reverse transcription. Transcript levels were amplified using specific primers on an iCycler IQ Optical System or a TFX96 Touch Real-Time System (Bio-Rad). Quantitative RT-PCR array was prepared according to manufacturer's instructions (SABiosciences/Qiagen) (Paper II, III).

Immunofluorescent staining (Paper II)
Immunohistochemistry was used to analyze protein expression in frozen tissue cryosections and single cells obtained by cytospin centrifugation. Immunofluorescence was documented using a confocal laser scanning microscope (Leica TCS SP2).

Electrophoresis and immunoblotting (Paper I)
Proteomic analysis was performed with 2-D electrophoresis, SDS page and immunoblotting.

Statistical analysis (Paper I-IV)
Data were analyzed with student's T-test, Wilcoxon's rank sum test or Mann-Whitney U test in order to test for differences between two groups. Mean values are given together with standard deviations. $P<0.05$ was considered significant.

Ethical considerations
All experiments involving animal procedures and use of human material were evaluated and approved by the local ethics committees and have been conducted according to the regulations for handling laboratory animals and patient data.

2.2 RESULTS AND COMMENTS

2.2.1 Absence of AIRE leads to increased B cell activation through defective STAT1 regulation

Aire$^{-/-}$ mTECs display reduced levels of TSA expression in the thymus, a defect that would lead to the presence of autoreactive T cells in the periphery. However, the majority of the manifestations in the periphery of *Aire*$^{-/-}$ mice including autoantibodies, liver infiltrates of B cells and development of MZB cell lymphoma imply an increased activation of B cells (Hässler et al., 2006). In this study, Paper I, the activation of B cells in the absence of AIRE was investigated. *Aire*$^{-/-}$ mice were immunized with T cell independent antigen type I, TNP-Ficoll. The response to TNP-Ficoll is mainly dependent on MZB cells, demonstrated by a reduction of specific antibodies in immunized MZB cell deficient mice (Guinamard et al., 2000). When compared to wild type mice, *Aire*$^{-/-}$ mice displayed a significant increase in the production of TNP-specific antibodies. Furthermore, the response was characterized by an increased activation of peripheral B cells, in particular the MZB cells. The distribution of all B cell subsets remained normal in the *Aire*$^{-/-}$ mice. Mice overexpressing the cytokine BAFF share the same characteristic B cell manifestations as observed in the *Aire*$^{-/-}$ mice (Mackay et al., 1999). In order to elucidate the mechanism behind the increased activation of MZB cells the levels of BAFF in *Aire*$^{-/-}$ mice and APS I patients was measured. Both mice and patients displayed significantly increased levels of BAFF in serum (Figure 3).

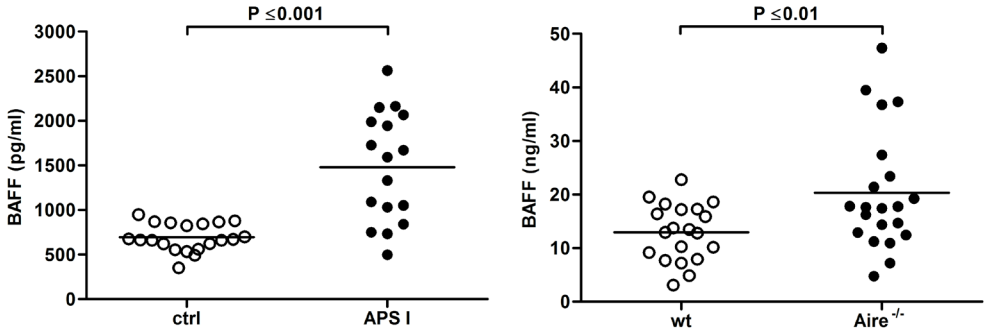

Figure 3. Increased BAFF in serum of APS I patients (left panel) and *Aire*$^{-/-}$ mice (right panel), measured with ELISA. Each dot represents one individual and mean values are indicated by horizontal lines.

BAFF has been shown to be secreted by DCs and radio resistant stromal cells when stimulated with IFN-γ and is important for the activation and maturation of B-cells (Schneider et al., 1999). To exclude that the increased levels of BAFF were due to autoreactive T cells, the levels of BAFF expressing DCs were assessed in athymic nude mice reconstituted with bone marrow from *Aire*$^{-/-}$ mice or wild type mice. These experiments confirmed the intrinsic increase of BAFF in *Aire*$^{-/-}$ mice and importantly demonstrated that the production of BAFF by DCs is T-cell independent. The levels of BAFF secreted by *Aire*$^{-/-}$ bone marrow-derived DCs (BMDCs) were found to be further increased upon IFN-γ stimulation *in vitro* (Figure 4). There were no differences in the

levels of IFN-γ in the spleen of *Aire*$^{-/-}$ mice and wild type mice. In addition, *in vitro* stimulation of *Aire*$^{-/-}$ BMDCs with IL-10 revealed no differences in BAFF secretion compared to wild type BMDC, suggesting that IFN-γ is the main inducer of BAFF in the *Aire*$^{-/-}$ mice.

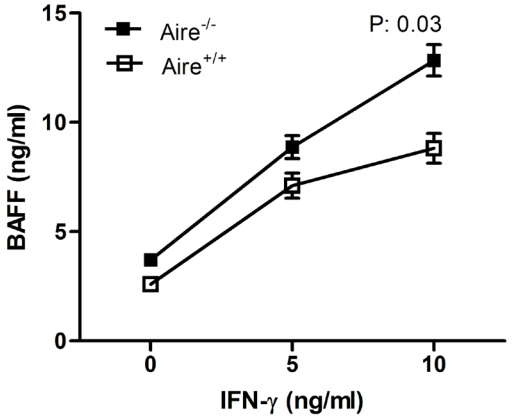

Figure 4. Production of BAFF from *Aire*$^{-/-}$ or wild type BMDCs after IFN-γ stimulation *in vitro*, measured with ELISA. Data represents mean values and SEM of four mice in each group and representative of two independent experiments.

Upon engagement, the IFN-γ receptor signals through the STAT1 pathway and regulates the expression of several genes. During the progress of this work it was shown that AIRE interacts functionally with the protein inhibitor of activated STAT1 (PIAS1) (Ilmarinen et al., 2008). This indicated that AIRE and PIAS1 might work together to inhibit downstream signaling of the IFN-γ receptor. We therefore suggest that altered signaling through STAT1 subsequently leads to increased secretion of BAFF in *Aire*$^{-/-}$ mice and in APS I patients. In line with this hypothesis, the STAT1 regulated gene *Gbp1* was found to be up-regulated in splenocytes from *Aire*$^{-/-}$ mice.

Comments

After the publication of this paper, the role of AIRE as a regulator of STAT1 signaling has been further investigated. The expression of several STAT1 regulated genes was found to be up-regulated in *Aire*$^{-/-}$ BMDCs (Figure 5). These genes have also been reported to be up-regulated in *Pias1*$^{-/-}$ mice (Liu et al., 2004). Furthermore, decreased levels of insulin has been reported in mTECs stimulated with IFN-γ (Levi and Polychronakos, 2009). More recently, another article by the same authors demonstrated an increase in the expression of several autoantigens, including insulin, in mTECs isolated from IFN-γ deficient mice. The expression of AIRE in these mice was also found to be increased (Levi and Polychronakos, 2013). The mechanism behind AIREs role in the regulation of TSAs in mTECs has not yet been fully established. However, these data suggest that AIRE regulates the IFN-γ signaling pathway and perhaps IFN-γ also regulates AIRE expression.

The role of BAFF in mediating B cell survival and autoreactive B cells has been demonstrated in several autoimmune diseases and recently the human neutralizing anti-BAFF monoclonal antibody, Belimumab, was approved for the

treatment of SLE. However the treatment has so far only yielded moderate results and alternative inhibitors are currently under investigation (Stohl, 2012).

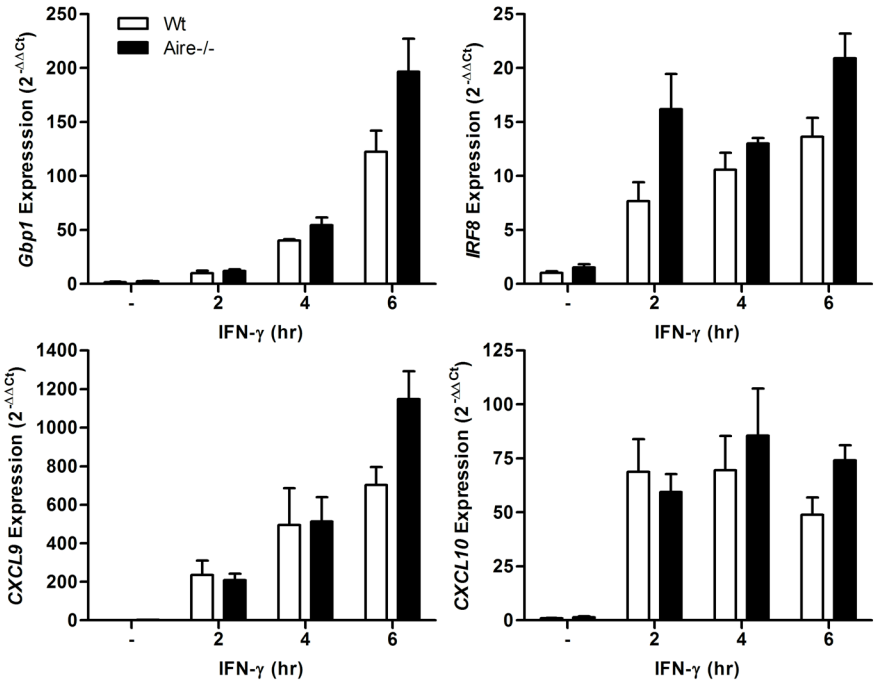

Figure 5. Expression of the STAT1-targeted genes *Gbp1*, *IRF8*, *CXCL9* and *CXCL10* in *Aire*$^{-/-}$ and wild type BMDCs after IFN-γ stimulation measured by quantitative RT-PCR. Bars represent mean of 3 mice and errors bars represent SEM.

2.2.2 AIRE in marginal zone dendritic cells regulate peripheral immunity

In this study (Paper II), the role of AIRE in peripheral DCs was further investigated. The expression profile of *Aire* in murine peripheral DCs subsets was investigated and revealed that AIRE is specifically expressed by DCs bearing the surface marker 33D1. 33D1 is specifically expressed by DCs residing in the marginal zone of the spleen and bridging channels, areas that have been shown to influence both T and B cell tolerance (Dudziak et al., 2007; Finkelman et al., 1996). In support of this, it was found that the marginal zone DC subset expresses the autoantigen insulin, which was decreased in *Aire*$^{-/-}$ 33D1^{+} DCs (Figure 6). Furthermore, the inducible co-stimulator (ICOS) ligand was found to be up-regulated on *Aire*$^{-/-}$ 33D1^{+} DCs. These features are seen in AIRE expressing mTECs in the thymus and in AIRE expressing stromal cells in the spleen and lymph nodes, which strengthen the role of the 33D1^{+} DCs in peripheral tolerance mechanisms.

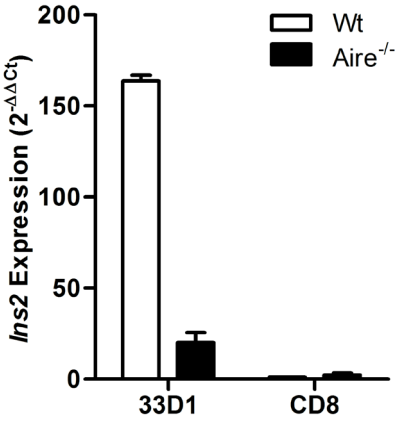

Figure 6. Expression of *Ins2* in *Aire*$^{-/-}$ and wild type 33D1^{+} and CD8^{+} DCs measured by quantitative RT-PCR. Bars summarise three independent experiments, error bars represent s.d.

The 33D1^{+} DC subset has also been implicated to be important for the maturation of plasmablasts in the extrafollicular areas of the spleen (García De Vinuesa et al., 1999). In accordance with this function, the chemokine CXCL12, known to be involved in the migration of plasma cells was found to be expressed by the 33D1^{+} DCs and down-regulated in *Aire*$^{-/-}$ 33D1^{+} DCs. In order to elucidate whether the decreased levels of CXCL12 affect the movement of cells within the structures of the spleen, *Aire*$^{-/-}$ mice were immunized with the T-cell dependent antigen, NP-CGG. The response against this antigen was found to be decreased in *Aire*$^{-/-}$ mice, which displayed a reduction of specific antibodies against IgG1 and IgG3. Also, the same antibodies were found to reside within germinal centers in the follicles of the spleen and not in the extrafollicular areas as seen in the spleens of wild type mice. Thus, we speculated that the low production of CXCL12 by *Aire*$^{-/-}$ 33D1^{+} DCs in the marginal zone was unable to support plasma cell migration out of the GC, which resulted in a haltered extrafollicular response to NP-CGG. Further, it was revealed that the *Aire*$^{-/-}$ mice displayed increased levels of Tfh cells and GCB cells after NP-CGG injection. The staggering of B cells in the follicles of *Aire*$^{-/-}$ mice, as well as the increased levels of BAFF and ICOSL on *Aire*$^{-/-}$ DCs may be the reason for the exaggerated numbers of B cells and T cells found in germinal centers. Also, a number of co-stimulatory factors on

the Tfh cells and the GCB cells themselves likely contribute to the increased activation of these cells (Figure 7).

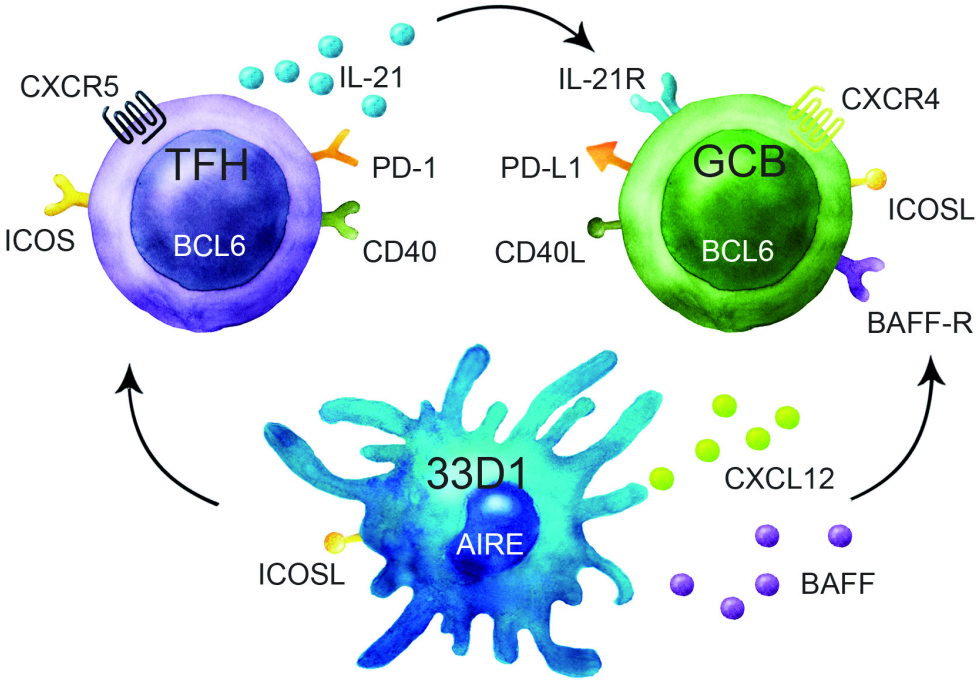

Figure 7. Schematic of the different cytokines, ligands and receptors connecting the 33D1$^+$ dendritic cells, the T-follicular helper cells and the germinal center B cells. Illustration courtesy of Dr. M. Winerdal.

Comments

Just before the publication of this paper, an article confirming the role of the 33D1$^+$ DCs in regulating extrafollicular T cell dependent responses was published (Chappell et al., 2012). Furthermore, mice that lack the receptor for CXCL12, CXCR4, display the same phenotype as the *Aire*$^{-/-}$ mice (Cyster, 2003). However, the mechanism behind AIREs regulation in these responses remains to be investigated. The localization of the 33D1$^+$ DCs in the marginal zone/red pulp area, makes it likely that this subset of DCs is involved in the exaggerated activation of MZB cells seen in aged *Aire*$^{-/-}$ mice (Hässler et al., 2006).

Interestingly, ICOS mediated mechanisms have been found to influence the development of insulin-dependent diabetes in NOD mice (Hawiger et al., 2008). Also, anti-ICOSL antibodies have been used to inhibit B cell mediated autoimmunity through blocking of Tfh cells in several autoimmune mouse models (Hu et al., 2009).

2.2.3 IFN-γ regulates the expression of *Aire* in marginal zone DCs

The previous findings, that AIRE is involved in regulating the STAT1 signaling pathway and that stimulation of *Aire*$^{-/-}$ BMDCs with IFN-γ leads to increased levels of BAFF, prompted the investigation for a similar role for the AIRE expressing 33D1^{+} DCs (Paper III). Similar to *Aire*$^{-/-}$ BMDCs, the 33D1^{+} DCs also displayed an increase of STAT1 targeted genes upon IFN-γ stimulation. The relationship between AIRE and IFN-γ have been further investigated by Levi and Polychronakos which have reported decreased levels of insulin in mTECs after stimulation with IFN-γ (Levi and Polychronakos, 2009). Since AIRE deficiency leads to decreased levels of insulin transcript (*Ins2*) similar to what is seen in wild type mice stimulated with IFN-γ, it was tempting to speculate that IFN-γ also affected the expression levels of *Aire*. Thus, IFN-γ stimulation of 33D1^{+} DCs *in vitro* resulted in both decreased levels of *Aire* and *Ins2* transcripts (Figure 8).

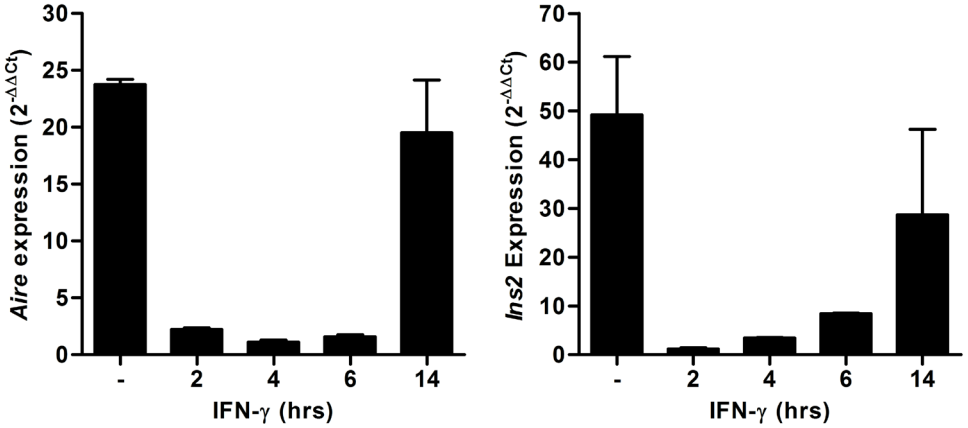

Figure 8. Relative mRNA levels of *Aire* and *Ins2* in 33D1^{+} DCs after IFN-γ stimulation *in vitro*, analyzed by quantitative RT-PCR. Data represents mean values of three independent experiments and error bars represent s.d.

Furthermore, the down-regulation of *Aire* and *Ins2* after IFN-γ was found to be transient, with a quick drop in expression two hours after stimulation followed by a restoration in expression after eight hours. In order to elucidate whether the down-regulation of *Aire* and *Ins2* in 33D1^{+} DCs is truly caused by IFN-γ, the transcript level of *Aire* was investigated in IFN-γ receptor deficient 33D1^{+} DCs. The expression of *Aire* was not down-regulated in the IFN-γ receptor deficient 33D1^{+} DCs, in fact, the levels were instead increased compared to wild type levels (Figure 9). This observation suggests that the levels of Aire in C57BL/6 mice are under the control of naturally occurring IFN-γ in steady state. Since the down-regulation of *Ins2* followed the down-regulation of *Aire*, the possibility that a converse situation may be found in the IFN-γ receptor deficient mice. In line with this hypothesis, an increased level of insulin was found in these mice.

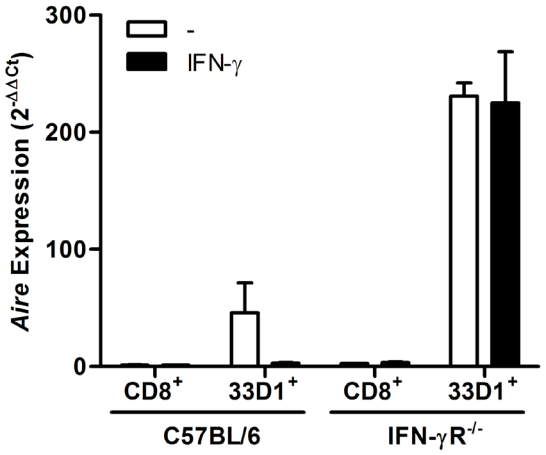

Figure 9. Relative expression of *Aire* in 33D1$^+$ and CD8$^+$ DCs sorted from spleen of wild type mice and IFN-γ receptor$^{-/-}$ mice analyzed by quantitative RT-PCR. Data represents mean values of two independent experiments and error bars represent s.d.

Comments

What regulates the expression of *Aire* has remained elusive for many years and so far no consisting data on this matter has been found. These results suggest that, not only does AIRE regulate the signaling of IFN-γ through the STAT1 pathway, but IFN-γ also regulates the level of *Aire* expression itself. Interestingly, a very recent publication demonstrated that mTECs from IFN-γ$^{-/-}$ mice do display increased levels of both *Aire* and *Ins2* (Levi and Polychronakos, 2013).

IFN-γ is mainly released by activated CD4$^+$ T cells, NKT cells and NK cells during inflammatory responses. These findings suggest that *Aire* positive 33D1$^+$ DCs present self-antigens during steady state, but upon activation *Aire* is down-regulated and the DCs will shift to an immunogenic state and instead up-regulate the expression of chemokines and co-stimulatory molecules to aid in the response against foreign pathogens (Blanco et al., 2008; Torres-Aguilar et al., 2010). It remains to be investigated whether this situation is true during an inflammatory response *in vivo*.

2.2.4 *Aire* deficiency affects the expression of developmental genes in DCs

In this study, paper IV, the role of *Aire* in the bone marrow precursors of dendritic cells was investigated. AIRE has been reported to be expressed in murine bone marrow and thus, raised the question whether the expression of *Aire* in the 33D1$^+$ DCs is acquired already at a precursor stage in the bone marrow (Halonen et al., 2001). The tissue resident DCs and plasmacytoid DCs (pDCs) are derived from the common dendritic cell precursor (CDP) where they diverge into pre-pDCs and pre-cDCs, possibly under the influence of a set of transcription factors. The pre-cDCs are further delineated into CD8$^+$, 33D1$^+$ and DN DCs upon maturation in the spleen, however the fate of development into either one of these subsets is thought to be pre-programmed in the bone marrow. This decision is thought to be influenced by the induction of certain lineage specific transcription factors (Satpathy et al., 2011). The DC precursor stages were sorted out from murine bone marrow according to their expression of specific surface markers (Figure 10).

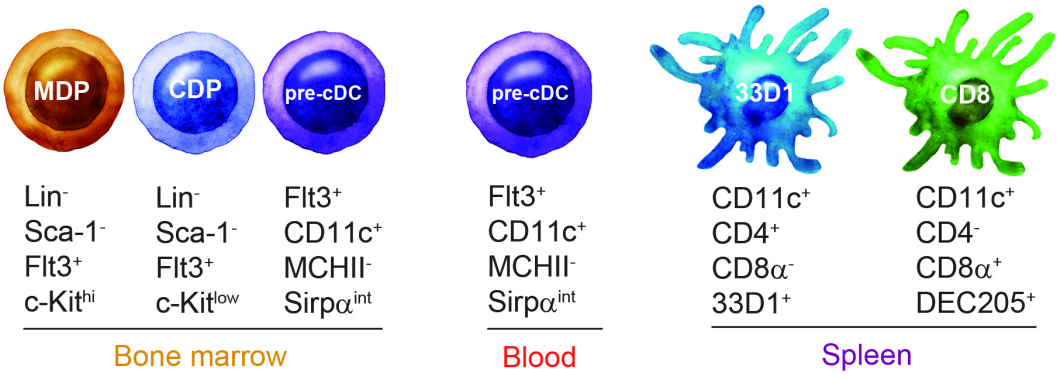

Figure 10. Schematic overview of the subtypes of dendritic cells and the markers used to identify each cell investigated in this study. MDPs, CDPs and pre-cDCs were sorted out from murine bone marrow. 33D1$^+$ and CD8$^+$ were sorted out from the spleen. Illustration courtesy of Dr. M. Winerdal.

Although lower than the expression in the fully differentiated 33D1$^+$ DC subset, *Aire* expression was found in the pre-cDC subset in the bone marrow. However, the frequency and distribution of the pre-cDC subset appeared to be normal in the *Aire*$^{-/-}$ mice. Furthermore, the population of 33D1$^+$ DCs in the spleen was found to be slightly decreased in the *Aire*$^{-/-}$ mice. This indicated that an alteration in the developmental step between the pre-cDC and 33D1$^+$ population might be the problem. Therefore, the transcriptional factors thought to influence the development of splenic DC subsets in the bone marrow were investigated. Two of the transcription factors thought to be important for the development of the 33D1$^+$ subset were found to be decreased in the *Aire*$^{-/-}$ 33D1$^+$ DCs (Figure 11). Mice deficient in either of these transcription factors, i.e. IRF4 or RelB, display a marked reduction of the 33D1$^+$ DC subset (Suzuki et al., 2004; Wu et al., 1998). However, the reduction of these genes in the *Aire*$^{-/-}$ 33D1$^+$ DCs were only about 50%, but might explain the reduction of 33D1$^+$ DCs in the spleen of *Aire*$^{-/-}$ mice.

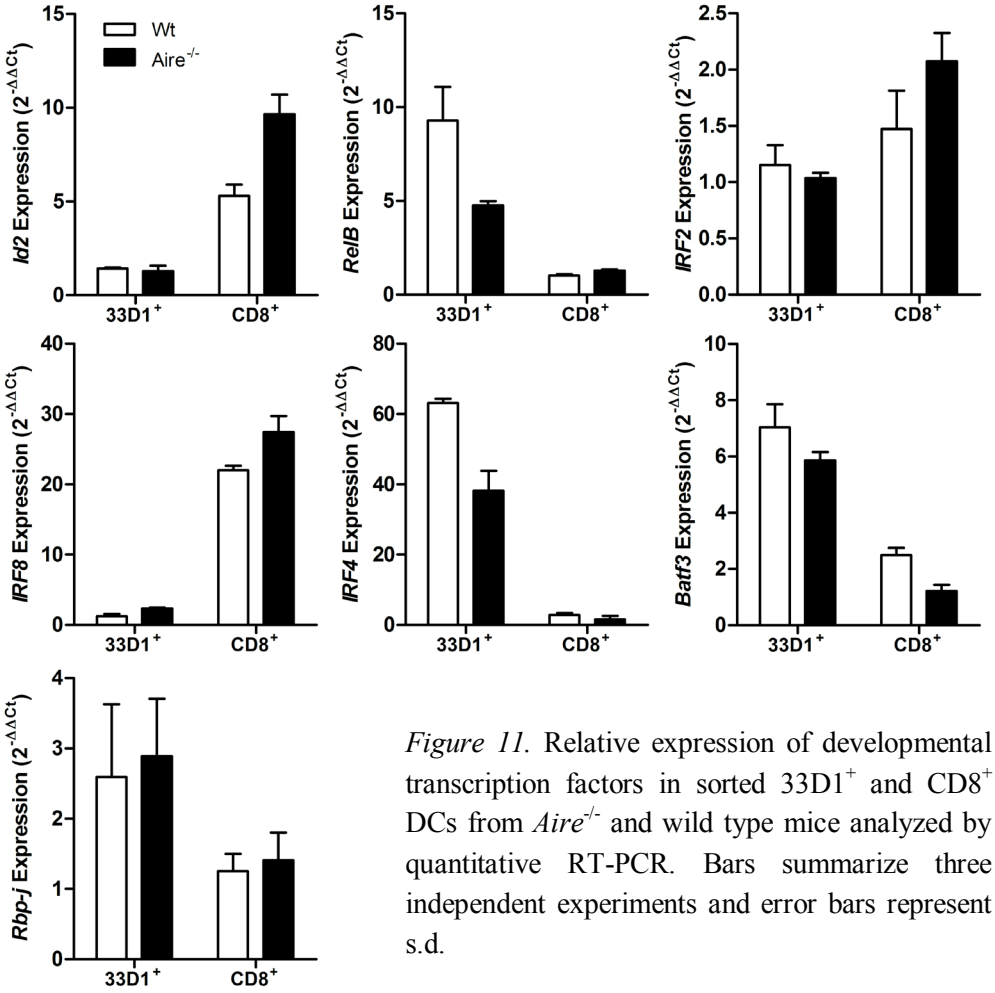

Figure 11. Relative expression of developmental transcription factors in sorted 33D1$^+$ and CD8$^+$ DCs from *Aire*$^{-/-}$ and wild type mice analyzed by quantitative RT-PCR. Bars summarize three independent experiments and error bars represent s.d.

Also, one gene that has not, so far, been shown to influence the development of 33D1$^+$ DCs is IRF8. IRF8 is instead thought to be important for development of the CD8$^+$ DC subset. Interestingly, *IRF8* is a STAT1-targeted gene and thus induced by IFN-γ (Liu et al., 2004). The previous findings, that *Aire*$^{-/-}$ 33D1$^+$ DCs display increased levels of STAT1-targeted genes upon IFN-γ, lead us to investigate the expression of *IRF8* in the 33D1$^+$ DC subset after IFN-γ stimulation. In accordance with our hypothesis, IRF8 was found to be up-regulated in the *Aire*$^{-/-}$ 33D1$^+$ DCs after IFN-γ stimulation *in vitro*. Thus, we speculate that the slight reduction of 33D1$^+$ DCs in the Aire$^{-/-}$ mice might be due to a rescue by the induced *IRF8* expression in this subset.

Comments

Neither the induction of *Aire*, nor the factors affecting the regulation of dendritic cell commitment and differentiation have been well studied. In particular, how lineage transcription factors are regulated to determine DC diversification is largely unknown (Fierabracci, 2011; Satpathy et al., 2011). These data point at a role for AIRE in the regulation of lineage commitment transcription factors at the later stages of DC development in the bone marrow. Further investigation of AIREs role in regulating these transcription factors is needed. Also, investigations of the pre-cDC subset in blood of *Aire*$^{-/-}$ mice may reveal some clues.

Interestingly, the transcription factor *RelB*, which was found to be down-regulated in the *Aire*$^{-/-}$ 33D1^{+} DCs, has also been implicated to influence the development of mTECs in the thymus. Further, *RelB*$^{-/-}$ mice lack AIRE expression and have an abnormal thymic architecture that lacks the medullary structure (Heino et al., 2000).

2.3 GENERAL DISCUSSION

Since the mouse *Aire* gene was discovered and the generation of *Aire* deficient mice began nearly 15 years ago, only a small part of the story behind the severe autoimmunity in APS I has been unraveled. When it comes to the function of AIRE it has been clearly established that AIRE is involved in the negative selection of autoreactive T cells in the thymus. Transfer studies in mice revealed that AIRE expression in mTECs is crucial for negative selection of developing T-lymphocytes in the thymus and that AIRE mediate this by the expression of tissue specific antigens (TSAs) that are normally expressed in the periphery (Anderson et al., 2002).

However, taking a major leap forward in the understanding of AIREs function, this story has a few flaws that may not fully explain the disease in APS I patients. For example, the same group also performed a study where they used a double transgenic mouse where ovalbumin was expressed in the thymus under the control of the rat insulin promoter and the T-lymphocytes only recognized a specific ovalbumin peptide. This study revealed that the negative selection of ovalbumin specific T-lymphocytes was impaired in the *Aire*$^{-/-}$ mice, but the levels of ovalbumin in the thymus of these mice was not reduced (Anderson et al., 2005). Furthermore, others showed that the autoreactive T-lymphocytes in *Aire*$^{-/-}$ mice react to antigens that are not regulated by AIRE in the mTECs (Kuroda et al., 2005). Also, mice that lack *Aire* on a NOD background display severe exocrine pancreatitis, a manifestation that has never been found in APS I patients. These mice show no destruction of the β-cells in the pancreas although insulin is supposed to be differentially regulated by AIRE in the thymus (Jiang et al., 2005). Thus, the control of TSA expression by AIRE in mice does not seem to correlate with the peripheral autoantigens causing organ destruction in APS I patients and indicate that APS I may not only be caused by faulty negative selection of autoreactive lymphocytes by mTECs. These findings, together with data of AIRE expression in cells other than the mTECs has led to an emerging set of reports investigating the role of AIRE in peripheral organs.

One study revealed that *Aire*$^{-/-}$ peripheral dendritic cells (DCs) show differential expression of several genes, including the co-stimulatory molecule VCAM-1. The overexpression of VCAM-1 could be coupled to an increased ability of *Aire*$^{-/-}$ DCs to activate naïve T cells (Ramsey et al., 2006). Furthermore, *Aire*$^{-/-}$ mice also display a clear B cell phenotype, attributed by infiltrates of B cells in the liver and the development of marginal zone B cell lymphomas in aged mice (Hässler et al., 2006). The findings in Paper I reveal the mechanisms behind this phenotype of overactivated B cells that is unlikely to be caused by defective negative selection of autorective T cells in the thymus. In this paper it was demonstrated that increased activation of DCs in absence of AIRE affect the activation of B cells in a T-cell independent manner. Aire$^{-/-}$ mice injected with T cell-independent antigen TNP-Ficoll showed an increased response *in vivo* and an overall increased activation status, in particular of MZB cells. This increased activation was found to be caused by excessive release of BAFF cytokines mediating survival of B cells in both *Aire*$^{-/-}$ mice and APS I patients. Performing bone marrow transfer of *Aire*$^{-/-}$ bone marrow into T cell deficient nude mice revealed that the increased secretion of BAFF was independent on autoreactive T-lymphocytes. Instead, the increased levels were caused by a continuous signaling of IFN-γ through the STAT1 pathway. This was hypothesized when a publication demonstrated that AIRE and PIAS1 interacted functionally to inhibit STAT1 signaling

(Ilmarinen et al., 2008), suggesting that in the absence of AIRE, STAT1 signaling is not inhibited and excess levels of IFN-γ targeted genes, as BAFF, is being transcribed. These findings together with the fact that AIRE is expressed in dendritic cells in the periphery lead to the investigations of Paper II.

The localization of *AIRE*/AIRE expression has been widely debated over the years and the fact that no clear function of the role of AIRE in cells other than the mTECs has fueled the skeptics that AIRE has no role in peripheral tolerance. However, *Aire*/*AIRE* has been found to be expressed in a range of cells and tissues and the expression of AIRE in DCs would coincide with the cells function as a professional presenter of antigens and mediator of tolerance mechanisms. In Paper II, we investigated the subtypes of DCs in the spleen because of the previous findings of overactivated lymphocytes in this organ. Interestingly, we found AIRE expression in a subtype of DC that is situated in the marginal zone and red pulp areas of the spleen. This study demonstrated that this subset possess functions that can aid the processes of B and T-lymphocyte activation and germinal center selection. For example, the $33D1^+$ DCs express the co-stimulatory molecule ICOSL that was found to be regulated by AIRE and thus regulate the activation of T-follicular helper cells in germinal center reactions. It is tempting to speculate that the *Aire* regulation of $33D1^+$ DCs including higher expression of ICOSL in $Aire^{-/-}$ mice could add to the pathology also in APSI patients. Also, these DCs were found to express the hallmark TSA insulin in an AIRE dependent manner. Paper III further demonstrated that AIRE is connected to the STAT1 pathway not only by the inhibition of IFN-γ signaling but also that *Aire*/*AIRE* itself is regulated by IFN-γ. The down regulation of *Aire* in the $33D1^+$ DCs during IFN-γ stimulation also lead to the down-regulation of insulin expression. This effect of IFN-γ has also been demonstrated in mTECs in the thymus and strengthens the role of the $33D1^+$ DC subset as a regulator of tolerance (Levi and Polychronakos, 2009). Furthermore, this finding also suggests that the $33D1^+$ DCs are able shift from a tolerogenic function in steady state to an immunogenic function during an immune response.

In Paper IV the origin of the $33D1^+$ DCs with regard to *Aire* induction was investigated. This study revealed that *Aire* may influence the proper maturation of $33D1^+$ DCs in the bone marrow. Further studies on the maturation of $33D1^+$ DCs in the bone marrow is needed in order to elucidate whether AIRE is really affecting this part of development.

In summary, the findings of the work included in this thesis suggest that AIRE has a role in dendritic cell biology in the periphery, in particular in the subset of DCs in the marginal zone of the spleen. Although sharing some features with the mTECs, the marginal zone DCs seem to have some additional distinct functions that most probably have evolved from the fact that these cells are situated in the peripheral organs where the purpose of AIRE may be different. I hope that the work in this thesis help to move the focus from thymic AIRE in the medulla and further broaden the view that AIRE is influencing so much more.

3 POPULÄRVETENSKAPLIG SAMMANFATTNING

Kroppens immunförsvar har utvecklats för att skydda oss mot mikroorganismer som farliga bakterier, virus och svamp. Vår hud och våra slemhinnor skyddar oss från en del av dessa angrepp men specialiserade immunceller i kroppens organ, så som mjälte och lymfkörtlar, behövs för att kroppen skall kunna stå emot specifikt argsinta och återkommande angrepp. Dessa celler är dendritceller som är specialiserade på att suga upp mikrober och skylta med dem för T och B-celler. T-celler utvecklas i tymus och är specialiserade på att eliminera farliga mikroorganismer och B-celler utvecklas i benmärgen och har som uppgift att producera antikroppar. Antikroppar fungerar som varningssignaler som mycket snabbt upplyser övriga celler om att en viss mikroorganism har tagit sig in i kroppen. Under sin utveckling i respektive organ genomgår T och B-cellerna flera selektioner som ser till att de reagerar på mikroorganismer som har tagit sig in i kroppen utifrån och att de inte reagerar på de celler och mikroorganismer som redan finns i kroppen naturligt. Celler som reagerar på kroppens egna mikroorganismer kommer att bli eliminerade för att förhindra att dessa tar sig runt i kroppen och förstör organ. Förmågan att kunna skilja på eget och främmande kallas tolerans. När selektionen av T och B-celler inte fungerar som den ska kan själv-reaktiva, eller autoreaktiva celler, ta sig runt i kroppen och leda till autoimmun sjukdom.

Autoimmuna sjukdomar så som diabetes och multipel skleros blir allt vanligare. I nuläget finns inget botemedel för autoimmunitet men det går att behandla en del av de symptom som uppstår i samband med sjukdomen. I den här avhandlingen har jag studerat en gen som är viktig för att tolerans ska uppstå, AIRE (AutoImmune Regulator). Mutationer i denna gen leder till en svår autoimmun sjukdom som kallas APS I (Autoimmunt polyendokrint syndrom typ I). APS I är en monogen recessivt ärftlig sjukdom, vilket innebär att en individ vars föräldrar har en mutation i AIRE kommer att utveckla APS I med mycket stor sannolikhet. Patienter med APS I lider av flera symptom som kan bli livshotande om de inte kontrolleras och behandlas. De vanligaste förekommande symptomen kommer från skador på organ som producerar hormon, såsom bisköldkörteln, binjurarna och bukspottkörteln. Det är även mycket vanligt att patienterna drabbas av svåra kroniska svampinfektioner i munnen.

Det är inte helt klargjort vilken roll AIRE har i utvecklingen av tolerans. För att kunna studera detta använder vi oss av möss som har en defekt AIRE gen liknande den mutation som patienter med APS I har. Med hjälp av dessa möss har forskare kommit fram till att AIRE är viktig för att autoreaktiva T-celler i tymus ska kunna elimineras. I avsaknad av AIRE cirkulerar autoreaktiva celler runt i immunsystemet och förstör kroppens celler och vävnad.

I den första artikeln i den här avhandlingen visar vi att avsaknad av AIRE även leder till att B-celler blir överreaktiva och producerar ovanligt stora mängder av antikroppar. Detta tror vi beror på att dendrit-cellerna i de AIRE defekta mössen och i APS I patienterna producerar mycket höga nivåer av en faktor som aktiverar B-celler och kallas för BAFF.

I den andra artikeln visar vi att AIRE finns i en specifik typ av dendritcell i mjälte och lymfkörtlar. Den här dendrit-cellen producerar, förutom BAFF, även andra faktorer som är viktiga för aktivering av T-celler och för att B-celler ska röra sig i mjälten. När AIRE inte uttrycks där bidrar dendritcellen till att aktiverade T och B-celler blir

överaktiverade i mjälten och får svårt att röra sig som de ska för att kunna vara med och hjälpa till under immunsvaret.

I det tredje arbetet har vi studerat hur uttrycket av AIRE kan regleras i den specifika dendritcellen i mjälten. När dendritcellen stimuleras med en molekyl som kallas interferon-γ går uttrycket av AIRE ner. När AIRE går ner går även uttrycket av gener som är viktiga för tolerans ner. Interferon-γ utsöndras bland annat av T-celler för att varna om att till exempel ett virus har kommit in i kroppen. Våra data tyder på att denna dendritcell möjligen har olika uppgifter i kroppen som bidrar till tolerans i en frisk kropp, men kan även prioritera att verka i försvaret mot invaderande mikroorganismer under en infektion.

I det fjärde arbetet undersöker vi hur dendritcellerna utvecklas i benmärgen utan AIRE och även om AIRE normalt uttrycks i tidiga dendritceller i benmärgen. Vi fann att AIRE uttrycks redan i ett förstadie till dendritcellen i mjälten och att när AIRE inte finns i benmärgen så saknas några av de gener som behövs för att dendritcellen ska fungera korrekt.

Sammanfattningsvis så bidrar våra upptäcker till att öka förståelsen om hur AIRE är viktig för att skapa tolerans på flera sätt. Genom att studera hur AIRE fungerar hoppas vi kunna hjälpa till att förstå hur autoimmunitet uppstår och förhoppningsvis finna nya angreppspunkter för behandling.

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5 REFERENCES

Abbas, A.K., J. Lohr, B. Knoechel, and V. Nagabhushanam. 2004. T cell tolerance and autoimmunity. *Autoimmun Rev* 3:471-475.

Abramson, J., M. Giraud, C. Benoist, and D. Mathis. 2010. Aire's partners in the molecular control of immunological tolerance. *Cell* 140:123-135.

Adamson, K.A., S.H. Pearce, J.R. Lamb, J.R. Seckl, and S.E. Howie. 2004. A comparative study of mRNA and protein expression of the autoimmune regulator gene (Aire) in embryonic and adult murine tissues. *J Pathol* 202:180-187.

Ahonen, P., S. Myllärniemi, I. Sipilä, and J. Perheentupa. 1990. Clinical variation of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) in a series of 68 patients. *N Engl J Med* 322:1829-1836.

Akirav, E.M., N.H. Ruddle, and K.C. Herold. 2011. The role of AIRE in human autoimmune disease. *Nat Rev Endocrinol* 7:25-33.

Akiyama, T., Y. Shimo, H. Yanai, J. Qin, D. Ohshima, Y. Maruyama, Y. Asaumi, J. Kitazawa, H. Takayanagi, J.M. Penninger, M. Matsumoto, T. Nitta, Y. Takahama, and J. Inoue. 2008. The tumor necrosis factor family receptors RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. *Immunity* 29:423-437.

Allen, C.D., T. Okada, H.L. Tang, and J.G. Cyster. 2007. Imaging of germinal center selection events during affinity maturation. *Science* 315:528-531.

Allman, D., and S. Pillai. 2008. Peripheral B cell subsets. *Curr Opin Immunol* 20:149-157.

Anderson, G., W.E. Jenkinson, T. Jones, S.M. Parnell, F.A. Kinsella, A.J. White, J.E. Pongrac'z, S.W. Rossi, and E.J. Jenkinson. 2006. Establishment and functioning of intrathymic microenvironments. *Immunol Rev* 209:10-27.

Anderson, M.S., E.S. Venanzi, Z. Chen, S.P. Berzins, C. Benoist, and D. Mathis. 2005. The cellular mechanism of Aire control of T cell tolerance. *Immunity* 23:227-239.

Anderson, M.S., E.S. Venanzi, L. Klein, Z. Chen, S.P. Berzins, S.J. Turley, H. von Boehmer, R. Bronson, A. Dierich, C. Benoist, and D. Mathis. 2002. Projection of an immunological self shadow within the thymus by the aire protein. *Science* 298:1395-1401.

Annunziato, F., P. Romagnani, L. Cosmi, E. Lazzeri, and S. Romagnani. 2001. Chemokines and lymphopoiesis in human thymus. *Trends Immunol* 22:277-281.

Aschenbrenner, K., L.M. D'Cruz, E.H. Vollmann, M. Hinterberger, J. Emmerich, L.K. Swee, A. Rolink, and L. Klein. 2007. Selection of Foxp3+ regulatory T cells specific for self antigen expressed and presented by Aire+ medullary thymic epithelial cells. *Nat Immunol* 8:351-358.

Bachem, A., S. Güttler, E. Hartung, F. Ebstein, M. Schaefer, A. Tannert, A. Salama, K. Movassaghi, C. Opitz, H.W. Mages, V. Henn, P.M. Kloetzel, S. Gurka, and R.A. Kroczek. 2010. Superior antigen cross-presentation and XCR1 expression define human CD11c+CD141+ cells as homologues of mouse CD8+ dendritic cells. *J Exp Med* 207:1273-1281.

Banchereau, J., and R.M. Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392:245-252.

Batten, M., J. Groom, T.G. Cachero, F. Qian, P. Schneider, J. Tschopp, J.L. Browning, and F. Mackay. 2000. BAFF mediates survival of peripheral immature B lymphocytes. *J Exp Med* 192:1453-1466.

Bennett, C.L., J. Christie, F. Ramsdell, M.E. Brunkow, P.J. Ferguson, L. Whitesell, T.E. Kelly, F.T. Saulsbury, P.F. Chance, and H.D. Ochs. 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat Genet* 27:20-21.

Betterle, C., N.A. Greggio, and M. Volpato. 1998. Clinical review 93: Autoimmune polyglandular syndrome type 1. *J Clin Endocrinol Metab* 83:1049-1055.
Betterle, C., and R. Zanchetta. 2003. Update on autoimmune polyendocrine syndromes (APS). *Acta Biomed* 74:9-33.
Björses, P., J. Aaltonen, N. Horelli-Kuitunen, M.L. Yaspo, and L. Peltonen. 1998. Gene defect behind APECED: a new clue to autoimmunity. *Hum Mol Genet* 7:1547-1553.
Björses, P., M. Halonen, J.J. Palvimo, M. Kolmer, J. Aaltonen, P. Ellonen, J. Perheentupa, I. Ulmanen, and L. Peltonen. 2000. Mutations in the AIRE gene: effects on subcellular location and transactivation function of the autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protein. *Am J Hum Genet* 66:378-392.
Björses, P., M. Pelto-Huikko, J. Kaukonen, J. Aaltonen, L. Peltonen, and I. Ulmanen. 1999. Localization of the APECED protein in distinct nuclear structures. *Hum Mol Genet* 8:259-266.
Blanco, P., A.K. Palucka, V. Pascual, and J. Banchereau. 2008. Dendritic cells and cytokines in human inflammatory and autoimmune diseases. *Cytokine Growth Factor Rev* 19:41-52.
Blechschmidt, K., M. Schweiger, K. Wertz, R. Poulson, H.M. Christensen, A. Rosenthal, H. Lehrach, and M.L. Yaspo. 1999. The mouse Aire gene: comparative genomic sequencing, gene organization, and expression. *Genome Res* 9:158-166.
Blizzard, R.M., and M. Kyle. 1963. Studies of the adrenal antigens and antibodies in addisons's disease. *J Clin Invest* 42:1653-1660.
Boehm, T., S. Scheu, K. Pfeffer, and C.C. Bleul. 2003. Thymic medullary epithelial cell differentiation, thymocyte emigration, and the control of autoimmunity require lympho-epithelial cross talk via LTbetaR. *The Journal of experimental medicine* 198:757-769.
Bouneaud, C., P. Kourilsky, and P. Bousso. 2000. Impact of negative selection on the T cell repertoire reactive to a self-peptide: a large fraction of T cell clones escapes clonal deletion. *Immunity* 13:829-840.
Buzi, F., R. Badolato, C. Mazza, S. Giliani, L.D. Notarangelo, G. Radetti, and A. Plebani. 2003. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome: time to review diagnostic criteria? *J Clin Endocrinol Metab* 88:3146-3148.
Capalbo, D., G. Giardino, L.D. Martino, L. Palamaro, R. Romano, V. Gallo, E. Cirillo, M. Salerno, and C. Pignata. 2012. Genetic basis of altered central tolerance and autoimmune diseases: a lesson from AIRE mutations. *Int Rev Immunol* 31:344-362.
Casellas, R., T.A. Shih, M. Kleinewietfeld, J. Rakonjac, D. Nemazee, K. Rajewsky, and M.C. Nussenzweig. 2001. Contribution of receptor editing to the antibody repertoire. *Science* 291:1541-1544.
Chappell, C.P., K.E. Draves, N.V. Giltiay, and E.A. Clark. 2012. Extrafollicular B cell activation by marginal zone dendritic cells drives T cell-dependent antibody responses. *J Exp Med* 209:1825-1840.
Chen, Z., C. Benoist, and D. Mathis. 2005. How defects in central tolerance impinge on a deficiency in regulatory T cells. *Proc Natl Acad Sci U S A* 102:14735-14740.
Chin, R.K., J.C. Lo, O. Kim, S.E. Blink, P.A. Christiansen, P. Peterson, Y. Wang, C. Ware, and Y.X. Fu. 2003. Lymphotoxin pathway directs thymic Aire expression. *Nature immunology* 4:1121-1127.
Consortium, F.-G.A. 1997. An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains. *Nat Genet* 17:399-403.
Crotty, S. 2011. Follicular helper CD4 T cells (TFH). *Annu Rev Immunol* 29:621-663.
Crozat, K., R. Guiton, V. Contreras, V. Feuillet, C.A. Dutertre, E. Ventre, T.P. Vu Manh, T. Baranek, A.K. Storset, J. Marvel, P. Boudinot, A. Hosmalin, I. Schwartz-Cornil, and M. Dalod. 2010. The XC chemokine receptor 1 is a conserved selective marker of mammalian cells homologous to mouse CD8alpha+ dendritic cells. *J Exp Med* 207:1283-1292.

Culina, S., V. Brezar, and R. Mallone. 2013. Insulin and type 1 diabetes: immune connections. *Eur J Endocrinol* 168:R19-31.
Cyster, J.G. 2003. Homing of antibody secreting cells. *Immunol Rev* 194:48-60.
Davidson, A., and B. Diamond. 2001. Autoimmune diseases. *N Engl J Med* 345:340-350.
den Haan, J.M., S.M. Lehar, and M.J. Bevan. 2000. CD8(+) but not CD8(-) dendritic cells cross-prime cytotoxic T cells in vivo. *J Exp Med* 192:1685-1696.
Derbinski, J., J. Gäbler, B. Brors, S. Tierling, S. Jonnakuty, M. Hergenhahn, L. Peltonen, J. Walter, and B. Kyewski. 2005. Promiscuous gene expression in thymic epithelial cells is regulated at multiple levels. *J Exp Med* 202:33-45.
Derbinski, J., S. Pinto, S. Rösch, K. Hexel, and B. Kyewski. 2008. Promiscuous gene expression patterns in single medullary thymic epithelial cells argue for a stochastic mechanism. *Proc Natl Acad Sci U S A* 105:657-662.
Derbinski, J., A. Schulte, B. Kyewski, and L. Klein. 2001. Promiscuous gene expression in medullary thymic epithelial cells mirrors the peripheral self. *Nat Immunol* 2:1032-1039.
DeVoss, J., Y. Hou, K. Johannes, W. Lu, G.I. Liou, J. Rinn, H. Chang, R.R. Caspi, R. Caspi, L. Fong, and M.S. Anderson. 2006. Spontaneous autoimmunity prevented by thymic expression of a single self-antigen. *J Exp Med* 203:2727-2735.
Ding, D., H. Mehta, W.J. McCune, and M.J. Kaplan. 2006. Aberrant phenotype and function of myeloid dendritic cells in systemic lupus erythematosus. *J Immunol* 177:5878-5889.
Dudziak, D., A.O. Kamphorst, G.F. Heidkamp, V.R. Buchholz, C. Trumpfheller, S. Yamazaki, C. Cheong, K. Liu, H.W. Lee, C.G. Park, R.M. Steinman, and M.C. Nussenzweig. 2007. Differential antigen processing by dendritic cell subsets in vivo. *Science* 315:107-111.
Eisenbarth, G.S. 2004. Autoimmune polyendocrine syndromes. *Adv Exp Med Biol* 552:204-218.
Eisenbarth, G.S., and P.A. Gottlieb. 2004. Autoimmune polyendocrine syndromes. *N Engl J Med* 350:2068-2079.
Ekwall, O., H. Hedstrand, L. Grimelius, J. Haavik, J. Perheentupa, J. Gustafsson, E. Husebye, O. Kämpe, and F. Rorsman. 1998. Identification of tryptophan hydroxylase as an intestinal autoantigen. *Lancet* 352:279-283.
Ernst, B., D.S. Lee, J.M. Chang, J. Sprent, and C.D. Surh. 1999. The peptide ligands mediating positive selection in the thymus control T cell survival and homeostatic proliferation in the periphery. *Immunity* 11:173-181.
Ferguson, B.J., C. Alexander, S.W. Rossi, I. Liiv, A. Rebane, C.L. Worth, J. Wong, M. Laan, P. Peterson, E.J. Jenkinson, G. Anderson, H.S. Scott, A. Cooke, and T. Rich. 2008. AIRE's CARD revealed, a new structure for central tolerance provokes transcriptional plasticity. *J Biol Chem* 283:1723-1731.
Fierabracci, A. 2011. Recent insights into the role and molecular mechanisms of the autoimmune regulator (AIRE) gene in autoimmunity. *Autoimmun Rev* 10:137-143.
Finkelman, F.D., A. Lees, R. Birnbaum, W.C. Gause, and S.C. Morris. 1996. Dendritic cells can present antigen in vivo in a tolerogenic or immunogenic fashion. *J Immunol* 157:1406-1414.
Fletcher, A.L., V. Lukacs-Kornek, E.D. Reynoso, S.E. Pinner, A. Bellemare-Pelletier, M.S. Curry, A.R. Collier, R.L. Boyd, and S.J. Turley. 2010. Lymph node fibroblastic reticular cells directly present peripheral tissue antigen under steady-state and inflammatory conditions. *J Exp Med* 207:689-697.
Foy, T.M., D.M. Page, T.J. Waldschmidt, A. Schoneveld, J.D. Laman, S.R. Masters, L. Tygrett, J.A. Ledbetter, A. Aruffo, E. Claassen, J.C. Xu, R.A. Flavell, S. Oehen, S.M. Hedrick, and R.J. Noelle. 1995. An essential role for gp39, the ligand for CD40, in thymic selection. *J Exp Med* 182:1377-1388.
Freeman, G.J., A.J. Long, Y. Iwai, K. Bourque, T. Chernova, H. Nishimura, L.J. Fitz, N. Malenkovich, T. Okazaki, M.C. Byrne, H.F. Horton, L. Fouser, L. Carter, V. Ling, M.R. Bowman, B.M. Carreno, M. Collins, C.R. Wood, and T. Honjo. 2000. Engagement of the PD-1 immunoinhibitory receptor by a novel B7

family member leads to negative regulation of lymphocyte activation. *J Exp Med* 192:1027-1034.
Gallo, V., G. Giardino, D. Capalbo, L. Palamaro, R. Romano, F. Santamaria, F. Maio, M. Salerno, P. Vajro, and C. Pignata. 2013. Alterations of the autoimmune regulator transcription factor and failure of central tolerance: APECED as a model. *Expert Rev Clin Immunol* 9:43-51.
García De Vinuesa, C., A. Gulbranson-Judge, M. Khan, P. O'Leary, M. Cascalho, M. Wabl, G.G. Klaus, M.J. Owen, and I.C. MacLennan. 1999. Dendritic cells associated with plasmablast survival. *Eur J Immunol* 29:3712-3721.
Gardner, J.M., J.J. Devoss, R.S. Friedman, D.J. Wong, Y.X. Tan, X. Zhou, K.P. Johannes, M.A. Su, H.Y. Chang, M.F. Krummel, and M.S. Anderson. 2008. Deletional tolerance mediated by extrathymic Aire-expressing cells. *Science* 321:843-847.
Gavanescu, I., C. Benoist, and D. Mathis. 2008. B cells are required for Aire-deficient mice to develop multi-organ autoinflammation: A therapeutic approach for APECED patients. *Proc Natl Acad Sci U S A* 105:13009-13014.
Gavanescu, I., B. Kessler, H. Ploegh, C. Benoist, and D. Mathis. 2007. Loss of Aire-dependent thymic expression of a peripheral tissue antigen renders it a target of autoimmunity. *Proc Natl Acad Sci U S A* 104:4583-4587.
Gay, D., T. Saunders, S. Camper, and M. Weigert. 1993. Receptor editing: an approach by autoreactive B cells to escape tolerance. *J Exp Med* 177:999-1008.
Gebre-Medhin, G., E.S. Husebye, J. Gustafsson, O. Winqvist, A. Goksøyr, F. Rorsman, and O. Kämpe. 1997. Cytochrome P450IA2 and aromatic L-amino acid decarboxylase are hepatic autoantigens in autoimmune polyendocrine syndrome type I. *FEBS Lett* 412:439-445.
Germain, R.N. 2001. The art of the probable: system control in the adaptive immune system. *Science* 293:240-245.
Ghia, P., E. ten Boekel, A.G. Rolink, and F. Melchers. 1998. B-cell development: a comparison between mouse and man. *Immunol Today* 19:480-485.
Goodnow, C.C., J. Crosbie, S. Adelstein, T.B. Lavoie, S.J. Smith-Gill, R.A. Brink, H. Pritchard-Briscoe, J.S. Wotherspoon, R.H. Loblay, and K. Raphael. 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature* 334:676-682.
Gross, C.C., and H. Wiendl. 2013. Dendritic cell vaccination in autoimmune disease. *Curr Opin Rheumatol* 25:268-274.
Gross, J.A., J. Johnston, S. Mudri, R. Enselman, S.R. Dillon, K. Madden, W. Xu, J. Parrish-Novak, D. Foster, C. Lofton-Day, M. Moore, A. Littau, A. Grossman, H. Haugen, K. Foley, H. Blumberg, K. Harrison, W. Kindsvogel, and C.H. Clegg. 2000. TACI and BCMA are receptors for a TNF homologue implicated in B-cell autoimmune disease. *Nature* 404:995-999.
Groux, H., A. O'Garra, M. Bigler, M. Rouleau, S. Antonenko, J.E. de Vries, and M.G. Roncarolo. 1997. A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389:737-742.
Grupillo, M., G. Gualtierotti, J. He, G. Sisino, R. Bottino, W.A. Rudert, M. Trucco, and Y. Fan. 2012. Essential roles of insulin expression in Aire+ tolerogenic dendritic cells in maintaining peripheral self-tolerance of islet β-cells. *Cell Immunol* 273:115-123.
Guilherme, L., E. Cunha-Neto, V. Coelho, R. Snitcowsky, P.M. Pomerantzeff, R.V. Assis, F. Pedra, J. Neumann, A. Goldberg, and M.E. Patarroyo. 1995. Human heart-infiltrating T-cell clones from rheumatic heart disease patients recognize both streptococcal and cardiac proteins. *Circulation* 92:415-420.
Guinamard, R., M. Okigaki, J. Schlessinger, and J.V. Ravetch. 2000. Absence of marginal zone B cells in Pyk-2-deficient mice defines their role in the humoral response. *Nat Immunol* 1:31-36.
Gylling, M., E. Kääriäinen, R. Väisänen, L. Kerosuo, M.L. Solin, L. Halme, S. Saari, M. Halonen, O. Kämpe, J. Perheentupa, and A. Miettinen. 2003. The hypoparathyroidism of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy protective effect of male sex. *J Clin Endocrinol Metab* 88:4602-4608.

Halonen, M., M. Pelto-Huikko, P. Eskelin, L. Peltonen, I. Ulmanen, and M. Kolmer. 2001. Subcellular location and expression pattern of autoimmune regulator (Aire), the mouse orthologue for human gene defective in autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED). *J Histochem Cytochem* 49:197-208.

Harrington, L.E., R.D. Hatton, P.R. Mangan, H. Turner, T.L. Murphy, K.M. Murphy, and C.T. Weaver. 2005. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 6:1123-1132.

Hawiger, D., E. Tran, W. Du, C.J. Booth, L. Wen, C. Dong, and R.A. Flavell. 2008. ICOS mediates the development of insulin-dependent diabetes mellitus in nonobese diabetic mice. *J Immunol* 180:3140-3147.

Hayter, S.M., and M.C. Cook. 2012. Updated assessment of the prevalence, spectrum and case definition of autoimmune disease. *Autoimmun Rev* 11:754-765.

Hedstrand, H., O. Ekwall, J. Haavik, E. Landgren, C. Betterle, J. Perheentupa, J. Gustafsson, E. Husebye, F. Rorsman, and O. Kämpe. 2000. Identification of tyrosine hydroxylase as an autoantigen in autoimmune polyendocrine syndrome type I. *Biochem Biophys Res Commun* 267:456-461.

Heino, M., P. Peterson, J. Kudoh, K. Nagamine, A. Lagerstedt, V. Ovod, A. Ranki, I. Rantala, M. Nieminen, J. Tuukkanen, H.S. Scott, S.E. Antonarakis, N. Shimizu, and K. Krohn. 1999a. Autoimmune regulator is expressed in the cells regulating immune tolerance in thymus medulla. *Biochem Biophys Res Commun* 257:821-825.

Heino, M., P. Peterson, N. Sillanpää, S. Guérin, L. Wu, G. Anderson, H.S. Scott, S.E. Antonarakis, J. Kudoh, N. Shimizu, E.J. Jenkinson, P. Naquet, and K.J. Krohn. 2000. RNA and protein expression of the murine autoimmune regulator gene (Aire) in normal, RelB-deficient and in NOD mouse. *Eur J Immunol* 30:1884-1893.

Heino, M., H.S. Scott, Q. Chen, P. Peterson, U. Mäebpää, M.P. Papasavvas, L. Mittaz, C. Barras, C. Rossier, G.P. Chrousos, C.A. Stratakis, K. Nagamine, J. Kudoh, N. Shimizu, N. Maclaren, S.E. Antonarakis, and K. Krohn. 1999b. Mutation analyses of North American APS-1 patients. *Hum Mutat* 13:69-74.

Hikosaka, Y., T. Nitta, I. Ohigashi, K. Yano, N. Ishimaru, Y. Hayashi, M. Matsumoto, K. Matsuo, J.M. Penninger, H. Takayanagi, Y. Yokota, H. Yamada, Y. Yoshikai, J. Inoue, T. Akiyama, and Y. Takahama. 2008. The cytokine RANKL produced by positively selected thymocytes fosters medullary thymic epithelial cells that express autoimmune regulator. *Immunity* 29:438-450.

Holländer, G.A., B. Wang, A. Nichogiannopoulou, P.P. Platenburg, W. van Ewijk, S.J. Burakoff, J.C. Gutierrez-Ramos, and C. Terhorst. 1995. Developmental control point in induction of thymic cortex regulated by a subpopulation of prothymocytes. *Nature* 373:350-353.

Holopainen, P., M. Arvas, P. Sistonen, K. Mustalahti, P. Collin, M. Mäki, and J. Partanen. 1999. CD28/CTLA4 gene region on chromosome 2q33 confers genetic susceptibility to celiac disease. A linkage and family-based association study. *Tissue Antigens* 53:470-475.

Hu, Y.L., D.P. Metz, J. Chung, G. Siu, and M. Zhang. 2009. B7RP-1 blockade ameliorates autoimmunity through regulation of follicular helper T cells. *J Immunol* 182:1421-1428.

Hubert, F.X., S.A. Kinkel, P.E. Crewther, P.Z. Cannon, K.E. Webster, M. Link, R. Uibo, M.K. O'Bryan, A. Meager, S.P. Forehan, G.K. Smyth, L. Mittaz, S.E. Antonarakis, P. Peterson, W.R. Heath, and H.S. Scott. 2009. Aire-deficient C57BL/6 mice mimicking the common human 13-base pair deletion mutation present with only a mild autoimmune phenotype. *J Immunol* 182:3902-3918.

Hubert, F.X., S.A. Kinkel, K.E. Webster, P. Cannon, P.E. Crewther, A.I. Proeitto, L. Wu, W.R. Heath, and H.S. Scott. 2008. A specific anti-Aire antibody reveals aire expression is restricted to medullary thymic epithelial cells and not expressed in periphery. *J Immunol* 180:3824-3832.

Husebye, E.S., and M.S. Anderson. 2010. Autoimmune polyendocrine syndromes: clues to type 1 diabetes pathogenesis. *Immunity* 32:479-487.

Husebye, E.S., J. Perheentupa, R. Rautemaa, and O. Kämpe. 2009. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. *J Intern Med* 265:514-529.

Hässler, S., C. Ramsey, M.C. Karlsson, D. Larsson, B. Herrmann, B. Rozell, M. Backheden, L. Peltonen, O. Kämpe, and O. Winqvist. 2006. Aire-deficient mice develop hematopoetic irregularities and marginal zone B-cell lymphoma. *Blood* 108:1941-1948.

Ikegami, H. 2002. Animal models of autoimmune polyglandular syndrome. *Endocrinol Metab Clin North Am* 31:431-439, viii.

Ilmarinen, T., P. Eskelin, M. Halonen, T. Rüppell, R. Kilpikari, G.D. Torres, H. Kangas, and I. Ulmanen. 2005. Functional analysis of SAND mutations in AIRE supports dominant inheritance of the G228W mutation. *Hum Mutat* 26:322-331.

Ilmarinen, T., H. Kangas, T. Kytömaa, P. Eskelin, J. Saharinen, J.S. Seeler, K. Tanhuanpää, F.Y. Chan, R.M. Slattery, K. Alakurtti, J.J. Palvimo, and I. Ulmanen. 2008. Functional interaction of AIRE with PIAS1 in transcriptional regulation. *Mol Immunol* 45:1847-1862.

Jacobson, D.L., S.J. Gange, N.R. Rose, and N.M. Graham. 1997. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol* 84:223-243.

Jameson, S.C., K.A. Hogquist, and M.J. Bevan. 1995. Positive selection of thymocytes. *Annu Rev Immunol* 13:93-126.

Jiang, W., M.S. Anderson, R. Bronson, D. Mathis, and C. Benoist. 2005. Modifier loci condition autoimmunity provoked by Aire deficiency. *J Exp Med* 202:805-815.

Jongbloed, S.L., A.J. Kassianos, K.J. McDonald, G.J. Clark, X. Ju, C.E. Angel, C.J. Chen, P.R. Dunbar, R.B. Wadley, V. Jeet, A.J. Vulink, D.N. Hart, and K.J. Radford. 2010. Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *J Exp Med* 207:1247-1260.

Karni, A., M. Abraham, A. Monsonego, G. Cai, G.J. Freeman, D. Hafler, S.J. Khoury, and H.L. Weiner. 2006. Innate immunity in multiple sclerosis: myeloid dendritic cells in secondary progressive multiple sclerosis are activated and drive a proinflammatory immune response. *J Immunol* 177:4196-4202.

Kekäläinen, E., H. Tuovinen, J. Joensuu, M. Gylling, R. Franssila, N. Pöntynen, K. Talvensaari, J. Perheentupa, A. Miettinen, and T.P. Arstila. 2007. A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Immunol* 178:1208-1215.

Kindt, T., R. Goldsby, and B. Osborne. 2007. Kuby immunology. W.H. Freeman and Company, New York.

Kinnunen, T., N. Chamberlain, H. Morbach, J. Choi, S. Kim, J. Craft, L. Mayer, C. Cancrini, L. Passerini, R. Bacchetta, H.D. Ochs, T.R. Torgerson, and E. Meffre. 2013. Accumulation of peripheral autoreactive B cells in the absence of functional human regulatory T cells. *Blood* 121:1595-1603.

Kisand, K., A.S. Bøe Wolff, K.T. Podkrajsek, L. Tserel, M. Link, K.V. Kisand, E. Ersvaer, J. Perheentupa, M.M. Erichsen, N. Bratanic, A. Meloni, F. Cetani, R. Perniola, B. Ergun-Longmire, N. Maclaren, K.J. Krohn, M. Pura, B. Schalke, P. Ströbel, M.I. Leite, T. Battelino, E.S. Husebye, P. Peterson, N. Willcox, and A. Meager. 2010. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. *J Exp Med* 207:299-308.

Kisand, K., and P. Peterson. 2011. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy: known and novel aspects of the syndrome. *Ann N Y Acad Sci* 1246:77-91.

Kisielow, P., H.S. Teh, H. Blüthmann, and H. von Boehmer. 1988. Positive selection of antigen-specific T cells in thymus by restricting MHC molecules. *Nature* 335:730-733.

Klein, J., and A. Sato. 2000. The HLA system. First of two parts. *N Engl J Med* 343:702-709.

Koble, C., and B. Kyewski. 2009. The thymic medulla: a unique microenvironment for intercellular self-antigen transfer. *J Exp Med* 206:1505-1513.

Kogawa, K., S. Nagafuchi, H. Katsuta, J. Kudoh, S. Tamiya, Y. Sakai, N. Shimizu, and M. Harada. 2002. Expression of AIRE gene in peripheral monocyte/dendritic cell lineage. *Immunol Lett* 80:195-198.
Krohn, K., R. Uibo, E. Aavik, P. Peterson, and K. Savilahti. 1992. Identification by molecular cloning of an autoantigen associated with Addison's disease as steroid 17 alpha-hydroxylase. *Lancet* 339:770-773.
Kukreja, A., and N.K. Maclaren. 2000. Current cases in which epitope mimicry is considered as a component cause of autoimmune disease: immune-mediated (type 1) diabetes. *Cell Mol Life Sci* 57:534-541.
Kuroda, N., T. Mitani, N. Takeda, N. Ishimaru, R. Arakaki, Y. Hayashi, Y. Bando, K. Izumi, T. Takahashi, T. Nomura, S. Sakaguchi, T. Ueno, Y. Takahama, D. Uchida, S. Sun, F. Kajiura, Y. Mouri, H. Han, A. Matsushima, G. Yamada, and M. Matsumoto. 2005. Development of autoimmunity against transcriptionally unrepressed target antigen in the thymus of Aire-deficient mice. *J Immunol* 174:1862-1870.
Lee, J.W., M. Epardaud, J. Sun, J.E. Becker, A.C. Cheng, A.R. Yonekura, J.K. Heath, and S.J. Turley. 2007. Peripheral antigen display by lymph node stroma promotes T cell tolerance to intestinal self. *Nat Immunol* 8:181-190.
Lei, Y., A.M. Ripen, N. Ishimaru, I. Ohigashi, T. Nagasawa, L.T. Jeker, M.R. Bösl, G.A. Holländer, Y. Hayashi, R.e.W. Malefyt, T. Nitta, and Y. Takahama. 2011. Aire-dependent production of XCL1 mediates medullary accumulation of thymic dendritic cells and contributes to regulatory T cell development. *J Exp Med* 208:383-394.
Lernmark A. 2001. Autoimmune diseases: are markers ready for prediction? *J Clin Invest* 108:1091-1096.
Lernmark, A., and H.E. Larsson. 2013. Immune therapy in type 1 diabetes mellitus. *Nat Rev Endocrinol* 9:92-103.
Leslie, D., P. Lipsky, and A.L. Notkins. 2001. Autoantibodies as predictors of disease. *J Clin Invest* 108:1417-1422.
Levi, D., and C. Polychronakos. 2009. Regulation of insulin gene expression by cytokines and cell-cell interactions in mouse medullary thymic epithelial cells. *Diabetologia* 52:2151-2158.
Levi, D., and C. Polychronakos. 2013. Self-antigen expression in thymic epithelial cells in Ifn-γ or Tnf-α deficiency. *Cytokine*
Li, J., Y. Li, J.Y. Yao, R. Jin, M.Z. Zhu, X.P. Qian, J. Zhang, Y.X. Fu, L. Wu, Y. Zhang, and W.F. Chen. 2007. Developmental pathway of CD4+CD8- medullary thymocytes during mouse ontogeny and its defect in Aire-/- mice. *Proc Natl Acad Sci U S A* 104:18175-18180.
Liiv, I., A. Rebane, T. Org, M. Saare, J. Maslovskaja, K. Kisand, E. Juronen, L. Valmu, M.J. Bottomley, N. Kalkkinen, and P. Peterson. 2008. DNA-PK contributes to the phosphorylation of AIRE: importance in transcriptional activity. *Biochim Biophys Acta* 1783:74-83.
Lindh, E., J. Brännström, P. Jones, F. Wermeling, S. Hässler, C. Betterle, B.Z. Garty, M. Stridsberg, B. Herrmann, M.C. Karlsson, and O. Winqvist. 2012. Autoimmunity and cystatin SA1 deficiency behind chronic mucocutaneous candidiasis in autoimmune polyendocrine syndrome type 1. *J Autoimmun*
Lindh, E., E. Rosmaraki, L. Berg, H. Brauner, M.C. Karlsson, L. Peltonen, P. Höglund, and O. Winqvist. 2010. AIRE deficiency leads to impaired iNKT cell development. *J Autoimmun* 34:66-72.
Liston, A., D.H. Gray, S. Lesage, A.L. Fletcher, J. Wilson, K.E. Webster, H.S. Scott, R.L. Boyd, L. Peltonen, and C.C. Goodnow. 2004. Gene dosage--limiting role of Aire in thymic expression, clonal deletion, and organ-specific autoimmunity. *J Exp Med* 200:1015-1026.
Liston, A., S. Lesage, J. Wilson, L. Peltonen, and C.C. Goodnow. 2003. Aire regulates negative selection of organ-specific T cells. *Nat Immunol* 4:350-354.
Liu, B., S. Mink, K.A. Wong, N. Stein, C. Getman, P.W. Dempsey, H. Wu, and K. Shuai. 2004. PIAS1 selectively inhibits interferon-inducible genes and is important in innate immunity. *Nat Immunol* 5:891-898.
Liu, Y.J. 2005. IPC: professional type 1 interferon-producing cells and plasmacytoid dendritic cell precursors. *Annu Rev Immunol* 23:275-306.

Mackay, F., S.A. Woodcock, P. Lawton, C. Ambrose, M. Baetscher, P. Schneider, J. Tschopp, and J.L. Browning. 1999. Mice transgenic for BAFF develop lymphocytic disorders along with autoimmune manifestations. *J Exp Med* 190:1697-1710.

Mackay, I.R. 2005. The etiopathogenesis of autoimmunity. *Semin Liver Dis* 25:239-250.

MacLennan, I.C. 1994. Germinal centers. *Annu Rev Immunol* 12:117-139.

MacLennan, I.C., K.M. Toellner, A.F. Cunningham, K. Serre, D.M. Sze, E. Zúñiga, M.C. Cook, and C.G. Vinuesa. 2003. Extrafollicular antibody responses. *Immunol Rev* 194:8-18.

Malchow, S., D.S. Leventhal, S. Nishi, B.I. Fischer, L. Shen, G.P. Paner, A.S. Amit, C. Kang, J.E. Geddes, J.P. Allison, N.D. Socci, and P.A. Savage. 2013. Aire-dependent thymic development of tumor-associated regulatory T cells. *Science* 339:1219-1224.

Maldonado, R.A., and U.H. von Andrian. 2010. How tolerogenic dendritic cells induce regulatory T cells. *Adv Immunol* 108:111-165.

Manavalan, J.S., P.C. Rossi, G. Vlad, F. Piazza, A. Yarilina, R. Cortesini, D. Mancini, and N. Suciu-Foca. 2003. High expression of ILT3 and ILT4 is a general feature of tolerogenic dendritic cells. *Transpl Immunol* 11:245-258.

Mannon, P.J., I.J. Fuss, L. Mayer, C.O. Elson, W.J. Sandborn, D. Present, B. Dolin, N. Goodman, C. Groden, R.L. Hornung, M. Quezado, Z. Yang, M.F. Neurath, J. Salfeld, G.M. Veldman, U. Schwertschlag, W. Strober, and A.-I.-C.s.D.S. Group. 2004. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* 351:2069-2079.

Marrack, P., J. Kappler, and B.L. Kotzin. 2001. Autoimmune disease: why and where it occurs. *Nat Med* 7:899-905.

Martin, F., and J.F. Kearney. 2002. Marginal-zone B cells. *Nat Rev Immunol* 2:323-335.

Mathis, D., and C. Benoist. 2009. Aire. *Annu Rev Immunol* 27:287-312.

Matsui, T., J.E. Connolly, M. Michnevitz, D. Chaussabel, C.I. Yu, C. Glaser, S. Tindle, M. Pypaert, H. Freitas, B. Piqueras, J. Banchereau, and A.K. Palucka. 2009. CD2 distinguishes two subsets of human plasmacytoid dendritic cells with distinct phenotype and functions. *J Immunol* 182:6815-6823.

Matzinger, P. 1994. Tolerance, danger, and the extended family. *Annu Rev Immunol* 12:991-1045.

Matzinger, P. 2002. The danger model: a renewed sense of self. *Science* 296:301-305.

Meager, A., K. Visvalingam, P. Peterson, K. Moll, A. Murumagi, K. Krohn, P. Eskelin, J. Perheentupa, E. Husebye, Y. Kadota, and N. Willcox. 2006a. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS medicine* 3:e289.

Meager, A., K. Visvalingam, P. Peterson, K. Möll, A. Murumägi, K. Krohn, P. Eskelin, J. Perheentupa, E. Husebye, Y. Kadota, and N. Willcox. 2006b. Anti-interferon autoantibodies in autoimmune polyendocrinopathy syndrome type 1. *PLoS Med* 3:e289.

Meffre, E. 2011. The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. *Ann N Y Acad Sci* 1246:1-10.

Meffre, E., A. Schaefer, H. Wardemann, P. Wilson, E. Davis, and M.C. Nussenzweig. 2004. Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. *J Exp Med* 199:145-150.

Melchers, F. 2005. The pre-B-cell receptor: selector of fitting immunoglobulin heavy chains for the B-cell repertoire. *Nat Rev Immunol* 5:578-584.

Merad, M., and M.G. Manz. 2009. Dendritic cell homeostasis. *Blood* 113:3418-3427.

Murphy, K.M., and S.L. Reiner. 2002. The lineage decisions of helper T cells. *Nat Rev Immunol* 2:933-944.

Myhre, A.G., M. Halonen, P. Eskelin, O. Ekwall, H. Hedstrand, F. Rorsman, O. Kämpe, and E.S. Husebye. 2001. Autoimmune polyendocrine syndrome type 1 (APS I) in Norway. *Clin Endocrinol (Oxf)* 54:211-217.

Nagamine, K., P. Peterson, H.S. Scott, J. Kudoh, S. Minoshima, M. Heino, K.J. Krohn, M.D. Lalioti, P.E. Mullis, S.E. Antonarakis, K. Kawasaki, S. Asakawa, F. Ito,

and N. Shimizu. 1997. Positional cloning of the APECED gene. *Nat Genet* 17:393-398.
Neufeld, M., N.K. Maclaren, and R.M. Blizzard. 1981. Two types of autoimmune Addison's disease associated with different polyglandular autoimmune (PGA) syndromes. *Medicine (Baltimore)* 60:355-362.
Niki, S., K. Oshikawa, Y. Mouri, F. Hirota, A. Matsushima, M. Yano, H. Han, Y. Bando, K. Izumi, M. Matsumoto, K.I. Nakayama, and N. Kuroda. 2006. Alteration of intra-pancreatic target-organ specificity by abrogation of Aire in NOD mice. *J Clin Invest* 116:1292-1301.
Org, T., F. Chignola, C. Hetényi, M. Gaetani, A. Rebane, I. Liiv, U. Maran, L. Mollica, M.J. Bottomley, G. Musco, and P. Peterson. 2008. The autoimmune regulator PHD finger binds to non-methylated histone H3K4 to activate gene expression. *EMBO Rep* 9:370-376.
Oven, I., N. Brdicková, J. Kohoutek, T. Vaupotic, M. Narat, and B.M. Peterlin. 2007. AIRE recruits P-TEFb for transcriptional elongation of target genes in medullary thymic epithelial cells. *Mol Cell Biol* 27:8815-8823.
Palmer, E. 2003. Negative selection--clearing out the bad apples from the T-cell repertoire. *Nat Rev Immunol* 3:383-391.
Pearce, S.H., T. Cheetham, H. Imrie, B. Vaidya, N.D. Barnes, R.W. Bilous, D. Carr, K. Meeran, N.J. Shaw, C.S. Smith, A.D. Toft, G. Williams, and P. Kendall-Taylor. 1998. A common and recurrent 13-bp deletion in the autoimmune regulator gene in British kindreds with autoimmune polyendocrinopathy type 1. *Am J Hum Genet* 63:1675-1684.
Pennell, L.M., C.L. Galligan, and E.N. Fish. 2012. Sex affects immunity. *J Autoimmun* 38:J282-291.
Perez, V.L., L. Van Parijs, A. Biuckians, X.X. Zheng, T.B. Strom, and A.K. Abbas. 1997. Induction of peripheral T cell tolerance in vivo requires CTLA-4 engagement. *Immunity* 6:411-417.
Perheentupa, J. 2006. Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *J Clin Endocrinol Metab* 91:2843-2850.
Peterson, P., J. Pitkänen, N. Sillanpää, and K. Krohn. 2004. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy (APECED): a model disease to study molecular aspects of endocrine autoimmunity. *Clin Exp Immunol* 135:348-357.
Pieper, K., B. Grimbacher, and H. Eibel. 2013. B-cell biology and development. *J Allergy Clin Immunol*
Pitkänen, J., V. Doucas, T. Sternsdorf, T. Nakajima, S. Aratani, K. Jensen, H. Will, P. Vähämurto, J. Ollila, M. Vihinen, H.S. Scott, S.E. Antonarakis, J. Kudoh, N. Shimizu, K. Krohn, and P. Peterson. 2000. The autoimmune regulator protein has transcriptional transactivating properties and interacts with the common coactivator CREB-binding protein. *J Biol Chem* 275:16802-16809.
Plotz, P.H. 2003. The autoantibody repertoire: searching for order. *Nat Rev Immunol* 3:73-78.
Podkrajsek, K.T., N. Bratanic, C. Krzisnik, and T. Battelino. 2005. Autoimmune regulator-1 messenger ribonucleic acid analysis in a novel intronic mutation and two additional novel AIRE gene mutations in a cohort of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. *J Clin Endocrinol Metab* 90:4930-4935.
Poulin, L.F., M. Salio, E. Griessinger, F. Anjos-Afonso, L. Craciun, J.L. Chen, A.M. Keller, O. Joffre, S. Zelenay, E. Nye, A. Le Moine, F. Faure, V. Donckier, D. Sancho, V. Cerundolo, D. Bonnet, and C. Reis e Sousa. 2010. Characterization of human DNGR-1+ BDCA3+ leukocytes as putative equivalents of mouse CD8alpha+ dendritic cells. *J Exp Med* 207:1261-1271.
Puel, A., R. Döffinger, A. Natividad, M. Chrabieh, G. Barcenas-Morales, C. Picard, A. Cobat, M. Ouachée-Chardin, A. Toulon, J. Bustamante, S. Al-Muhsen, M. Al-Owain, P.D. Arkwright, C. Costigan, V. McConnell, A.J. Cant, M. Abinun, M. Polak, P.F. Bougnères, D. Kumararatne, L. Marodi, A. Nahum, C. Roifman, S. Blanche, A. Fischer, C. Bodemer, L. Abel, D. Lilic, and J.L. Casanova. 2010. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic

mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J Exp Med* 207:291-297.
Punt, J.A., B.A. Osborne, Y. Takahama, S.O. Sharrow, and A. Singer. 1994. Negative selection of CD4+CD8+ thymocytes by T cell receptor-induced apoptosis requires a costimulatory signal that can be provided by CD28. *J Exp Med* 179:709-713.
Pöntynen, N., A. Miettinen, T.P. Arstila, O. Kämpe, M. Alimohammadi, O. Vaarala, L. Peltonen, and I. Ulmanen. 2006. Aire deficient mice do not develop the same profile of tissue-specific autoantibodies as APECED patients. *J Autoimmun* 27:96-104.
Pöntynen, N., M. Strengell, N. Sillanpää, J. Saharinen, I. Ulmanen, I. Julkunen, and L. Peltonen. 2008. Critical immunological pathways are downregulated in APECED patient dendritic cells. *J Mol Med (Berl)* 86:1139-1152.
Ramsey, C., S. Hässler, P. Marits, O. Kämpe, C.D. Surh, L. Peltonen, and O. Winqvist. 2006. Increased antigen presenting cell-mediated T cell activation in mice and patients without the autoimmune regulator. *Eur J Immunol* 36:305-317.
Ramsey, C., O. Winqvist, L. Puhakka, M. Halonen, A. Moro, O. Kämpe, P. Eskelin, M. Pelto-Huikko, and L. Peltonen. 2002. Aire deficient mice develop multiple features of APECED phenotype and show altered immune response. *Hum Mol Genet* 11:397-409.
Rautemaa, R., J. Hietanen, S. Niissalo, S. Pirinen, and J. Perheentupa. 2007. Oral and oesophageal squamous cell carcinoma--a complication or component of autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED, APS-I). *Oral Oncol* 43:607-613.
Rieux-Laucat, F., F. Le Deist, C. Hivroz, I.A. Roberts, K.M. Debatin, A. Fischer, and J.P. de Villartay. 1995. Mutations in Fas associated with human lymphoproliferative syndrome and autoimmunity. *Science* 268:1347-1349.
Rosatelli, M.C., A. Meloni, M. Devoto, A. Cao, H.S. Scott, P. Peterson, M. Heino, K.J. Krohn, K. Nagamine, J. Kudoh, N. Shimizu, and S.E. Antonarakis. 1998. A common mutation in Sardinian autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy patients. *Hum Genet* 103:428-434.
Rose, N.R., and C. Bona. 1993. Defining criteria for autoimmune diseases (Witebsky's postulates revisited). *Immunol Today* 14:426-430.
Rossjohn, J., D.G. Pellicci, O. Patel, L. Gapin, and D.I. Godfrey. 2012. Recognition of CD1d-restricted antigens by natural killer T cells. *Nat Rev Immunol* 12:845-857.
Ruan, Q.G., C.Y. Wang, J.D. Shi, and J.X. She. 1999. Expression and alternative splicing of the mouse autoimmune regulator gene (Aire). *J Autoimmun* 13:307-313.
Sakaguchi, S., T. Yamaguchi, T. Nomura, and M. Ono. 2008. Regulatory T cells and immune tolerance. *Cell* 133:775-787.
Sallusto, F., D. Lenig, R. Förster, M. Lipp, and A. Lanzavecchia. 1999. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 401:708-712.
Salvetti, M., G. Ristori, R. Bomprezzi, P. Pozzilli, and R.D. Leslie. 2000. Twins: mirrors of the immune system. *Immunol Today* 21:342-347.
Santiago-Schwarz, F. 2004. Dendritic cells: friend or foe in autoimmunity? *Rheum Dis Clin North Am* 30:115-134.
Satpathy, A.T., K.M. Murphy, and W. KC. 2011. Transcription factor networks in dendritic cell development. *Semin Immunol* 23:388-397.
Savkur, R.S., and T.P. Burris. 2004. The coactivator LXXLL nuclear receptor recognition motif. *J Pept Res* 63:207-212.
Schneider, P., F. MacKay, V. Steiner, K. Hofmann, J.L. Bodmer, N. Holler, C. Ambrose, P. Lawton, S. Bixler, H. Acha-Orbea, D. Valmori, P. Romero, C. Werner-Favre, R.H. Zubler, J.L. Browning, and J. Tschopp. 1999. BAFF, a novel ligand of the tumor necrosis factor family, stimulates B cell growth. *J Exp Med* 189:1747-1756.
Schubert, L.A., E. Jeffery, Y. Zhang, F. Ramsdell, and S.F. Ziegler. 2001. Scurfin (FOXP3) acts as a repressor of transcription and regulates T cell activation. *J Biol Chem* 276:37672-37679.

Seach, N., T. Ueno, A.L. Fletcher, T. Lowen, M. Mattesich, C.R. Engwerda, H.S. Scott, C.F. Ware, A.P. Chidgey, D.H. Gray, and R.L. Boyd. 2008. The lymphotoxin pathway regulates Aire-independent expression of ectopic genes and chemokines in thymic stromal cells. *J Immunol* 180:5384-5392.
Shevach, E.M. 2009. Mechanisms of foxp3+ T regulatory cell-mediated suppression. *Immunity* 30:636-645.
Shlomchik, M.J., and F. Weisel. 2012. Germinal center selection and the development of memory B and plasma cells. *Immunol Rev* 247:52-63.
Shortman, K., and Y.J. Liu. 2002. Mouse and human dendritic cell subtypes. *Nat Rev Immunol* 2:151-161.
Skyler, J.S. 2013. The year in immune intervention for type 1 diabetes. *Diabetes Technol Ther* 15 Suppl 1:S88-95.
Steinman, L. 1995. Escape from "horror autotoxicus": pathogenesis and treatment of autoimmune disease. *Cell* 80:7-10.
Steinman, R.M., and J. Banchereau. 2007. Taking dendritic cells into medicine. *Nature* 449:419-426.
Steinman, R.M., and Z.A. Cohn. 1973. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J Exp Med* 137:1142-1162.
Steinman, R.M., D. Hawiger, and M.C. Nussenzweig. 2003. Tolerogenic dendritic cells. *Annu Rev Immunol* 21:685-711.
Stohl, W. 2012. Biologic differences between various inhibitors of the BLyS/BAFF pathway: should we expect differences between belimumab and other inhibitors in development? *Curr Rheumatol Rep* 14:303-309.
Stolarski, B., E. Pronicka, L. Korniszewski, A. Pollak, G. Kostrzewa, E. Rowińska, P. Włodarski, A. Skórka, M. Gremida, P. Krajewski, and R. Ploski. 2006. Molecular background of polyendocrinopathy-candidiasis-ectodermal dystrophy syndrome in a Polish population: novel AIRE mutations and an estimate of disease prevalence. *Clin Genet* 70:348-354.
Su, M.A., K. Giang, K. Zumer, H. Jiang, I. Oven, J.L. Rinn, J.J. Devoss, K.P. Johannes, W. Lu, J. Gardner, A. Chang, P. Bubulya, H.Y. Chang, B.M. Peterlin, and M.S. Anderson. 2008. Mechanisms of an autoimmunity syndrome in mice caused by a dominant mutation in Aire. *J Clin Invest* 118:1712-1726.
Surh, C.D., and J. Sprent. 2002. Regulation of naïve and memory T-cell homeostasis. *Microbes Infect* 4:51-56.
Suzuki, E., Y. Kobayashi, O. Kawano, K. Endo, H. Haneda, H. Yukiue, H. Sasaki, M. Yano, M. Maeda, and Y. Fujii. 2008. Expression of AIRE in thymocytes and peripheral lymphocytes. *Autoimmunity* 41:133-139.
Suzuki, S., K. Honma, T. Matsuyama, K. Suzuki, K. Toriyama, I. Akitoyo, K. Yamamoto, T. Suematsu, M. Nakamura, K. Yui, and A. Kumatori. 2004. Critical roles of interferon regulatory factor 4 in CD11bhighCD8alpha-dendritic cell development. *Proc Natl Acad Sci U S A* 101:8981-8986.
Söderbergh, A., A.G. Myhre, O. Ekwall, G. Gebre-Medhin, H. Hedstrand, E. Landgren, A. Miettinen, P. Eskelin, M. Halonen, T. Tuomi, J. Gustafsson, E.S. Husebye, J. Perheentupa, M. Gylling, M.P. Manns, F. Rorsman, O. Kämpe, and T. Nilsson. 2004. Prevalence and clinical associations of 10 defined autoantibodies in autoimmune polyendocrine syndrome type I. *J Clin Endocrinol Metab* 89:557-562.
Tangye, S.G., V.L. Bryant, A.K. Cuss, and K.L. Good. 2006. BAFF, APRIL and human B cell disorders. *Semin Immunol* 18:305-317.
Tauber, A.I. 2003. Metchnikoff and the phagocytosis theory. *Nat Rev Mol Cell Biol* 4:897-901.
ten Boekel, E., F. Melchers, and A.G. Rolink. 1998. Precursor B cells showing H chain allelic inclusion display allelic exclusion at the level of pre-B cell receptor surface expression. *Immunity* 8:199-207.
Tiniakou, E., K.H. Costenbader, and M.A. Kriegel. 2013. Sex-specific environmental influences on the development of autoimmune diseases. *Clin Immunol*
Torres-Aguilar, H., M. Blank, L.J. Jara, and Y. Shoenfeld. 2010. Tolerogenic dendritic cells in autoimmune diseases: crucial players in induction and prevention of autoimmunity. *Autoimmun Rev* 10:8-17.

Ueno, H., E. Klechevsky, N. Schmitt, L. Ni, A.L. Flamar, S. Zurawski, G. Zurawski, K. Palucka, J. Banchereau, and S. Oh. 2011. Targeting human dendritic cell subsets for improved vaccines. *Semin Immunol* 23:21-27.

Uibo, R., E. Aavik, P. Peterson, J. Perheentupa, S. Aranko, R. Pelkonen, and K.J. Krohn. 1994. Autoantibodies to cytochrome P450 enzymes P450scc, P450c17, and P450c21 in autoimmune polyglandular disease types I and II and in isolated Addison's disease. *J Clin Endocrinol Metab* 78:323-328.

Valladeau, J., and S. Saeland. 2005. Cutaneous dendritic cells. *Semin Immunol* 17:273-283.

Velloso, L.A., O. Winqvist, J. Gustafsson, O. Kämpe, and F.A. Karlsson. 1994. Autoantibodies against a novel 51 kDa islet antigen and glutamate decarboxylase isoforms in autoimmune polyendocrine syndrome type I. *Diabetologia* 37:61-69.

Venanzi, E.S., D.H. Gray, C. Benoist, and D. Mathis. 2007. Lymphotoxin pathway and Aire influences on thymic medullary epithelial cells are unconnected. *J Immunol* 179:5693-5700.

Vignali, D.A., L.W. Collison, and C.J. Workman. 2008. How regulatory T cells work. *Nat Rev Immunol* 8:523-532.

von Boehmer, H., and H.J. Fehling. 1997. Structure and function of the pre-T cell receptor. *Annu Rev Immunol* 15:433-452.

Wakkach, A., N. Fournier, V. Brun, J.P. Breittmayer, F. Cottrez, and H. Groux. 2003. Characterization of dendritic cells that induce tolerance and T regulatory 1 cell differentiation in vivo. *Immunity* 18:605-617.

Walker, L.S., and A.K. Abbas. 2002. The enemy within: keeping self-reactive T cells at bay in the periphery. *Nat Rev Immunol* 2:11-19.

Wang, C.Y., J.D. Shi, A. Davoodi-Semiromi, and J.X. She. 1999. Cloning of Aire, the mouse homologue of the autoimmune regulator (AIRE) gene responsible for autoimmune polyglandular syndrome type 1 (ASP1). *Genomics* 55:322-326.

Wardemann, H., and M.C. Nussenzweig. 2007. B-cell self-tolerance in humans. *Adv Immunol* 95:83-110.

Wardemann, H., S. Yurasov, A. Schaefer, J.W. Young, E. Meffre, and M.C. Nussenzweig. 2003. Predominant autoantibody production by early human B cell precursors. *Science* 301:1374-1377.

Watanabe, N., Y.H. Wang, H.K. Lee, T. Ito, W. Cao, and Y.J. Liu. 2005. Hassall's corpuscles instruct dendritic cells to induce CD4+CD25+ regulatory T cells in human thymus. *Nature* 436:1181-1185.

Winqvist, O., J. Gustafsson, F. Rorsman, F.A. Karlsson, and O. Kämpe. 1993. Two different cytochrome P450 enzymes are the adrenal antigens in autoimmune polyendocrine syndrome type I and Addison's disease. *J Clin Invest* 92:2377-2385.

Winqvist, O., F.A. Karlsson, and O. Kämpe. 1992. 21-Hydroxylase, a major autoantigen in idiopathic Addison's disease. *Lancet* 339:1559-1562.

Witebsky, E., N.R. Rose, K. Terplan, J.R. Paine, and R.W. Egan. 1957. Chronic thyroiditis and autoimmunization. *J Am Med Assoc* 164:1439-1447.

Wu, L., A. D'Amico, K.D. Winkel, M. Suter, D. Lo, and K. Shortman. 1998. RelB is essential for the development of myeloid-related CD8alpha- dendritic cells but not of lymphoid-related CD8alpha+ dendritic cells. *Immunity* 9:839-847.

Wu, Y., W. Wu, W.M. Wong, E. Ward, A.J. Thrasher, D. Goldblatt, M. Osman, P. Digard, D.H. Canaday, and K. Gustafsson. 2009. Human gamma delta T cells: a lymphoid lineage cell capable of professional phagocytosis. *J Immunol* 183:5622-5629.

Yamazaki, S., D. Dudziak, G.F. Heidkamp, C. Fiorese, A.J. Bonito, K. Inaba, M.C. Nussenzweig, and R.M. Steinman. 2008. CD8+ CD205+ splenic dendritic cells are specialized to induce Foxp3+ regulatory T cells. *J Immunol* 181:6923-6933.

Zhu, M., R.K. Chin, P.A. Christiansen, J.C. Lo, X. Liu, C. Ware, U. Siebenlist, and Y.X. Fu. 2006. NF-kappaB2 is required for the establishment of central tolerance through an Aire-dependent pathway. *The Journal of clinical investigation* 116:2964-2971.

Zinkernagel, R.M., G.N. Callahan, J. Klein, and G. Dennert. 1978. Cytotoxic T cells learn specificity for self H-2 during differentiation in the thymus. *Nature* 271:251-253.

Zlotogora, J., and M.S. Shapiro. 1992. Polyglandular autoimmune syndrome type I among Iranian Jews. *J Med Genet* 29:824-826.

Zuklys, S., G. Balciunaite, A. Agarwal, E. Fasler-Kan, E. Palmer, and G.A. Holländer. 2000. Normal thymic architecture and negative selection are associated with Aire expression, the gene defective in the autoimmune-polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). *J Immunol* 165:1976-1983.