Immunological Studies using Human and Canine Model Disorders

KERSTIN M. AHLGREN
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Abstract

The studies presented in this thesis focus on human and canine models for autoimmune disease, with the main aim to gain new knowledge about disease mechanisms and to further evaluate the dog as a model for autoimmune disease.

Autoimmune Polyendocrine Syndrome type 1 (APS-1) is a hereditary human multiorgan disease caused by mutations in the autoimmune regulator (AIRE) gene. Hallmarks of APS-1 are chronic mucocutaneous candidiasis caused by Candida albicans, together with the autoimmune endocrine disorders hypoparathyroidism and adrenocortical failure. Many human diseases have an equivalent disease in dogs. Because humans share environment, and in part life style with the dogs they provide an interesting model for further genetic studies.

Immune responses to Candida albicans in APS-1 patients displayed an increased secretion of the proinflammatory cytokine IL-17A and similar results were also found in AIRE deficient mice. Anticytokine autoantibodies to IL-17A, IL-17F and IL-22 were detected in APS-1 patients, and a radioligand binding assay for measuring these autoantibodies was developed and evaluated.

In the canine studies we investigated whether canine diabetes mellitus could serve as a model for human autoimmune diabetes mellitus. Furthermore, we investigated type I IFN responses in Nova Scotia duck tolling retriever dogs with a systemic autoimmune disease resembling human SLE.

Four assays were used in search for signs of humoral autoimmunity in diabetic dogs. However, no evidence for a type 1 diabetes-like phenotype in dogs was found. Sera from Nova Scotia duck tolling retrievers suffering from steroid-responsive meningitis arteritis elicited an increased expression of IFN-inducible genes in the canine MDCK cell line. This suggests that these dogs have an IFN signature, as seen in human SLE.

Keywords: Autoimmunity, T cell, T helper cell, B cell, Autoantibodies, Interferon, Interleukin, Dogs, APS-1, Candida albicans, fungus

Kerstin M. Ahlgren, Uppsala University, Department of Medical Sciences, Akademiska sjukhuset, SE-751 85 Uppsala, Sweden.

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In memory of Bertil Ahlgren
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Abbreviations

APS-1 | Autoimmune polyendocrine syndrome type 1
---|---
DM | Diabetes mellitus
SLE | Systemic lupus erythematosus
PRR | Pattern Recognition Receptor
TLR | Toll-like receptor
TCR | T cell receptor
Th | T helper cell
mTEC | Medullary thymic epithelial cell
AIRE | Autoimmune regulator
IPEX | Immune dysregulation, endocrinopathy, enteropathy, X-linked syndrome
Treg | Regulatory T cell
IL | Interleukin
IFN | Interferon
TGF | Transforming growth factor
IL-17RA | Interleukin-17 receptor A
*C. albicans* | *Candida albicans*
APC | Antigen presenting cell
BCR | B cell receptor
T1D | Type 1 diabetes mellitus
APECED | Autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy
TSA | Tissue-specific self-antigens
moDC | Monocyte derived dendritic cell
LADA | Latent autoimmune diabetes of the adult
GAD65 | Glutamic acid decarboxylase 65 kDa
IAA | Insulin autoantibody
ICA | Cytoplasmic islet cell antibodies
ZnT8A | ZnT8 autoantibody
GADA | GAD autoantibody
IA2A | IA-2 autoantibody
MIDD | Maternally inherited diabetes and deafness
MODY | Maturity-onset diabetes of young people
pDC | Plasmacytoid dendritic cell
IRF | Interferon regulatory factor
NSDTR | Nova Scotia duck tolling retriever
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tbody>
<tr>
<td>IMRD</td>
<td>Immune-mediated rheumatic disease</td>
</tr>
<tr>
<td>ANA</td>
<td>Antinuclear antibody</td>
</tr>
<tr>
<td>SRMA</td>
<td>Steroid responsive meningitis-arteritis</td>
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<tr>
<td>IIF</td>
<td>Indirect Immunofluorescence</td>
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<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cells</td>
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<tr>
<td>ELISpot</td>
<td>Enzyme-linked immunosorbent spot</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>RPE</td>
<td>R-Phycoerythrin</td>
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<td>ITT</td>
<td><em>In vitro</em> transcription and translation</td>
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<tr>
<td>MDCK</td>
<td>Madin-Darby canine kidney</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>RLBA</td>
<td>Radioligand binding assays</td>
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</table>
Introduction

Many different diseases are considered to be of autoimmune origin. Autoimmunity can lead to pathological changes such as tissue destruction. In this thesis studies focus on human and canine models for autoimmune disorders. Autoimmune Polyendocrine Syndrome type 1 (APS-1) is a human multiorgan autoimmune disorder that serves as a model for autoimmune disease. Hallmarks of APS-1 are chronic mucocutaneous candidiasis together with the autoimmune endocrine disorders hypoparathyroidism and adrenal failure. Dogs are generally considered to be a suitable model for several human diseases, but a careful characterization of the disease of interest is essential. Canine models for autoimmune disorders in this thesis are canine diabetes mellitus (DM) and a canine systemic autoimmune disease resembling human Systemic Lupus Erythematosus (SLE). Studies on autoimmune disease mechanisms allow for development of new therapies and for early identification of affected individuals.
Background

The immune system
The immune system has a remarkable ability to eliminate potentially harmful microbes but at the same time to tolerate self. It comprises of receptors that recognises an almost infinite number of antigens. In humans the immune system generates self-specific receptors, which can cause an autoimmune reaction if not controlled. Both central and peripheral tolerance mechanisms are important to control self-reactivity.

The immune system can be divided into innate immunity which reacts with a rapid but nonspecific response to a pathogen, and adaptive immunity which is specific and characterized by immunological memory. Innate immune mechanisms are needed to activate adaptive responses. Innate immunity includes phagocytes, antimicrobial peptides, the complement system and pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs)[1]. Adaptive immunity includes lymphocytes, i.e. T and B cells, and cytokines and chemokines are important for regulation of adaptive immunity. Factors of both innate and adaptive immunity contribute to autoimmunity[2].

Cells of the immune system
The different cell types in the immune system are derived from a common hematopoietic stem cell in the bone marrow[3,4]. These pluripotent stem cells can form both lymphoid progenitor cells and myeloid progenitor cells[5]. Myeloid progenitor cells can develop into dendritic cells, monocytes and macrophages, megakaryocytes, and granulocytes. This thesis will, however, mainly focus on features of cells of the lymphoid lineage which includes T cells, B cells and NK cells.

T cells
T cell receptor (TCR) recognition of specific peptides on MHC activates T cells if they receive concomitant co-stimulatory signals. CD4+ T helper (Th) cells recognize peptides loaded on MHC II molecules and CD8+ T cells recognize peptides loaded on MHC I molecules[6]. Upon activation T cells start
to differentiate into effector T cells (Figure 1). The primary T cell response is followed by generation of memory T cells[7]. These processes are tightly regulated to avoid pathological responses to self-antigens.

Individual lymphocytes carry several copies of a single TCR with a unique antigen-binding site. Somatic DNA recombination generates a diversity of receptor genes by combining multiple gene segments and by modifying the junctional sequences. Diversity of the TCR due to amino acid sequence variations at the antigen binding site enables responses to many different antigens.

The majority of T cells have TCRs made up of α:β heterodimers, but a minority carries γ:δ TCRs. These two TCR types are structurally similar, but it seems that γ:δ TCRs are not restricted to antigen recognition on MHC, i.e. they may bind free antigens[8].

Central and peripheral T cell tolerance

Central tolerance develops primarily during perinatal life in the thymus[9]. Thymocytes carrying TCRs that recognize peptides presented on MHC undergo positive selection in the thymus. Medullary thymic epithelial cells (mTECs) enable expression of tissue-specific self-antigens, i.e. promiscuous expression, during negative selection[10]. The transcription factor autoimmune regulator (AIRE) controls the differentiation of mTECs and the promiscuous expression of self-antigens[11,12]. Mutations in the AIRE gene cause the disease APS-1[13,14]. Thymocytes expressing TCR with high affinity to intrathymic autoantigens are deleted whereas low affinity thymocytes mature and enter the periphery.

Peripheral tolerance mechanisms prevent activation of potentially self-reactive lymphocytes[15]. Anergy, or hyporesponsiveness, is induced when a lymphocyte recognises an antigen without receiving concomitant co-stimulation[16]. Tregs play an important role in the maintenance of immune homeostasis and self-tolerance. Tregs possess the ability to down-regulate T cell responses to foreign antigens, and importantly, to self-antigens. The T cell response can be suppressed by Tregs in different ways, e.g. via CTLA-4:CD80 interactions[17] and by cytokine production. FOXP3 is the key transcription factor driving Treg differentiation and mutated FOXP3 gives rise to the X-linked recessive disease immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX). The autoimmune disorders APS-1 and IPEX both exemplify the importance of central and peripheral tolerance mechanisms[18].

CD4+ T cells

Based on the pattern of cytokine secretion CD4+ T cells can be divided into Th1 cells, Th2 cells, Th17 cells, Th22 cells and regulatory T (Treg) cells
Several recent studies however report functional plasticity within the CD4$^+$ effector T cell program\cite{19,20,21,22,23,24}. Lineage differentiation is influenced by the strength of antigenic stimulation and by activated transcription factors. The lineage commitment also largely depends on the cytokine environment e.g. members of the interleukin (IL)-12 family. IL-12 comprises a p40 unit together with a p35 unit and is pivotal for Th1 differentiation. IL-23 is a member of the same family and consist of a p19 subunit and the p40 unit that is common to IL-23 and IL-12. IL-23 promotes differentiation of Th cells into pathogenic Th17 cells\cite{20,25,26}.

<table>
<thead>
<tr>
<th>Table 1. CD4$^+$ effector T cell subsets</th>
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<tbody>
<tr>
<td>Cell subset</td>
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<tr>
<td>Th1</td>
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<tr>
<td>Th2</td>
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<tr>
<td>Treg</td>
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<tr>
<td>Th17</td>
</tr>
<tr>
<td>Th22</td>
</tr>
</tbody>
</table>

T-bet is the key transcription factor for Th1 cell lineage commitment\cite{27}. Th1 cells are implicated in the defense to intracellular bacteria by producing interferon (IFN)-γ that activates macrophages. Th1 cells also produce IL-2 and tumor necrosis factor.

The key transcription factor for Th2 cells is GATA-3 and Th2 cells require IL-4 for differentiation\cite{28}. Th2 cells mediate humoral responses and protection against extracellular parasites through the production of IL-4, IL-5, IL-10 and IL-13. In addition Th2 cells mediate allergic reactions.

Differentiation of the Th17 lineage requires the transcription factors RORγt and RORα\cite{29}. IL-6 and transforming growth factor (TGF)-β are required for differentiation of Th17 cells but IL-1β\cite{30} and IL-23\cite{31} can further induce IL-17 expression. Th17 cell development is negatively regulated by IL-4, IL-27, IL-2 and IFN-γ in mice\cite{32,33,34,35,36}. Th17 cells are implicated in protection against infections caused by extracellular bacteria and fungus by production of pro-inflammatory cytokines and recruitment of neutrophil granulocytes to the site of infection. IL-17A, IL-17F and IL-22 are expressed by Th17 cells and subsets of NK cells, γδ-T cells and NKT cells\cite{37}. Chronic mucocutaneous candidiasis has been associated to mutations of the genes encoding IL-17 and the IL-17 receptor (IL-17RA) and defects in the IL-17 signaling pathway\cite{38,39,40,41}. The exact role of IL-17 in antifungal immunity is, however, still controversial because both positive and negative effects have been described\cite{42}. A subset of Th17 cells that express GM-CSF drives autoimmune neuroinflammation in mice\cite{25,26}.

It is under debate whether Th22 cells are a unique lineage or a subset of Th17 cells. The proposed Th22 cells reside in the skin, produce IL-22 and are implicated in skin inflammations\cite{43,44,45}. Furthermore it is suggested
that Th22 cells restrict growth of the commensal *Candida albicans* (*C. albicans*)[46]. Indeed *C. albicans*-specific Th cells that produce IL-22 are found in healthy individuals[46].

Tregs express the transcription factor FOXP3 and contribute to maintaining immune homeostasis by secreting suppressive cytokines IL-10 and TGF-β. Tregs can withdraw IL-2 from the microenvironment, which leads to cytokine deprivation-induced apoptosis of effector T cells[47]. Another mechanism is trans-endocytosis of CD80/CD86 via CTLA-4[17]. The Treg and the Th17 cell lineages are able to interconvert depending on the concentrations of retinoic acid, IL-6, IL-23 and TGF-β[22,24].

![Figure 1. Outcomes of T cell activation.](image)

**Figure 1.** Outcomes of T cell activation. Antigen presenting cells (APCs) circulate the body and engulf antigens that are subsequently degraded in endocytic vesicles and loaded as peptides on MHC molecules. Innate immune mechanisms control the activation of antigen-specific immune responses[48,49]. APCs express low levels of MHC and co-stimulatory molecules. Hence, their capacity to activate naïve T cells is limited. As APCs encounter pathogens, their expression of MHC and co-stimulatory molecules increase, they start to produce cytokines and thereby their ability to prime T cells progresses[49]. Activation of transcription factors downstream of the TCR leads to IL-2 expression and clonal expansion. Lineage differentiation depend on antigenic stimuli, activated transcription factors and cytokines.

**Cytokines**

Cytokines play an important role in regulation of immune responses, but are also implicated in autoimmunity[50]. Although very potent, no single cytokine can alone cause tissue damage in autoimmunity or antifungal immunity, but instead cytokines are likely secreted in concert with each other and the cytokine milieu determine the outcome of the processes. Chemokines are a family of chemo-attracting cytokines.
Interleukins can be produced by mononuclear phagocytes and tissue cells but are primarily secreted from APC and T cells and promote T cell differentiation and effector functions.

Interferons

The interferon system affects both innate and adaptive immunity and it is the first line defense against virus infections [51]. Interferons are divided into three groups characterized by the type of receptor and signal transduction pathway (Table 2).

**Type I Interferons**

In systemic autoimmune diseases type I IFN plays a pivotal role. Type I IFNs include IFN-α, IFN-β, IFN-ε, IFN-ω and IFN-κ. In humans there are 13 IFN-α genes, one IFN-β gene and one IFN-ω gene, located on chromosome 9. The genes encoding type I IFNs lack introns. The main producers of IFN-α are the plasmacytoid dendritic cells[52,53]. Patients with SLE display increased serum levels of IFN-α which correlates with disease activity and number of organs targeted by disease. An increased expression of type I IFN regulated genes, i.e. an IFN signature, is present in SLE and some other autoimmune diseases[54].

**Type II Interferons**

IFN-γ is a type II IFN encoded by one gene located on human chromosome 12. IFN-γ is expressed by activated Th cells and NK cells[55]. IFN-γ is a Th1 cytokine and contributes to cell-mediated immune responses by activating macrophages and by increasing the antigen processing and antigen presenting ability of APCs[56].

**Type III Interferons**

IFN-λ is the only known Type III IFN at present.

<table>
<thead>
<tr>
<th>Type</th>
<th>Interferons</th>
<th>Receptors</th>
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</thead>
<tbody>
<tr>
<td>Type I</td>
<td>IFN-α, IFN-β, IFN-ε, IFN-ω, IFN-κ</td>
<td>IFNAR1: IFNAR2</td>
</tr>
<tr>
<td>Type II</td>
<td>IFN-γ</td>
<td>IFNGR1: IFNGR2</td>
</tr>
<tr>
<td>Type III</td>
<td>IFN-λ</td>
<td>IL-10R2: IFNLR1</td>
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</table>
B cells

In lymphoid tissues dendritic cells bind to and activate CD4+ T cells that in turn interact with antigen-specific B cells. Activated B cells subsequently form germinal centers where they continue to receive help from follicular T helper cells and turn into antibody-secreting plasma cells[57]. Each B cell expresses a receptor specific for a particular antigen. Antibodies are secreted B cell receptors (BCRs). Similar to T cells and TCRs, diversity in BCR specificity is enabled by somatic DNA recombination. In addition B cells undergo somatic hypermutation after encounter with and activation by an antigen. B cells that have become activated can undergo yet another somatic recombination, called isotype switching which confers antibodies of the same antigen specificity with different isotypes.

Immature B cells in the bone marrow are resistant to apoptosis, but their development can be blocked by interactions with self-antigen. Self-reactive mature B cells that cannot undergo receptor editing are susceptible to elimination by apoptosis[58]. However a pool of autoreactive B cells appears in the peripheral lymphoid organs.

Autoimmunity

Autoimmunity is characterized by an inappropriate activation of pathogenic T cells, B cells or both against self-antigens. Genetic factors, immune deficiencies, hormones and environment can contribute to autoimmunity[59]. Many autoimmune diseases are associated to certain HLA alleles, e.g. type I diabetes (T1D) to HLA-DR and -DQ alleles[60]. Immune defects associated with autoimmunity include IgA deficiency, deficiency of complement components and regulatory T cell defects[59]. Hormonal and environmental factors associated with autoimmunity include sex hormones, ultraviolet radiation[61], infections[62] and pharmaceuticals. Therapeutic IFN-α treatment can induce autoimmune thyroiditis[63].

Autoimmune diseases

There are more than 80 different autoimmune diseases. They are often classified as organ-specific or systemic. In organ-specific diseases the autoimmune reactions are localized to isolated organs such as thyroid, adrenals, stomach and pancreas whereas in systemic autoimmunity reactivity is widespread in the body. Autoimmune responses engage both T cells, B cells and APCs for recognition of self-antigens. However, one cell type may have a prominent role for causing disease. This is exemplified by the decisive roles of autoantibodies and B cells in SLE and autoreactive T cells in T1D[64].
Autoimmune disease models

1. Autoimmune Polyendocrine Syndrome type 1 as a model disorder

APS-1, or autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED) is a rare autoimmune disease caused by mutations in the AIRE gene which is located on chromosome 21[13,14]. APS-1 serves as a useful model for elucidating the mechanism of central tolerance[65]. More than 50 AIRE mutations have been described in association with APS-1[66]. Functional studies of the AIRE protein have implicated a function as a transcription factor (Figure 2).

![Figure 2. AIRE controls promiscuous expression of otherwise tissue-specific self-antigens (TSA) in thymus. CD4⁺ and CD8⁺ thymocytes that bind peptide-MHC complexes with high affinity will be deleted. Extrathymic AIRE-expressing cells can form longterm contacts with naive autoreactive T cells entering the lymph node. Such interaction leads to deletion of these cells[15].](image)

Diagnosis of APS-1 is based on clinical features (Table 3). The three cardinal manifestations are chronic mucocutaneous candidiasis, adrenal failure and hypoparathyroidism. In 1990, Ahonen et al. described the clinical presentation in 68 patients, with one to eight different disease components. Although the spectrum varied, most (63%) had three to five disease components[67]. A typical feature of APS-1 patients is autoantibodies to endocrine tissue antigens. In recent years new autoantigens have been discovered[68,69]. Interestingly, anti-cytokine antibodies were recently detected in APS-1 patients[41,70,71,72].

The animal model of APS-1, AIRE-deficient mice, does not display the same repertoire of autoantibodies as APS-1 patients[73]. Furthermore AIRE-
deficient mice lack spontaneous candidiasis and autoimmune tissue damage[73]. On the other hand AIRE-deficient mice exhibit an exacerbated fungal infection in an actively induced candidiasis model compared to wild-type mice.

Table 3. Clinical signs of Autoimmune Polyendocrine Syndrome type I

<table>
<thead>
<tr>
<th>Disease manifestations</th>
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<tr>
<td>Mucocutaneous candidiasis</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
</tr>
<tr>
<td>Adrenal insufficiency</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Pernicious anemia</td>
</tr>
<tr>
<td>Chronic active hepatitis</td>
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<tr>
<td>Growth hormone deficiency</td>
</tr>
<tr>
<td>Asplenia</td>
</tr>
<tr>
<td>Dental enamel hypoplasia</td>
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<tr>
<td>Autoimmune thyroid disease</td>
</tr>
<tr>
<td>Gonadal insufficiency</td>
</tr>
<tr>
<td>Vitiligo</td>
</tr>
<tr>
<td>Alopecia</td>
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<tr>
<td>Malabsorbation</td>
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**Chronic mucocutaneous candidiasis**

*C. albicans* is a commensal fungus, usually not causing clinically important infections. In individuals with a compromised immune system the fungi can undergo a phenotypic switch from yeast and start spreading and grow invasively as hyphae, causing considerable harm to the host[74]. Patients with APS-1 are not known to be prone to infections by other microorganisms, such as bacteria, but often suffer from chronic mucocutaneous candidiasis, which is characterized as recurrent or persistent superficial infections of skin, mucous membranes and nails by *C. albicans*[75].

![Figure 3. *C. albicans* as yeast (left) and hyphae (right).](image)

Typically, the *C. albicans* mucositis is pseudomembranotic, painful and with erosions on the oral and esophageal mucosa and may contribute to development of oral and squamous cell carcinoma in APS-1 patients[76].
*C. albicans* infection usually is the first sign of disease in APS-1, it may be hypothesized that mechanisms involved in the fungal defense or infection drives the development of other manifestations of APS-1.

**Immune cell deviations in AIRE deficiency**

AIRE contributes to negative selection of thymocytes by regulating expression of tissue-restricted self-antigens by mTECs. Mutations in AIRE contributes to aberrant αβ-thymocyte selection in thymus; however, the γδ-thymocytes seem unaffected by mutations in AIRE[77]. Accumulation of DC in the thymus is also dependent on AIRE which contributes to development and selection of natural Tregs[78]. Indeed mutations in AIRE negatively affect the homeostasis and activity of Treg[79,80].

Although AIRE is primarily expressed in thymus, effects of AIRE-mutations can be observed in the periphery. Reduced maturation and impaired function of monocyte derived DCs (moDC) has been reported[81].

Studies of iNKT cells, another cell type with immunoregulatory functions, are however inconclusive. One study claims that the numbers of iNKT cells are not affected by AIRE-mutations[80], while in other studies decreased number of circulating iNKT cells was demonstrated in APS-1 and AIRE-deficient mice[82,83]. These aberrations may however be secondary to AIRE’s function in the thymus.

**2. The dog as a model for human disease**

The human share environment, many genetic factors and in part life style with the dog. That, in combination with the unique structure of the canine genome, makes the dog an interesting model for genetic studies of complex human diseases[84]. Before using the dog as a model for human disease, a careful characterization of the canine disease is essential. In these studies the aim was to investigate whether dogs with DM could serve as a model for human autoimmune DM and to analyze the expression of type I IFN in dogs with a systemic autoimmune disease resembling human SLE.

**Human diabetes mellitus**

DM is a group of disorders, collectively being a major threat to public health worldwide. In the year 2000 the global prevalence of diabetes for all age-groups was estimated to 2.8% of the population. Recent WHO calculations indicate that almost 3 million deaths per year worldwide are attributable to DM and the numbers of afflicted persons are estimated to be doubled by the year 2030 solely taking into account the demographic changes. In humans the two most common forms are T1D and T2D.

The human forms of DM that are considered to be of autoimmune origin are T1D and latent autoimmune diabetes of the adult (LADA). LADA is a
slowly progressive form of T1D with onset during adulthood in contrast to T1D, which have a more abrupt onset earlier in life. Their difference in onset may indicate separate disease mechanisms[85]. Autoantibodies in autoimmune DM are directed to targets in the pancreas (Figure 4). Most prevalent are autoantibodies to the 65 kDa isoform of glutamic acid decarboxylase, (GAD65), the protein tyrosine phosphatase Islet antigen 2 (IA2) and recently identified zinc transporter protein ZnT8[86]. Present at lower frequencies are antibodies to insulin (IAA) and cytoplasmic islet antibodies (ICA). Measuring ZnT8 antibodies (ZnT8A), GAD65 antibodies (GADA), Islet antigen 2 antibodies (IA2A), and IAA in combination detects 98% patients at disease onset[86]. GAD65 is an enzyme expressed in brain and pancreas. GAD65 catalyse the decarboxylation of glutamic acid to GABA and CO2[87]. The function of GABA in pancreatic β-cells remains to be elucidated, but it has been suggested that GABA has a paracrine suppressive effect on glucagon secretion[88].

T2D is characterized by peripheral insulin resistance and is part of the metabolic syndrome[89]. Other forms of diabetes are secondary forms of diabetes, related to pregnancy, medical disorders or surgical removal of the pancreas. Further, several monogenic forms of diabetes have been identified, such as maternally inherited diabetes and deafness (MIDD), maturity-onset diabetes of young people (MODY)[90] and Wolfram syndrome[91].

Figure 4. The insulin producing β-cells are located in pancreatic islets of Langerhans. To the left binding of monoclonal antibodies to GAD65 in tissue section from canine pancreas is depicted in red. (In blue, nuclear DAPI staining). To the right isolated islets of Langerhans from a euthanized dog.

a) Canine Diabetes mellitus

DM develops spontaneously in dogs, but the disease mechanisms have not yet been identified. Like in humans, the disease is complex and includes several subtypes. Diabetes is one of the most common endocrine disorders in dogs[92].
The clinical symptoms of human and canine DM are similar but the etiologies may differ. The diagnosis of canine DM is often based on its clinical signs, including polyuria and polydipsia, a persistent fasting hyperglycemia of more than 8 mmol/l and glycosuria. Prolonged periods of hyperglycemia have a negative effect on many tissues. Capillary epithelial cells in the retina, mesangial cells in the renal glomerulus and neurons and Schwann cells in the peripheral nerves can be damaged in human DM[93]. Similar to human DM, long-term complications have been reported in dogs[94].

Canine DM may be divided into insulin-resistant and insulin-deficient DM. Canine insulin resistance can occur as a consequence of hormonal disturbances and can lead to insulin deficiency by means of glucotoxicity. Insulin deficiency may additionally be caused by autoimmunity or develop because of β-cell loss associated with exocrine pancreatic disease[95]. Autoantibodies to canine GAD65, IA2 and recombinant canine proinsulin have been reported at low frequency in English DM dogs[96,97].

**Human Systemic Lupus Erythematosus**

SLE is a human systemic autoimmune disease with a female predominance. It is often relapsing-remitting but also chronic active, and long quiescent disease activity patterns occur[98]. Disease manifestations include cutaneous rash, fever, arthritis, anemia, serositis, glomerulonephritis, pancytopenia and seizures[61,99]. A majority of the patients have autoantibodies to nucleic acid[61]. SLE patient sera containing immune complexes can activate plasmacytoid DCs (pDCs) - the main producers of type I IFN in vivo[100]. In SLE patients pDCs migrate to tissues where IFN-α is secreted[101].

**Interferon-induced genes in SLE**

IFNs induce expression of hundreds of genes. Transcriptome analysis by mRNA amplification on hybridization chips can determine genes activated in response to IFNs. This analysis shows that SLE patients have a so-called IFN signature[54]. Some genes are induced by only one IFN, such as IRF1, preferentially induced by IFN-γ, whereas other genes such as MX1 can be induced by type I and type III IFN. The genes in the IFN signature encodes cytokines, chemokines, membrane receptors, signal transduction proteins, growth factors, apoptotic factors, adhesion molecules and antimicrobial proteins[102]. Interferon regulatory factors (IRFs) possess DNA binding domains that recognise interferon stimulated response elements, located at the promoter region of interferon inducible genes. Large-scale genetic association studies have shown that genetic variants of IRF5 is associated with risk of SLE[103,104,105,106] and a recent study showed that IRF5 activation is altered in monocytes from SLE patients[107].
b) Canine systemic autoimmune diseases

Dogs of the breed Nova Scotia duck tolling retriever (NSDTR) have a high incidence of immune-mediated diseases. Some of the dogs develop a condition termed immune-mediated rheumatic disease (IMRD), a disease complex resembling the human autoimmune disease SLE. These dogs may also develop anti-nuclear antibodies (ANAs) but do not develop autoAb to double-stranded DNA. In a study of 33 diseased NSDTRs 23 of them were ANA positive[108]. Another condition appearing at higher frequency in NSDTRs than other breeds is called steroid responsive meningitis-arteritis (SRMA), also known as aseptic meningitis[108]. Familial predisposition and a genome-wide association study show that genetic factors are involved in the etiology of disease[109,110].

IMRD

Dogs that develop IMRD are usually between two and six years. Clinical signs of IMRD are symmetrical nonerosive polyarthritis, stiffness, mainly after resting and signs of pain from several joints of extremities. A proportion of dogs with IMRD are ANA positive, i.e. antinuclear antibodies can be detected in sera from these dogs by indirect immunofluorescence (IIF). Based on this test it is possible to divide ANA-positive dogs into different subgroups, where 25% display a homogeneous fluorescence pattern and 75% speckled staining pattern[111].

SRMA

Dogs suffering from SRMA typically display fever, neck pain, stiffness and reluctance to move. Infectious causes to the clinical signs of disease are ruled out by test of the cerebrospinal fluid. The prognosis for young dogs in acute stage of disease is relatively good with early and aggressive anti-inflammatory or immunosuppressive therapy. Age of onset of SRMA is typically between 6 and 18 months with a range from 4 months to 7 years[112].
Autoimmunity is defined as an inappropriate immune reaction to self-tissue and subsequent tissue damage. Chronic mucocutaneous candidiasis is often the first sign of APS-1 but the underlying mechanisms are not yet clear. Dogs are generally considered to be a suitable model for several immunological human diseases, but a careful characterization of the disease of interest is essential. The principal aims of this thesis was to gain knowledge about mechanisms involved with autoimmune diseases and to further evaluate the dog as a model for autoimmune diseases.

Aims of this thesis

- To investigate disease mechanisms of chronic mucocutaneous candidiasis in APS-1 (Paper I and II)
- To investigate if diabetic dogs can be used as model organism for studies of T1D (Paper III)
- Develop an assay for measuring canine type I IFN-induced gene transcription and investigate if an IFN signature is present in dogs with systemic autoimmunity (Paper IV)
Material and methods

Here follows a brief description of the material and methods used within the studies. For more detailed information, see Paper I-IV.

Patients

Paper I & II
In Paper I and II human APS-1 patients, patients with isolated chronic mucocutaneous candidiasis and thyroiditis, and healthy control persons were included. Additionally, AIRE-deficient and wild-type mice were included in Paper I. Peripheral blood mononuclear cells (PBMC) from nine APS-1 patients were included in Paper I, two females and seven males, aged 18-58 years. The mean age at onset was ten years. All patients had chronic mucocutaneous candidiasis, eight of nine adrenal insufficiencies, seven of nine hypoparathyroidism; five had alopecia, four vitiligo, four malabsorption, three nail pitting, three dental enamel hypoplasia, two diabetes, two chronic active hepatitis, two growth hormone deficiency, two gonadal insufficiencies, two thyroid disease and two had pernicious anemia. The healthy controls were sex- and age-matched with each patient. Serum samples from 11 Swedish and 57 Finnish APS-1 patients, and 70 healthy blood donors were used for autoantibody assays in Paper I and serum samples from 25 Norwegian APS-1 patients were used in Paper II. All the Swedish/Finnish patients and 21 of 25 Norwegian patients had chronic mucocutaneous candidiasis.

Dogs

Paper III
In total dogs from 64 different breeds diagnosed with diabetes, dogs with Addison’s disease, thyroiditis or healthy control dogs were included in the study. Diabetes diagnoses were based on the clinical signs polyuria and polydipsia and a persistent fasting hyperglycaemia >8 mmol/l. Addison’s disease was diagnosed based on clinical signs, hyponatremia, hyperkalemia
and on ACTH-stimulation test. The thyroiditis dogs had increased levels of thyrotropin-stimulating hormone or autoantibodies to thyreoglobulin or both.

**Paper IV**

All dogs included in Paper IV are of the breed Nova Scotia duck tolling retriever. Dogs with IMRD showed signs of pain from several joint of extremities, stiffness, mainly after resting and symmetrical nonerosive polyarthritis. Dogs with SRMA displayed fever, neck pain, stiffness and reluctance to move. Cerebrospinal fluid sample analyses were performed for most of SRMA dogs, and ruled out infectious cause of disease. Healthy control dogs had no history of autoimmune disease.

**Cell isolation**

Venous blood was collected to Sodium-heparinized tubes. In Paper I human PBMC was isolated and in Paper IV dog PBMC was isolated, both using a density centrifugation method. Due to differences in density between human and canine PBMC, Ficoll-Paque PLUS was used for the human samples while Ficoll-Paque Premium 1.084 was used for canine samples (both from GE Healthcare, Uppsala, Sweden).

**Recall assay**

PBMC were cultured in 24-well plates in 37 °C, 5% CO₂ in humidified atmosphere. Cell density was 2X10⁶ cells/ml. Human PBMC were cultured in TB-1 lymphocyte medium, 1% HS. The cells were stimulated by *C. albicans*, *Mycobacterium tuberculosis*, Zymosan or peptidoglycan. Cells and supernatants were collected at one, two and three days of culture.

**Intracellular cytokine staining**

Th1 cells were stained for intracellular IFN-γ and investigated using flow cytometry. Cells were stimulated to produce cytokine and stained for surface molecules CD3 and CD4. After staining of surface molecules cells were permeabilized in order for anti-cytokine mAb to be able to enter cells. Next, after intracellular cytokine staining cells were washed, fixated and analyzed using a FACS Canto II cytometer (BD Biosciences, San Jose, CA).
Enzyme-linked immunosorbent spot (ELISPOT)

Human IL-17A secreting cells were detected using ELISPOT (Mabtech, Nacka, Sweden). The cells were cultured in antibody pre-coated ELISPOT plates for 72 h in the presence of stimulatory antigens. Spots were developed and counted manually in a dissection microscope.

Enzyme-linked immunosorbent assay (ELISA)

Based on the quantitative sandwich enzyme immunoassay technique ELISAs were used for measuring the contents of cytokines from cultured cells. Microtiter plates precoated with monoclonal antibodies were used. Standards and samples were added to the plate and cytokine of interest was bound by the immobilized antibodies. After washing unbound molecules, enzyme-linked polyclonal antibodies directed to cytokine of interest were added. Unbound antibody-enzyme reagents were washed off and substrate added. Color, in proportion to the amount of cytokine of interest in samples and standards was developed and measured.

Human IL-17A, murine IFN-γ, IL-4, IL-10, IL-17A, IL-6 and IL-15 were from R&D systems, Minneapolis, MN. Murine IL-23 ELISA was from eBioscience, Rome, Italy. ELISA was used as a reference method for measuring autoantibodies in Paper II.

Multiplex bead immunoassay

Cell supernatants were analyzed for multiple cytokines using BMD’s FIDIS Human Cytokine 25-plex kit (BMD, Marne la Valée, France). The method is based on a solid phase sandwich technique in which the spectral properties of different bead regions are monitored to achieve simultaneous measurement of multiple cytokines. A solution of beads with different spectral properties conjugated to analyte-specific capture antibodies were mixed and incubated with samples, controls and standards for 2 h in filter bottom microplates. After washing the beads using a vacuum manifold, analyte-specific biotinylated detector antibodies were added and incubated for 1 h. Excessive detector antibodies were then removed and streptavidin conjugated to the fluorescent protein R-Phycoerythrin (RPE) added forming the solid phase sandwich with the immune complexed bead. The beads were then analyzed using the FIDISTM100 instrument. By monitoring the spectral properties of the beads and the amount of associated RPE fluorescence, the concentration of each analyte are determined. Four-parametric curve-fitting were used to calculate cytokine concentrations in the samples.
Quantitative Real-Time RT-PCR

Total RNA was isolated from cells using RNeasy micro or mini kit according to the manufacturer’s instructions (Qiagen, Hilden, Germany). Reverse transcription was performed with 10 µl or 0.2 µg total RNA random hexamer primers, dNTPs and Superscript III (Invitrogen, Carlsbad, CA).

Quantitative real time PCR of the cDNA was performed using primers designed based on human or canine DNA sequences. Relative expression to the housekeeping genes GUSB, GAPDH or 18s rRNA was calculated for all samples and qPCR was run on a MyiQ Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA) as previously described[113].

Immunoprecipitations of radioactively labeled protein

Plasmids containing genes of interest (encoding human IL-17A, IL-17F, IL-22, hetero and homodimers thereof, human IL-10, and canine and human GAD-65) were used for in vitro transcription and translation (ITT) into $^{35}$S labeled proteins. Patient sera, control sera and sera from healthy or diseased dogs were used for immunoprecipitations and subsequently the amount of antibody-bound protein was measured by assaying the radioactivity of precipitates. Indices were calculated and serum with an index mean-value plus 3 (Paper I-II) or 4 (Paper III) standard deviations were considered autoantibody-positive.

Figure 5. The principle for immunofluorescent staining. Patient sera or monoclonal antibodies directed to antigen of interest are added to tissue sections on microscope slides. Fluorophore labeled secondary antibodies are added and specific binding assessed in a microscope.

Immunofluorescence

Tissue sections of human and canine pancreata were used for autoantibody detection in canine sera. Tissues were snap frozen at harvest and stored in -70º C until cryosectioning. Human pancreata were stained in 3-µm sections while 6-µm sections were used for canine substrates. Sera and antibodies as
indicated in Paper III were used for IIF and fluorescence microscope or confocal microscope was used for assessing autoantibody binding (Figure 5).

**Antibody ELISA (Paper II)**

Immunosorbent plates were coated with IL-17F or IL-22 protein and non-specific binding sites were blocked with BSA (3%). After washing, samples diluted 1:50 were added, in duplicates. After subsequent washing alkaline phosphatase conjugated goat anti-human IgG was added. SIGMAFAST p-nitrophenol phosphate substrate solution was used for visualization of anti-protein binding. Absorbance was read at 405 nm in a spectrophotometer.

**Canine type I IFN assay**

Madin-Darby canine kidney (MDCK) cells are known to be responsive to IFN. Therefore these cells were cultured and stimulated with sera from dogs with IMRD, SRMA and healthy control dogs. After 6 h stimulation the cells were harvested and mRNA isolated. After cDNA synthesis the relative expression of the IFN responsive genes MX1, IFIT10 and CXCL-10 was measured by qPCR.

**Statistical analyses**

Students T test, nonparametric Mann Whitney test, Kruskal-Wallis test and Dunn’s multiple comparisons tests were used. One way analysis of variance (ANOVA) was calculated to determine whether there was a significant difference between groups in Paper III. Correlations of the result of the antibody assays by radioligand binding assays (RLBA) and ELISA were calculated using the Spearman r-test, confidence interval and p-value.
Results

Paper I

IL-17A secretion in response to *Candida albicans* is increased in AIRE deficiency

In Paper I we found that the IL-17A secretion in response to *ex vivo* recall stimulation with *C. albicans* is increased in PBMC from APS-1 patients and in AIRE-deficient mice. None of the control antigens - *Mycobacterium tuberculosis*, Zymosan or peptidoglycan - gave rise to increased secretion of IL-17A in APS-1 patients. This shows that the response observed is *C. albicans*-specific. AIRE deficiency appears to promote a strong Th17 response to *C. albicans*. In contrast, the IL-22 secretion was reduced.

In patients with isolated chronic mucocutaneous candidiasis without AIRE deficiency the IL-17A secretion was unaltered and the IL-22 secretion was reduced.

Recently, it was found that APS-1 patients carry autoantibodies to cytokines that are related to the *C. albicans*-infection[41,70]. We confirmed presence of antibodies to IL-17A, IL-17F and IL-22 by radioligand binding assay and immunoprecipitation. We also investigated whether patients suffering from other autoimmune diseases display anti-IL-22 autoantibodies, but found that the anti-IL-22 antibodies are specific to APS-1. We found high titers of anti-IL-22 autoantibodies in 89 % of the APS-1 patients.

*Main findings:*
- The IL-17A secretion in response to *C. albicans* is increased in both APS-1 patients and its animal model.
- PBMC from patients suffering from chronic mucocutaneous candidiasis, with or without AIRE deficiency, secrete less IL-22 than healthy controls.
- A strong antibody response to IL-22 is seen in almost all APS-1 patients.
Paper II

Radioligand binding assays based on IL-17F and IL-22 as monomers or dimers can be used for screening of autoantibodies in APS-1

In Paper I autoantibodies were measured by in vitro transcription and translation followed by immunoprecipitation with a radioactively-labeled protein. In Paper II this assay was improved and evaluated in comparison to ELISA. Fusion proteins of the cytokines IL-17F and IL-22 was tested, both as homo- and hetero-dimers because cytokines are small proteins and thus difficult to express in vitro. We found that the assays based on dimeric proteins are highly reproducible with low inter- and intra-variation. The method has high throughput and easy standardization compared to ELISA, thus providing excellent choices for the screening of IL-17F and IL-22 autoantibodies.

Main finding:
- Screening of autoantibodies to IL-17F and IL-22 can preferably be performed using radioligand-binding assay using fusion proteins of the two cytokines.

Paper III

No evidence for autoimmune disease in diabetic dogs

We used four assays to investigate signs of humoral autoimmunity in diabetic dogs. Sera from diabetic dogs were tested for islet cell antibodies and GAD65 antibodies using indirect immunofluorescence and in vitro transcription and translation followed by immunoprecipitation with a radioactively-labeled protein. Among a large cohort of diabetic dogs from many different breeds we found no evidence for humoral autoimmunity.

Main finding:
- DM in Swedish dogs does not appear to have an autoimmune etiology.

Paper IV

Nova Scotia duck tolling retrievers suffering from steroid responsive meningitis-arteritis exhibit a type I IFN signature

IFIT1, MX1 and CXCL10 are examples of IFN-inducible genes. By directly measuring the mRNA expression of these genes in PBMC from diseased and healthy NSDTRs, a higher expression of IFIT1 was detected in dogs suffering from IMRD compared to healthy dogs.
It is difficult to detect type I IFN directly in blood because the protein levels are below the detection limit with current techniques. Therefore indirect methods are used for measuring the type I IFN content in sera or plasma. A novel method for indirect measurement of canine type I IFN was developed. MDCK cells were stimulated with sera from diseased and healthy dogs. IFN-inducible gene expression was measured by real-time PCR by stimulated cells.

**Main findings:**
- PBMC from NSDTR suffering from IMRD display a higher mRNA expression of IFIT1 than healthy control dogs.
- Sera from NSDTRs suffering from SRMA can induce a higher expression of the IFN-inducible gene MX1 in cultured cells than sera from healthy dogs, indicating an IFN signature in dogs with SRMA.
Conclusions and general discussion

The precise sequence of events leading to chronic mucocutaneous candidiasis is not clear, though much progress in this research area has been achieved in recent years.

IL-22 has emerged as an important cytokine in protection from fungal infections. Healthy individuals carry IL-22-producing Th22 cells specific for *C. albicans*[46]. We and others have shown that IL-22 secretion is depressed in chronic mucocutaneous candidiasis, either isolated or caused by AIRE-deficiency or STAT1 mutations[70,114,115,116]. This argues for a prominent role for IL-22 in antifungal defense regardless of the underlying cause for susceptibility to the disease. In addition, protective immunity to candidiasis involves an early IL-22 response in mice[117]. IL-22 receptor engagement induces STAT3 phosphorylation and thereby release of antimicrobial peptides. This contributes to antifungal resistance at mucosal surfaces[117].

The role of Th17 cells in protection vs. pathology to fungal infection remains controversial. In Paper I we report a several-fold increase in IL-17A producing cells and secretion of IL-17A in response to *C. albicans* in PBMC from APS-1 patients. These results were corroborated in a British APS-1 patient group[115]. On the other hand, IL-17A and IL-17 receptor A deficiencies are associated to isolated chronic mucocutaneous candidiasis[40]. Reduced IL-17A and IL-17F expression in response to *C. albicans* has also been reported in isolated chronic mucocutaneous candidiasis patients[39]. STAT3 mutations lead to hyper-IgE syndrome, a condition characterized by chronic mucocutaneous candidiasis and other immune deficiencies. These patients fail to mount a Th17 response[118]. Thus APS-1 patients display an enhanced Th17 response to *C. albicans*, whereas patient groups with other underlying causes of candidiasis display an impaired Th17 response. It remains to establish whether the Th17 cells protect from fungal infection or are involved in the pathology[42].

A feature of chronic mucocutaneous candidiasis in APS-1 is the presence of autoantibodies to IL-22 as observed in Paper I. These antibodies can be the primary cause of candidiasis or a consequence of the enhanced Th17 responses. At present it is not known whether the autoantibodies to IL-22 cause chronic mucocutaneous candidiasis. Prospective studies in infants with APS-1 are required to solve this question.
Others have reported a normal frequency but an impaired function of Tregs from APS-1 patients[79]. Indeed the frequency of Tregs was unaltered in our study (data not included). In Paper I higher amount of IL-2 from C. albicans-stimulated PBMC from APS-1 patients was observed. Cytokine deprivation-induced apoptosis is a mechanism by which Tregs inhibit T cell responses[47]. I speculate that the increased amount of IL-2 is a consequence of impaired Treg-mediated IL-2 deprivation.

In conclusion, IL-22 responses to C. albicans are impaired in patients suffering from chronic mucocutaneous candidiasis, whereas the Th17 response to C. albicans is enhanced in APS-1. I hypothesize that IL-22 deficiency has a prominent role in the disease mechanism of chronic mucocutaneous candidiasis, whereas the increased Th17 response seen in Paper I is related to the AIRE-deficiency. This is supported by the enhanced Th17 responses observed in AIRE-deficient mice (Paper I).

In Paper I we report that nearly all APS-1 patients carry autoantibodies to IL-22 and one fifth of the patients carry antibodies to IL-17A or IL-17F. The anti-IL-22 antibodies were specific for APS-1. However the sensitivity and specificity of the autoantibody assays were not determined. In paper II a radioligand binding assay based on hetero- and homo-dimers of IL-22 and IL-17F was developed. We found that the assays based on dimeric proteins are highly reproducible with low inter- and intra-variation. Thus, the assays can be used as diagnostic methods for APS-1.

Several human diseases have an equivalent disease in dogs. Because humans share environment, and in part lifestyle with the dogs they are interesting for use in comparative studies. In Paper III and IV the main aim was to evaluate the dog as a model for autoimmune disease and gain knowledge about disease mechanisms in canine DM and in a systemic autoimmune disease resembling human SLE.

Although many studies have characterized canine DM, no consensus has been established for etiological classification of DM in dogs. According to WHO criteria DM in humans can be diagnosed by venous plasma glucose concentration is >11.1 mmol/l 2 h after a 75-g peroral glucose tolerance test or by two fasting glucose measurements >6.1 mmol/l. The diagnosis of canine DM is often based on its clinical signs, including polyuria and polydipsia, a persistent fasting hyperglycemia of more than 8 mmol/l and glycosuria. DM is more common in middle aged and older dogs, rather than young dogs and most dogs require exogenous insulin therapy [95].

Based on disease pathogenesis a system for classification of DM in dogs was suggested by the Royal Veterinary College in UK, and presented by Catchpole at al, 2005[95]. In this system the disease was divided into insulin deficient diabetes (IDD) and insulin resistant diabetes (IRD). IDD is characterized by a progressive loss of β-cells. Processes that lead to IDD include
congenital β-cells hypoplasia/abiotrophy, β-cells loss associated with exocrine pancreatic disease, immune-mediated destruction and idiopathic β-cells deficiency. IRD result from antagonism of insulin function by other hormones. This includes resistance due to diestrous/gestational DM or to other endocrine disorders or iatrogenic effects. Obesity may also contribute to IRD, but is not a primary cause of DM in dogs[95]. In 2009, Fall presented a new classification system in her doctoral thesis[119]. This system was based on new knowledge on causes for DM and included 7 classes:

- juvenile diabetes, including β-cell hypoplasia and combined β-cell deficiency and pancreatic acinar atrophy
- progesterone-related, i.e. gestational/diestrous
diabetes secondary to pancreatitis
diabetes caused by endocrine tumors
diabetes caused by iatrogenic effects
- immune-mediated diabetes
- idiopathic diabetes

In conclusion, DM has several etiologies in dogs. One of the proposed etiologies is immune-mediated DM. Autoimmunity in canine diabetes is however controversial. Antibodies to canine GAD65, IA2 and recombinant canine proinsulin have been reported at low frequency in English diabetic dogs [96,97], while we and others report absence of autoantibodies[120,121].

In autoimmune diseases such as APS-1 and T1D mature B cells turn into plasma cells and produce autoantibodies before clinical manifestations appear[122,123]. These autoantibodies offer a useful diagnostic and potentially predictive disease marker. If the autoantigen is known, radioligand immunoprecipitation assays or immunoglobulin ELISA can be used for measuring the quantity of antibodies in sera. Indirect immunofluorescence (IIF) staining of tissue sections can also detect autoantibodies. This method can be useful if the autoantigen is unknown, but the organ targeted by disease is known. In canine diabetes the clinical picture resembles the human disease, which indicates pancreas as the target for a potential autoimmune attack. Therefore we used IIF to look for signs of autoimmunity in canine pancreas. We also used a certified human ICA assay. RLBA for antibodies to human and canine GAD65 was used on a large cohort of diabetic and control dogs from different breeds. We found however no presence of humoral autoreactivity. Probably, the dogs included in our study suffer from other types of diabetes, including gestational diabetes[124], combined endocrine and exocrine pancreatic insufficiency and idiopathic diabetes. However, future studies of cellular responses to islet cell antigens are needed, since autoreactive T cells play an important role in the pathogenesis of human T1D[64,125]. In addition, future studies on histopathology of canine pancreata may shed further light on the etiology of diabetes in dogs.
Human SLE patients have overproduction of IFN-α and treatment with antibodies to IFN-α ameliorates disease. This suggests a detrimental role for IFN-α in the pathogenesis. Because NSDTR dogs often develop a disease complex resembling SLE, including IMRD and SRMA[108]. It is not known whether IMRD and SRMA are two different diseases or parts of the same disease. A genetic study implies a partially shared genetic background [110].

Increased type I IFN secretion was detected in SRMA dogs by an indirect method in Paper IV. PBMC from IMRD dogs expressed more IFIT1, an IFN-inducible gene. This may suggest a role for type I IFN in the pathogenesis of systemic autoimmune diseases in NSDTRs. It would be desirable to treat dogs with antibodies to IFN-α to confirm this association.

This thesis contributes to increased knowledge about mechanisms in autoimmune disease in man and dog. In addition the results presented herein can form the basis for future research with the aim to develop new therapies to autoimmune diseases. The results presented in Paper I has in addition increased our understanding of disease mechanisms in chronic mucocutaneous candidiasis.
Immunologiska studier av modellsjukdomar i människa och hund


I APS-1 är det ofta den kroniska eller frekvent återkommande och svårbehandlade kandida-infektionen som är det första tecknet på sjukdom, och uppstår redan i barndomen hos de flesta patienterna. Jag studerade immunsvaret mot kandida i blodceller från a) patienter med APS-1, b) patienter med kronisk mukokutan kandida infektion utan AIRE-mutation och c) friska ålders- och köns-matchade kontroller. Jag fann en förhöjd utsöndring av cytokinet IL-17A i APS-1 patienternas blodceller efter stimulering med kandida, jämfört med friska kontroller. Denna ökade utsöndring kunde inte observeras hos patienter med kronisk mukokutan kandida infektion utan AIRE-mutation. En annan signaleringsmolekyl som är viktig vid svampinfektioner, IL-22 var däremot lägre hos patienter än friska. Denna nedreglering var dock inte enbart observerad vid stimulering med kandida, utan även vid stimulering med kontrollantigenerna Mycobacterium tuberculosis, Zymosam och peptidoglykan. Patienter med kronisk mukokutan kandidainfektion utan AIRE-mutation hade också låg nivå av IL-22 i våra försök. Detta tyder på att...
IL-22 är viktigt för att skydda mot kronisk mukokutan kandida, och att AIRE mutationer ökar IL-17A-svaret mot kandida.

APS-1 patienter har autoantikroppar mot IL-22, IL-17A och IL-17F. Reaktiviteten mot IL-22 är stark, förekommer hos 89% av APS-1 patienterna, men inte i friska kontroller eller patienter med andra autoimmuna sjukdomar. I arbete 2 utvecklade vi en förbättrad metod för att mäta förekomst av autoantikroppar mot IL-22 och IL-17F. Vi fann att genom att använda fusionsprotein av IL-17F och IL-22 kan man få en mätmetod som är både reproducerbar och användbar för stort antal prover.


Denna avhandling bidrar till ökad kunskap om mekanismer i autoimmuna sjukdomar, i människa och hund. Dessa kunskaper har inte bara bidragit till ökad förståelse för de studerade sjukdomarna, utan kan också ligga till grund för vidare forskning med syfte att utveckla nya behandlingar mot autoimmuna sjukdomar. Resultaten i delarbete 1 har även bidragit till ökad förståelse om sjukdomsmekanismer vid kroniska svampinfektioner.
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