Clinical and Experimental Studies of Organ-Specific Autoimmune Diseases

With Special Reference to Addison's Disease and Autoimmune Hepatitis

BY

GENNET GEBRE-MEDHIN
ABSTRACT

Organ-specific autoimmunity constitutes a large health problem, where both the clinical management and our understanding of the pathogenetic mechanisms need to improve. Women with Addison’s disease have abnormally low levels of dehydroepiandrosterone (DHEA), its sulphate ester (DHEA-S) and androgens relative to age, and many patients complain of physical and mental fatigue and low stress tolerance. To define a suitable dose, the effect of oral DHEA replacement was evaluated in women with Addison’s disease.

DHEA was administered for three months to nine women with Addison's disease in either of two doses, 50 mg (n=5) or 200 mg (n=4). A dose of 50 mg restored the DHEA(S) and androgen levels to normal without altering the insulin sensitivity, body composition or serum lipid profile.

Autoimmune polyendocrine syndrome type I (APS I) is a rare but useful model disorder of autoimmunity, characterised by multiple organ-specific autoimmune manifestations and high-titre autoantibodies and with adrenocortical insufficiency, Addison's disease, as one of its cardinal manifestations. Approximately 10-20% of APS I patients suffer from autoimmune hepatitis, which carries a high mortality, if untreated. The presence of putative antigenic targets in the liver was investigated.

Cytochrome P4501A2 (CYP1A2) and aromatic L-amino acid decarboxylase (AADC) were identified as hepatic autoantigens with the use of APS I sera for immunofluorescent staining of normal human liver, Western blot of microsomal and cytosol fractions of human liver homogenate, and immunoprecipitation of in vitro transcribed and translated radioactively labelled proteins. The presence of CYP1A2- and AADC-antibodies was significantly correlated to AIH, and CYP1A2 antibodies inhibited enzyme activity in vitro.

In conclusion, a daily replacement dose of 50 mg of DHEA sufficiently restores levels of DHEA, DHEA(S) and androgens in women with Addison's disease, without severe side-effects. We have further identified CYP1A2 and AADC as hepatic autoantigens associated with autoimmune hepatitis in APS I.

Key words: Addison's disease, replacement therapy, DHEA, autoimmune hepatitis, APS I, autoantigen, cytochrome P4501A2, aromatic L-amino acid decarboxylase.

Gennet Gebre-Medhin, Department of Medical Sciences, University Hospital, SE-751 85 Uppsala, Sweden
gennet.gebre-medhin@medsci.uu.se

© Gennet Gebre-Medhin 2001

ISSN 0282-7476
ISBN 91-554-5043-1

Printed in Sweden by Eklundshofs Grafiska AB, Uppsala 2001

*Dag Hammarskjöld*

*To Per, David, Andreas, Jonatan and Simon*
This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:


Reprints were made with the permission of the publishers.
Clinical features 27
Serum biochemistry and histological features 28
 Therapeutic considerations 28
 Prognosis 28
IDIOPATHIC AUTOIMMUNE HEPATITIS (AIH) 29
 Susceptibility to AIH 30
 Triggering factors 30
 Mechanisms of autoimmune liver damage 32
 Autoantibodies in AIH 32
ADDISON'S DISEASE 34
 Clinical findings 34
 Treatment and prognosis 35
DEHYDROEPIANDROSTERONE (DHEA) 36
 Metabolism and physiology 36
 DHEA in health and disease 37
CURRENT INVESTIGATION
RESULTS 39
 Identification of CYP1A2 and AADC as hepatic autoantigens
 in APS I (I) 39
 AADC antibodies and disease-correlation in APS I (II) 40
 CYP1A2 and AADC antibodies in other autoimmune liver
 disorders (III) 40
 DHEA replacement in women with Addison's disease (IV) 41
DISCUSSION 42
GENERAL CONCLUSION AND FUTURE PERSPECTIVES 46
ACKNOWLEDGEMENTS 48
REFERENCES 51
ABBREVIATIONS

ACTH  Adrenocorticotropic hormone
AADC  Aromatic L-amino acid decarboxylase
ADCC  Antibody dependent cell cytotoxicity
AIH   Autoimmune hepatitis
AIRE  Autoimmune regulator (human)
Aire  Autoimmune regulator (mouse)
APC   Antigen presenting cell
APS   Autoimmune polyendocrine (polyglandular) syndrome
CAH   Chronic active hepatitis
CD    Cluster of differentiation
CTLA  Cytotoxic-T-lymphocyte-associated
CYP   Cytochrome P450
DHEA  Dehydroepiandrosterone
EAE   Experimental autoimmune encephalomyelitis
HBV   Hepatitis B virus
HCV   Hepatitis C virus
HSR   Homogeneously staining region
Ig    Immunoglobulin
IGF   Insulin-like growth factor
IL    Interleukin
IFN   Interferon
ITT   In vitro transcribed and translated
LKM   Liver kidney microsomal
MHC   Major histocompatibility complex
NOD   Non-obese diabetic
PHD   Plant homeodomain
SAND  (A sequence present in) Sp100, Aire, NucP41/75 and DEAF-1/suppressin
SLE   Systemic lupus erythematosus
TAP   Transporter associated with antigen processing
Th    T helper
TGF   Transforming growth factor
TNF   Tumour necrosis factor
TPH   Tryptophan hydroxylase
TSH-R Thyroid-stimulating hormone receptor
INTRODUCTION

In this work, selected aspects of organ-specific autoimmune disease have been studied from a clinical and an experimental point of view. Autoimmune diseases are common, affecting about three per cent of the population, and thus constitute a major public health problem. Although we have greatly increased our knowledge of the involvement of possible pathogenetic mechanisms in autoimmunity in the past fifty years, there are still large gaps to be filled and there is still no single autoimmune disorder with a known aetiology. This fact limits our possibility of curing these patients and leaves us to rely on replacement therapy and non-specific immunosuppressive treatment, when needed. As autoimmune diseases are life-long, often develop at a young age, affect vital organs and predispose to the development of other autoimmune disorders, the need for further research to reveal underlying aetiological and pathogenetic mechanisms is evident. Meanwhile, improvement of currently existing replacement therapy is also warranted.

In our research we have focused on idiopathic autoimmune adrenocortical insufficiency, Addison's disease and the rare autoimmune polyendocrine syndrome type I (APS I), in which multiple organ-specific autoimmune manifestations occur and with adrenal insufficiency as one of its cardinal manifestations. The aims of the present investigations were to evaluate the effect of androgen replacement therapy in women with Addison's disease and to use APS I as a model disorder in an attempt to identify pathogenetic mechanisms in the development of autoimmune hepatitis.

AUTOIMMUNITY

Autoimmunity, mediated by autoreactive T and B cells, is part of a healthy immune system and is often transiently seen in the response to infectious disease. It is only when the autoreactive responses are present in an uncontrolled and sustained manner with destruction and/or impaired function of the targeted organ(s) that an autoimmune disease occurs. A disease is commonly designated autoimmune on the basis of direct or indirect criteria, as follows:

Direct criteria
- The presence of pathogenetic autoreactive antibodies or T cells
- Transmissibility of disease by T cells from human to human, or human to animal
Organ-specific autoimmune diseases

Indirect criteria

- Immune activation in the absence of a known triggering agent
- Hyperimmunoglobulinaemia
- Disease-associated antibodies (even without proven pathogenicity)
- Association with known susceptibility alleles of the major histocompatibility complex
- Responsiveness to immunosuppressive treatment
- A valid animal model

Autoimmune diseases can be classified as destructive or non-destructive, systemic or organ-specific, depending on the nature of the antigenic target. In most autoimmune diseases autoreactivity results in destruction of the targeted organ, resulting in loss of function. In these diseases the antigenic target is an intracellular enzyme, as in Addison's disease, where 21-hydroxylase is the target autoantigen \(^ {257}\), and in type 1 diabetes mellitus, where glutamic acid decarboxylase (GAD) is targeted.\(^ {14}\) In the non-destructive form, an altered function due to interactions of the autoantibody with tissue-specific cell surface receptors is seen, as in Graves' thyrotoxicosis, where TSH receptor-stimulating autoantibodies are present,\(^ {204}\) and in myasthenia gravis, where acetylcholine receptor antibodies are present.\(^ {199}\) In organ-specific autoimmune diseases the antigenic targets are tissue-specific, e.g. H\(^ +/K^+\) ATPase in autoimmune gastritis\(^ {117}\) or thyroglobulin and thyroid peroxidase in autoimmune thyroiditis,\(^ {58}\) whereas in systemic disease, proteins present in more or less all cells are targeted, such as nuclear proteins and dsDNA in systemic lupus erythematosus (SLE). As mentioned earlier, the knowledge of the aetiological and pathogenetic mechanisms underlying autoimmune diseases is limited. A short summary of factors and mechanisms that are thought to be involved is presented below.

The concept of tolerance

For B and T cells not to react against self, efficient mechanisms of induction and maintenance of self-tolerance are needed. Both cell types display receptors that after gene rearrangements are present in \(~10^{15}\) different variants, each with its own antigen specificity, but without the ability to discriminate self from non-self. There is an obvious need to shape this generous repertoire into one devoid of self-reactive cells, and also to control the level of responsiveness in those cells surviving. To attain this, both B and T cells are subject to several steps of positive and negative selection, mainly based on reactivity against self-antigen. These steps take place in the bone-marrow and thymus (central tolerance) and in peripheral lymphoid organs such as the spleen and lymph nodes (peripheral tolerance).
The generation of tolerant B cells

Immature B cells are subjected to a first selection round in the bone marrow, where cells bearing Ig receptors that are able to recognise self-antigen are clonally deleted by apoptosis. Not all self-antigens are present in the bone marrow, which means that autoreactive B cells are able to slip through this first line of defence. Additional rearrangement of the Ig receptor, taking place during B cell maturation, might also lead to autoreactivity, necessitating further defence mechanisms. Remaining B cells enter the circulation and are transported to the T cell zones of the lymph nodes or spleen, where T helper (Th) cell-mediated activation is needed for further survival. This interaction between activated CD4+ T cells and B cells is mediated through co-receptor CD40 and its ligand. If this interaction does not take place, or antigen is present at a very high concentration, the B cell either becomes anergic, a state of unresponsiveness, or undergoes apoptosis. In the activated B cells, additional fine-tuning of the variable antigen-binding region of the Ig receptor then takes place, denoted somatic hypermutation. This results in a receptor with an antigen-specific high-affinity binding capacity.

The role of B cell tolerance in preventing autoimmunity is unclear, but experimental data indicate that it is mainly due to lack of help from T cells. Under some circumstances in experimental animal models, B cells may become activated in a T cell-independent manner, but the significance of this is unclear.

The generation of tolerant T cells

Immature T cells enter the thymus, where they encounter thymic cortical cells expressing peptides derived from self-antigens, presented in either MHC class I or II molecules. The immature T cells are predestined to undergo apoptosis, but those cells bearing T cell receptors that are able to recognise a self MHC-peptide complex will be rescued, a process called positive selection. On the other hand, T cells binding MHC-antigen complex with too strong affinity will be deleted. About 95% of the original cells are deleted in this first round, which is aimed at selecting T cells able to recognise self-MHC. The remaining five per cent of the T cells then migrate to the thymic medulla, where they encounter professional antigen-presenting cells (APC) such as dendritic cells and macrophages. This round of selection is mainly intended for deletion of T cells reacting with self-antigen, a process denoted negative selection, whereby another two to three per cent of the T cells are deleted. Included in the positive and negative selection mechanisms are the final rearrangement of the T cell receptor and shaping of necessary co-receptors, e.g. CD4 and CD8. Although it is mainly self MHC-restricted and self-tolerant T cells that finally leave the thymus, there is evidence that potentially autoreactive T cells are present in persons without overt autoimmune disease, e.g. T cells reactive against myelin basic protein not leading to multiple sclerosis or against insulin.
Organ-specific autoimmune diseases

without the development of diabetes, suggesting the existence of additional control mechanisms. Possible reasons why autoreactive T cells may slip through this first line of defence are that the antigen is not presented in the thymus, that the antigen is expressed in too low a concentration or that the affinity between antigen and T cell receptor is low. The following are some mechanisms involved in the attainment of peripheral T cell tolerance:

- Ignorance of autoreactive T cells due to an anatomical barrier, e.g. the blood-brain barrier or when the level of antigen is below the threshold required to induce activation or deletion.
- The 2-signal theory suggesting peripheral deletion of autoreactive T cells due to lack of a co-stimulatory signal from the APC, usually mediated by B7.1 (CD80) or B7.2 (CD86). These molecules are only expressed on the APC as a result of stimulation of pattern recognition receptors restricted to foreign antigens.
- Induction of apoptosis through Fas/Fas ligand interaction mediated by regulatory T cells or non-professional APCs.
- Induction of apoptosis through Fas/Fas ligand interaction mediated by regulatory T cells or non-professional APCs.
- Inhibition of activity through competitive binding of the inhibiting cytotoxic T lymphocyte-associated protein 4 (CTLA-4) on T cells with B7.1 and B7.2 on the APC.
- Suppression of activity through secretion of inhibiting cytokines, e.g. interleukin-10 (IL-10) and transforming growth factor-β (TGF-β), by regulatory T cells.

It is worthy of note that not all mechanisms involved in attainment of peripheral tolerance result in deletion of the autoreactive B or T cell, but some of these mechanisms rather inhibit them or put them in an unresponsive or resting state, with a potential for activation. Tolerance is recognised as the major protection against autoimmunity, and most of the known pathogenetic mechanisms are involved to varying degrees in the breaking of this tolerance.

The Danger hypothesis - an alternative view on immunoregulation

Polly Matzinger and co-workers have presented a modified view of mechanisms initiating an immune response. Their hypothesis suggests that the presence or not of danger in the tissue in question provides the on/off signal in an immune response. The need of self/non-self discrimination is thus eliminated. This is based on the assumption that the body cares more about what is dangerous (or beneficial) than what is non-self (or self). APCs are still viewed as the final activators of Th cells, delivering the second signal in the above mentioned two-signal system, but in this model APCs are activated by endogenous alarm signals from distressed or damaged tissues rather than foreign antigens. Activators come in two forms: Pre-packaged in the form of extracellular exposure of intracellular antigens, e.g. DNA, RNA and mitochondria, or inducible as
heat shock proteins and interferon (IFN) α. Thymic deletion of autoreactive T cells is recognised, but mainly as a mode of inducing tolerance against host APCs. Tolerance is thought to be provided by each tissue in that a second activation signal is not emitted as long as danger is not present.

**The role of genetic factors**

Genetic factors are said to contribute about one-third to one-half of the risk in most autoimmune disorders. The fact that many autoimmune diseases show familial clustering and higher concordance rates in monozygotic than in dizygotic twins lends further support to a genetic contribution. Most autoimmune diseases are polygenic traits. The strongest association with autoimmune diseases is found for genes encoded in the MHC, but genes outside this region are also thought to be involved, e.g. the insulin gene in humans and possibly the interleukin-2 gene in NOD mice, both of which are associated with type 1 diabetes mellitus. Genome-wide linkage studies performed in major human autoimmune diseases and associated murine models, such as type 1 diabetes mellitus, multiple sclerosis and rheumatoid arthritis, revealed distinct gene clusters to which all the diseases were associated in various degrees. Candidate genes proposed within these loci include the tumour necrosis factor (TNF)-receptor 1 gene, involved in the inflammatory response, and genes encoding the T cell receptor Vß-chain involved in antigen specificity. A candidate parameter of interest is the intra-thymic level of antigen expression, as one of the susceptibility genes in type 1 diabetes mellitus has been found to determine levels of insulin expression in the thymus.

In conclusion, most autoimmune diseases are polygenic and genes associated with autoimmune diseases seem to be involved in sustained inflammatory responses and loss of tolerance to self-antigens. There is no single gene that is sufficient for disease expression, but rather sets of genes render an individual person susceptible to autoimmunity. An exception to this is APS I, which is a monogenic disease inherited in an autosomal recessive way (see below). The gene was recently identified and homozygosity for the gene defect has been found to result in complete penetrance of the disorder. Further studies with the aim of revealing and characterising functional properties of the gene product will hopefully contribute important information on the aetiology of autoimmunity.

**Aberrant antigen expression and presentation**

The disruption of cell and tissue barriers as a consequence of infection, inflammation or trauma may result in the release of sequestered self-antigens against which no tolerance has been gained, and an autoimmune response may follow as in sympathetic ophthalmia. Furthermore, tissue damage and local necrosis during an infection may lead to the uncovering of cryptic epitopes and an autoimmune reaction may follow, as in
Organ-specific autoimmune diseases

Experimental Coxsackie virus-induced diabetes. Inflammatory cytokines may induce aberrant MHC class II expression in non-professional APCs, leading to inappropriate activation of autoreactive T cells. The covalent binding of a pathogen to a self-protein, resulting in a "neoantigen", is another form of altered antigen presentation. An immunological response to both the pathogen and the self-antigen may result as is proposed for drug-induced autoimmune hepatitis.

Molecular mimicry

The induction of cross-reactive antibodies or T cells due to the presence of shared epitopes, either identical or with similar shape and charge, between an infectious agent and a self-antigen is the basis for the phenomenon termed molecular mimicry. Once the autoimmune response has been elicited, the triggering factor is no longer necessary, as the tissue damage results in excessive exposure of self-antigen. Reported homologies between infectious agents and known autoantigens include: P2-C enzyme in Coxsackie virus B and glutamic acid decarboxylase involved in type 1 diabetes mellitus, heat-shock protein 65 in Mycobacterium tuberculosis and the human homologue heat shock protein 60 involved in rheumatoid arthritis, and Yersinia enterocolitica and thyrotropin receptor involved in Graves' disease.

Superantigens

Superantigens are microbial proteins with the ability to cross-bind the outer surface of MHC class II molecules to the less variable Vß chain of the T-cell receptor common to a whole family of T cells, resulting in an activation signal. In this way, a superantigen does not require intracellular processing and is not restricted to a particular MHC class II allele. Unlike a conventional antigen that can stimulate less than 0.01% of naive lymphocytes, a superantigen may be able to polyclonally stimulate 5-30% of circulating T cells, including potentially autoreactive cells, and Th cell-mediated activation of autoreactive B cells may follow. Examples of superantigens are Staphylococcus aureus-derived enterotoxins, and involvement of superantigens in the pathogenesis of rheumatoid arthritis and type 1 diabetes mellitus has been proposed.

Major histocompatibility complex

Major histocompatibility complex molecules, in humans also called human leukocyte antigen (HLA), are present on all nucleated cells, where they present peptides derived from degraded exogenous and endogenous proteins to T cells (Fig. 1). They play an important role in initiating the adaptive immune response, but also in positive and negative selection of T lymphocytes in the thymus and secondary lymphoid organs. There are two major groups of peptide-presenting molecules, designated MHC classes I and II (HLA classes I and II). These are encoded in the MHC, a cluster of genes located on the short arm of chromosome 6. Apart from the MHC class I and II genes, this
cluster of genes also encodes several other proteins involved in immunity, for example heat shock proteins, lymphotoxins, complement factors, transporter associated with antigen processing (TAP) 1 and 2, and cytokines such as TNF-α. There are several significant genetic associations between genes within the MHC and autoimmune diseases, and these will be commented on further on in this thesis.

Figure 1. MHC and antigen processing. To the left, principal pathways of generating peptides for loading onto MHC class I molecules are shown. Endogenous proteins are degraded in proteasomes and transported through the TAP molecule to the luminal surface of the endoplasmic reticulum where they are loaded onto MHC class I molecules. The MHC-peptide complex is then exported through the Golgi apparatus to the surface of the cell. To the right, the processing of extracellular antigens is shown. Proteins are translocated into the cell by endocytosis and harboured in early endosomes. Class II molecules are assembled on the luminal surface of the endoplasmic reticulum together with the invariant chain, a molecule preventing peptides from being prematurely loaded. The class II molecules in complex with the invariant chain are delivered by way of the Golgi apparatus into primary lysosomes, which fuse with early endosomes to form the MHC class II compartment, where the invariant chain is released and a peptide is loaded into the class II groove. The MHC-peptide complex is then transported to the cell surface. (Adopted from Klein and Sato. NEJM, 2000; 343(10):702-9)
Organ-specific autoimmune diseases

MHC class I

MHC class I is present on all nucleated cells in the body and is mainly involved in the presentation of peptides degraded from intracellularly derived viral or self-proteins.\textsuperscript{188, 213} The class I molecule is a heterodimer consisting of an $\alpha$-chain with five domains - two peptide-binding domains, one immunoglobulin-like domain, a transmembrane region and a cytoplasmic tail, and a smaller $\beta$-chain, $\beta_2$-microglobulin encoded by a gene on chromosome 15.\textsuperscript{185} Three polymorphic genes, HLA A, B and C, encode the $\alpha$-chain, rendering an individual person six different class I molecules if heterozygous. The MHC class I subunits are synthesised on polyribosomes, translocated through the endoplasmic reticulum, where they are assembled with $\beta_2$-microglobulin and loaded with a peptide. The loaded complex is then transported to the cell surface, where it is exposed to CD8+ T cells which upon positive matching become activated and differentiate into cytotoxic cells (Fig. 1).

MHC class II

In contrast to class I, class II molecules are only expressed on a subgroup of immune cells such as B cells, activated T cells, macrophages, thymic epithelial cells and dendritic cells. However, when an immune response is elicited and IFN$\gamma$ is secreted, an up-regulation and \textit{de novo} synthesis of class II molecules in other cells can occur, enhancing the immune response. Peptides presented in the class II groove are mainly derived from extracellular proteins.\textsuperscript{250} The class II molecule is also a heterodimer, but with two equally sized $\alpha$- and $\beta$-chains, both of which are encoded in the MHC. There are three genes encoding sets of class II $\alpha$ and $\beta$ chains: HLA-DR, DQ and DP. While there are single pairs of DQ and DP genes per haplotype, there are usually two genes encoding DR molecules. Class II genes also display a high degree of allelic polymorphism, with over 100 alleles in a single locus. The commonly used designations for class II loci are: D for class (II), P, Q or R for family and A or B for chain. Individual genes are noted in Arabic numbers followed by the allelic variant as a number preceded by an asterisk, e.g. HLA-DRB1*0401. In the same way as for class I, the class II subunits are synthesised separately on the cytosolic surface of the endoplasmic reticulum and then translocated across and assembled on the luminal surface together with the invariant chain, a molecule preventing peptides from being prematurely loaded in the endoplasmic reticulum.\textsuperscript{56} This complex is then transported to the cytoplasm enclosed in a membranous vesicle and fused with endosomes harbouring degraded extracellular proteins. The invariant chain is released and peptide is loaded into the class II groove. The complex is then transported to the cell surface, where it is exposed to CD4+ T cells, which, if activated, will differentiate into either Th1 or Th2 cells, depending on the presence of additional co-stimulatory factors (Fig. 1).
The peptide-harbouring groove

The nature of the peptide-harbouring groove is crucial in determining which peptide binds. In the class I molecule the ends of the groove are closed, limiting the peptide length to 8-10 residues, whereas in class II the groove is open, allowing longer peptides to bind. In both MHC class I and II molecules the groove displays cavities or pockets, of which two to three are particularly influential regarding what peptide binds. The amino acid residues lining these pockets anchor the peptide, and they are defined by the allele encoding each class I α- and II α- and β-chain (Fig. 2).

**Figure 2.** Interactions between HLA molecules and peptides. Panel A shows examples of peptides found in complex with the listed HLA class I molecules. The peptides that bind a specific HLA molecule may differ in their sequences, but share two or three amino acid residues that fit into the anchoring pockets, referred to as anchor residues (bold).

In panel B a longitudinal section through the peptide-binding groove of an HLA class I molecule is shown and also a peptide with the side-chains of amino acid residues 1 to 9 oriented either down into the pockets or upwards. Arrows indicate anchoring pockets.

The interaction of peptides with class II molecules is governed by similar principles.

(Adopted from Klein and Sato. N EJM, 2000; 343(10):702-9)
Organ-specific autoimmune diseases

The change of a single amino acid in these positions may totally alter the affinity properties and the selection of binding peptides. An example is aspartic acid in position 57 in HLA-DQB which is associated with protection against diabetes, while an uncharged residue in this position, such as alanine or serine, predisposes to the disease. This is probably the basis for the association of certain alleles with specific diseases as in pemphigus vulgaris in Ashkenazi Jews, where the disease-associated allele, HLA-DRB1*0402, preferentially binds peptides derived from the targeted autoantigen. Furthermore, protective HLA alleles have been identified that bind certain microbial-derived peptides efficiently, eliciting a greater immune response. This has been demonstrated in malaria-infected individuals, where certain class I and class II alleles are associated with resistance to severe malaria.

HLA alleles associated with different autoimmune diseases show a varying but often low strength of penetration, indicating that other genes are also involved. The strongest association is that seen for HLA-B27 and ankylosing spondylitis, where 90% of the patients are positive, compared to 10% of healthy individuals. Further evidence for a disease-causing role is that HLA-B27-transgenic animals spontaneously develop a similar disease.

Apoptosis
Programmed cell death, apoptosis, is a suicide machinery that is under genetic control. In contrast to necrosis, where disruption of the cell membrane occurs with spillage of cell contents, inducing an inflammatory response, in apoptosis the cell membrane is kept intact and no antigen presentation or inflammatory response occurs. Under normal conditions this process is involved in the death of useless or unwanted cells. In the immune system, apoptosis is involved in both the innate and adaptive immune responses. Among other things, it plays an important role in deletional tolerogenesis of B and T cells, in T cell-mediated cytotoxicity and in natural killer cell-mediated killing. Upon viral infection cell-autonomous apoptosis is induced to prevent viral spread.

Fas, a cell-surface receptor, and its ligand FasL, are important molecules that transduce cell-death signals. In studies in murine models of lymphoproliferative disease, acceleration of disease was observed due to spontaneous mutations of Fas and FasL. In humans, defective Fas or FasL is seen in association with the autoimmune lymphoproliferative syndrome. Dysregulation of apoptosis, with enhanced or decreased activity, may contribute to the development of autoimmunity and it is proposed that it may play a role in the pathogenesis of systemic autoimmune diseases such as SLE, rheumatoid arthritis and Sjögren's disease, although contradictory reports
Figure 3. T-cell subsets and cytokines. T-cell receptors recognise peptides presented by MHC molecules. Cytotoxic T cells (CTL) are positive for CD8 and recognise processed intracellular antigens of viral or endogenous origin presented by MHC class I. Activated cytotoxic T cells kill the infected cell and also secrete IFNγ, which renders adjacent cells resistant to infection. T-helper cells (Th) are positive for CD4 and recognise processed extracellular antigens presented by MHC class II. There are two major populations of Th cells. Activated Th1 cells secrete IL-2 and IFNγ, which activate macrophages and CTLs. Activated Th2 cells secrete IL-4, 5 and 6, which, together with a T cell receptor-mediated signal, activate B cells to proliferate into antibody-producing plasma cells. Cytokines secreted in the environment partly determine whether a Th1 or Th2 response occurs and similarly, cytokines secreted by Th1 cells inhibit Th2 cells and vice versa. (Adopted from Delves and Riott, NEJM, 2000; 343(2):108-17).

exist. It has also been suggested that disturbed activation-induced apoptosis may be involved in experimental autoimmune encephalomyelitis (EAE) and multiple sclerosis. Furthermore, apoptosis has been shown to be associated with changes in the subcellular localisation of specific intracellular antigens that are targets in systemic autoimmune disease. Defective apoptosis may contribute to excessive accumulation of intracellular material, resulting in altered degradation and expression of specific intracellular target autoantigens.
Organ-specific autoimmune diseases

T cell subsets and cytokines
Cytokines have been found to play a key role in the differentiation of T cells and in maintaining the balance of effector mechanisms in an immune response. Various cells in the immune system and target tissues express cytokines, and the local pattern of expression of these cytokines regulates the immune response, mainly by modulating the activation of distinct cell populations (Fig. 3). In the context of autoimmunity, attention has been focused on the ability of cytokines to directly regulate self-reactive T cells and on their effects on antigen presenting cells.77

Imbalance in cytokine production may result in a polarised Th1 or Th2 response. The cytokines secreted by a Th1 cell facilitates or promotes cell-mediated immunity, including activation of T cell-mediated cytotoxicity, whereas Th2 cells induce antibody production in B cells (Fig. 3).161 A Th1 cytokine profile is associated with organ-specific autoimmune diseases such as multiple sclerosis and type 1 diabetes mellitus, whilst a Th2 cytokine profile is seen in SLE. Studies on IL–12, a critical cytokine for induction of Th1 responses, have shown aggravation of autoimmune disease upon its administration, whereas inhibition of IL–12 has been found to ameliorate disease in animal models of type 1 diabetes mellitus,218 multiple sclerosis132 and autoimmune uveitis.230 Furthermore, IL–4 and TGF-β, cytokines belonging to the Th2 pathway, have been shown to protect against autoimmunity by down-regulating Th1 responses and polarising islet antigen responses toward a Th2 phenotype, when transgenically expressed in pancreatic β-islets of NOD mice.118, 163

The timing and duration of cytokine expression in an inflammatory response, mainly in experimental disease models, has also been proven important in the development of autoimmunity. For example, TNF has been shown to be protective against development of type 1 diabetes mellitus in NOD mice if expressed late or in a prolonged manner,53 whereas an early transgenic expression of TNF can induce type 1 diabetes mellitus.91

In conclusion, cytokines may mediate both protective and activating effects in T cell–mediated autoimmunity, depending on the timing and level of their production and the cell population with which they interact. By taking this into consideration, modulation of cytokine expression opens the way for new therapeutic strategies against autoimmunity. In humans, administration of monoclonal antibodies against TNF-α has already become an effective form of therapy in rheumatoid arthritis.
Autoantibodies in autoimmunity

A direct pathogenetic role of autoantibodies has been proven in a number of diseases and the main mechanisms proposed are as follows:

- Autoantibodies may be directed against cell-surface or matrix antigens, with consequent activation of complement factors and phagocytes, as in haemolytic anaemia and pemphigus vulgaris.
- Immune complex-mediated destruction (autoantibodies in complex with soluble antigen) through activation of complement factors and phagocytes, as in SLE.
- Autoantibodies may be directed against cell-surface receptors, either blocking or stimulating the function of the receptor in an uncontrolled manner, as in Graves' thyroiditis and myasthenia gravis.

The pathogenetic role of these autoantibodies is further strengthened by the fact that the disease is transferred to the foetus through placental transmission of IgG. In most organ-specific autoimmune diseases, however, the targeted antigen is located intracellularly, and apart from sporadic observations there is no evidence that autoantibodies cross cell surface membranes. The role of these autoantibodies still remains unknown. Might they merely reflect T cell reactivity? What we do know is that the autoantigenic target is usually an enzyme involved in important biosynthetic pathways, e.g. GAD in type 1 diabetes mellitus, 21-hydroxylase in Addison's disease and thyroid peroxidase in autoimmune thyroiditis. Furthermore, autoantibodies tend to be directed against conserved, functionally important sites, as implied by their ability to recognise antigens from a wide variety of species and to inhibit enzyme function in vitro. How these findings should be interpreted is still not clear. Identified disease-associated autoantibodies have, however, proven useful as early diagnostic markers of disease, since they usually present before clinical onset, and in some diseases antibody levels may be used to monitor disease activity, as for example, anti-asialoglycoprotein receptor antibodies in autoimmune hepatitis, anti-dsDNA in SLE nephritis and anti-proteinase-3 antibodies in Wegener's granulomatosis.
Autoimmune polyendocrine syndrome type I, also called autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED; OMIM 240300), is a rare disease featuring multiple organ-specific autoimmune manifestations, characterised by the presence of high titres of circulating organ-specific autoantibodies. APS I is a monogenic, autosomal, recessively inherited disease in which the disease-causing gene has recently been cloned and identified. These features make APS I an interesting model of organ-specific autoimmunity.

Prevalence of APS I
The prevalence of APS I is in general very low, but higher figures have been reported among Finns (1:25,000), Iranian Jews (1:6,500-9,000) and Sardinians (1:14,500), with known small founder populations and in the case of Iranian Jews a high rate of consanguinity as probable explanations.

Today's knowledge about APS I is mainly based on observations in four well-characterised APS I populations of Finnish, North American, Iranian Jewish, and Italian origin (Table 1). A female preponderance is a common feature in many autoimmune disorders and a slight overrepresentation of women is reported in the North American and Italian populations, whereas an equal sex distribution has been found in the Finnish and Iranian Jewish populations. There is wide variation in the age at clinical onset, the number and type of disorders involved and the course of the disease within the different populations, but some major differences between the populations are also seen and will be commented on in the following sections.

Clinical considerations
The clinical manifestations of APS I can be roughly divided into three groups: Endocrine disorders, non-endocrine disorders and ectodermal dystrophies (Table 2).

Among the endocrine disorders, hypoparathyroidism is most commonly present (76-93%), followed by adrenocortical insufficiency (67-73%), with gonadal insufficiency, type 1 diabetes mellitus and thyroid disease present in various degrees (2-50%). Single cases of growth hormone deficiency, ACTH deficiency and diabetes insipidus have also been reported.

Of the non-endocrine disorders, mucocutaneous candidiasis affecting the dermis, nails and oral, vaginal, and oesophageal mucous membranes is by far the most common manifestation, which is present in almost all patients. Other non-endocrine
disorders observed are alopecia, vitiligo, keratopathy, hepatitis, intestinal dysfunction and atrophic gastritis (9-37%). Here again single reports of additional disorders such as asplenia,83, 190 cholelithiasis,83 vasculitis, terminal renal failure and periodic hypercalcaemia are found.194

<table>
<thead>
<tr>
<th>Origin</th>
<th>North American</th>
<th>Iranian Jewish</th>
<th>Italian</th>
<th>Finnish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>106*</td>
<td>23</td>
<td>41</td>
<td>78</td>
</tr>
<tr>
<td>Female/male ratio</td>
<td>1.4</td>
<td>1.1</td>
<td>2.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 1. Clinical characteristics of different APS I populations

<table>
<thead>
<tr>
<th></th>
<th>North American</th>
<th>Iranian Jewish</th>
<th>Italian</th>
<th>Finnish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypoparathyroidism</td>
<td>82</td>
<td>96</td>
<td>93</td>
<td>85</td>
</tr>
<tr>
<td>Adrenal insufficiency</td>
<td>67</td>
<td>22</td>
<td>73</td>
<td>72</td>
</tr>
<tr>
<td>Gonadal insufficiency</td>
<td>12</td>
<td>26</td>
<td>43</td>
<td>39</td>
</tr>
<tr>
<td>Parietal-cell atrophy</td>
<td>15</td>
<td>9</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Thyroid disease</td>
<td>NR</td>
<td>4</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Type 1 diabetes mellitus</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>78</td>
<td>17</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td>Alopecia</td>
<td>26</td>
<td>13</td>
<td>37</td>
<td>27</td>
</tr>
<tr>
<td>Intestinal dysfunction</td>
<td>24</td>
<td>NR</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>11</td>
<td>NR</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td>Keratopathy</td>
<td>NR</td>
<td>NR</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>9</td>
<td>NR</td>
<td>15</td>
<td>13</td>
</tr>
</tbody>
</table>

References

Neufeld 1980
Zlotogora 1992
Betterle 1998
Perheentupa 1999

*Of which 56 derive from a literature search. NR=Not reported

Dental enamel hypoplasia affecting the permanent teeth and without association with hypoparathyroidism is seen in up to 70 % of the Finnish patients, and pitted nail dystrophy unrelated to nail candidiasis in 50 %.2, 4 Another reported ectodermal dystrophy is calcification of the tympanic membrane without a history of middle ear disease, present in approximately 30 % of Finnish patients.4

The accepted criteria for the diagnosis of APS I are the presence of at least two of the three major components, namely hypoparathyroidism, candidiasis and adrenal
insufficiency, or, in a sibling the presence of one of these. APS I usually develops in infancy and most patients are symptomatic by the age of five years, but cases with adult onset are also seen. The majority of the patients develop three to five manifestations, some of which may not appear until the fifth decade. Mucocutaneous candidiasis is generally the first disorder to appear, usually before the age of five years, followed by hypoparathyroidism before the age of 10 years and Addison's disease before the age of 15. It should be emphasised, though, that several other components such as vitiligo, keratopathy and hepatitis have each been the sole manifestation in some patients for many years, and often have not been recognised as part of the APS I syndrome until later. The earlier the first manifestation appears, the more likely it is that multiple disorders will follow, and vice versa. Patients with Addison's disease as their first manifestation apart from candidiasis tend to develop significantly fewer manifestations in total.

APS I patients of Iranian Jewish origin seem to differ from other APS I populations in that in the former patients Addison's disease is reported to have a later onset and to appear to a lesser extent. Candidiasis rarely occurs in this group and when present it exists in a milder form, and no cases of keratopathy have yet been reported.

**T cell-mediated immunity in APS I**

Chronic candidiasis, one of the major components of APS I, is regarded as a hallmark of defective T-cell function. There are reports of various types of T cell dysfunction, e.g. cutaneous anergy or impaired delayed type hypersensitivity reaction to *Candida* antigens, and altered suppressor T cell activity, although none of them seem conclusive. High levels of protective antibodies against major *Candida* antigens and normal vaccination responses, on the other hand, indicate an intact T helper cell function and antibody formation. When looking at APS I patients as a group, however, variable T cell abnormalities are found in significantly higher degrees than in healthy controls, suggestive of a limited T-cell disorder.

**Humoral immunity in APS I**

Most studies of autoimmunity in APS I patients have focused on humoral aspects, i.e. the occurrence of autoantibodies and identification of the corresponding antigen. All autoantigens identified to date are summarised in Table 2. Autoantigenic targets can be divided into two major groups, namely enzymes involved in steroid synthesis in the adrenal cortex and gonads and those involved in the synthesis of neurotransmitters. All enzymes in the former group are members of the cytochrome P450 family, whereas in the second group both pyridoxal phosphate-dependent decarboxylases and pteridine-dependent hydroxylases have been identified. In a recent study, two
transcription factors were identified for the first time as autoantigens in APS I, namely SOX9 and SOX10, and an association with vitiligo was shown.99

Table 2. Clinical manifestations in APS I and their related autoantigens

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>Related autoantigen(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adrenal insufficiency</td>
<td>21-OH, SCC, 17-OH</td>
<td>124, 256-257</td>
</tr>
<tr>
<td>Gonadal insufficiency</td>
<td>SCC</td>
<td>255</td>
</tr>
<tr>
<td>Parietal cell atrophy</td>
<td>H⁺/K⁺ ATPase</td>
<td>117</td>
</tr>
<tr>
<td>Thyroid disease</td>
<td>TPO, thyroglobulin</td>
<td>28</td>
</tr>
<tr>
<td>Type 1 diabetes mellitus</td>
<td>GAD65, ICA, insulin, IA-2</td>
<td>2, 95, 243</td>
</tr>
<tr>
<td>Non-endocrine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Candidiasis</td>
<td>-</td>
<td>98</td>
</tr>
<tr>
<td>Alopecia</td>
<td>TH</td>
<td></td>
</tr>
<tr>
<td>Intestinal dysfunction</td>
<td>TPH, GAD65</td>
<td>73, 228</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>CYP1A2, AADC, CYP2A6</td>
<td>Papers I-II, 49</td>
</tr>
<tr>
<td>Keratopathy</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Vitiligo</td>
<td>SOX9, SOX10, AADC</td>
<td>99</td>
</tr>
<tr>
<td>Ectodermal dystrophies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enamel hypoplasia</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tympanic membrane calcification</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Nail dystrophy</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: 21-OH, 21-hydroxylase; SCC, side chain cleavage enzyme; 17-OH, 17-hydroxylase; TPO, thyroid peroxidase; GAD65, glutamic acid decarboxylase; CYP, cytochrome P450; AADC, aromatic L-amino acid decarboxylase; SOX, SRY box.

All autoantigens identified so far are intracellularly located. There is no evidence that the APS I-associated autoantibodies have a primary disease-causing role in vivo and there are only a few reports to suggest that autoantibodies are able to cross the cell membrane.206 Interesting information on the normal physiology and possible disease-associated mechanisms has been obtained, as the target antigens have all been shown to be tissue-specific key enzymes that are involved in important intracellular biosynthetic pathways.
Genetics in APS I

APS I is inherited in an autosomal recessive mode. No clear association with HLA haplotypes has been found. In 1994 the disease-associated locus was mapped to chromosome 21q22.3, based on linkage analysis of Finnish families, and in 1997 two individual groups identified the responsible gene named AIRE, which stands for autoimmune regulator. AIRE is one of the first well-characterised genes outside the HLA locus that is known to be involved in the regulation of autoimmunity.

The gene encodes a 545 amino acid long, proline-rich protein with a molecular weight of 58 kD and a subcellular localisation in mammalian cells, mainly in distinct speckles of the nucleus, although cytoplasmic expression has also been seen to a lesser extent. Studies of the protein structure have shown features observed in proteins involved in regulation of transcription, such as two PHD-type zinc fingers, a DNA binding motif SAND, an HSR domain involved in protein dimerisation, and four nuclear receptor-binding motifs.

In humans, limited expression of AIRE has been found in immunologically active tissues such as the thymic medulla, lymph nodes, foetal liver and spleen and in restricted cell populations such as epithelial cells in the thymus and peripheral blood lymphocytes. In the mouse, however, the homologue Aire is expressed in additional tissues, e.g. the brain, liver, kidney, pancreas, intestine, gonads, pituitary, thyroid and adrenal gland.

The overall function of AIRE protein is not yet understood, but recent experimental studies of transiently expressed AIRE have shown stimulation of transcription activity in vitro and concerning the murine Aire protein, involvement in normal thymic architecture and negative selection of thymocytes. These results indicate that AIRE protein has a role in the generation of tolerance, an interesting observation with regard to the observed multi-system autoimmune manifestations of APS I.

To date, 29 different disease-causing mutations have been identified in the different ethnic groups, but it is not yet possible to draw firm conclusions regarding genotype-phenotype correlations. Mutations have been found to cluster in four hotspots, all of which are located within the regions encoding the regulatory domains mentioned above, and they result in truncated proteins lacking one or more of these domains. Experimental studies with mutated AIRE proteins have shown reduced or eliminated transcription activity and an altered subcellular localisation, to the cytoplasm.

In the different populations of APS I patients, one predominant haplotype is associated with the disease, indicating a founder effect. Among the Finnish APS I patients 89%
have the major Finnish mutation, R257X, and of the Sardinian patients 92% have the common Sardinian mutation, R139X, both resulting in a truncated protein. All Iranian Jewish APS I patients share the same disease-causing point mutation, Y85C, resulting in a single amino acid change and a protein with an unaltered transcriptional activity and subcellular location. This might at least partially explain the milder phenotype presented by these patients compared to that of other APS I patients. Most APS I patients are homozygous, but in 5-7% only one affected allele can be detected and about the same proportion of patients lack any of the identified mutations. A possible explanation for this is that these patients have mutations, not yet identified, located in the promoter region or introns of the AIRE gene. None of the known mutations are found in healthy controls, but some apparently unaffected relatives have been found to be heterozygous.

There are no other known diseases associated with AIRE dysfunction. But it has been proposed that AIRE might play a role in the occurrence of autoimmunity seen in patients with Down's syndrome. The critical region of this disease includes the location of AIRE, and a gene dosage effect is suggested. However, no conclusive evidence has been produced.

LIVER DISEASE IN APS I

Reports of the occurrence of liver disease in patients with APS I date back to the mid-twentieth century. The association was initially based mainly on autopsy findings and isolated case reports. Histological findings consisted mainly of perportal fibrosis and cirrhosis, and were generally interpreted as post-infectious. Before APS I was recognised as an autoimmune disease, these findings led to the suggestion that APS I might be a sequel of subclinical viral hepatitis. Since then, however, retrospective studies of large APS I populations of different geo-ethnic origin have added to our knowledge and today the general view is that most of the disease manifestations have an autoimmune origin. The liver disease associated with APS I is poorly described in the literature. It has been characterised as chronic active hepatitis (CAH) on the basis of biopsy findings, but the term autoimmune hepatitis (AIH) is also often used. Although it has been suspected to have an autoimmune origin, no conclusive evidence of this had been produced and no disease-specific autoantibodies had been identified at the commencement of this study. A few published case reports, which will be summarised below, have given a somewhat more detailed description of the liver disease in APS I patients, supporting an autoimmune aetiology. In the following the term AIH will be used when referring to APS I-associated liver disease.
Organ-specific autoimmune diseases

Prevalence and genetics
According to the reports, AIH is present in 10-20% of APS I patients. This may be an underestimation, since in a study of autopsy findings in APS I patients, significant pathological changes in the liver were present in eight of nine cases. There is no information on the male/female distribution or possible HLA associations.

Clinical features
AIH is the most feared disease manifestation in APS I. It has an early onset, the first signs of disease usually presenting before the age of ten years (range 0-20), and a high mortality. The clinical picture at diagnosis ranges from asymptomatic to that of a fulminant hepatitis, with lethargy, jaundice and gross ascites. The clinical course also varies. In some patients there is recurrent elevation of liver enzymes with concomitant jaundice, but with spontaneous recovery, whereas in other cases deranged liver enzymes progress to an intractable fulminant hepatitis within a few days to weeks, with a lethal outcome. There are also reports of a more slow progress, with cirrhosis developing over a 6- to 10-year period.

Serum biochemistry and histological features
A 3- to 10-fold increase in serum aminotransferase activity is observed in most patients. No immunoreactivity against known AIH-associated markers (Tables 3 and 4) is seen and the Ig levels are reported to be normal. Viral serology has been negative when performed. In one case immunohistochemical staining revealed a reactivity in the patient serum against hepatic epithelial cells that is not further described. The morphological findings are unanimous and consistent with AIH. The most common feature is piecemeal necrosis with lymphoplasmocytic infiltration, but no further characterisation or sub-typing of involved cells has been performed. Panlobular necrosis and end-stage cirrhosis are also reported.

Therapeutic considerations
When treatment has been applied, a combination of immunosuppressive and corticosteroid therapy has proven useful. Usually, if the treatment is started early, normalisation of both the clinical and biochemical derangement is seen, as well as a significant histological improvement. In the more aggressive and therapy-resistant form of AIH, liver transplantation has been carried out with varying results.

Prognosis
No comprehensive information on the prognosis is available, but a high mortality is anticipated. In a total of 29 APS I patients with AIH, reported in the literature, seven died at a young age of fulminant therapy-resistant hepatitis. Often there were no preceding clinical symptoms or subclinical biochemical alterations.
remaining cases remission was either spontaneous or therapy-induced, but with relapses usually occurring within a few years.

The picture evolving is that of a severe form of early-onset CAH carrying significant mortality and with several features pointing to an autoimmune pathogenesis. Apart from periodic screening for transaminase elevation, an early marker for liver disease would be useful in identifying early cases of AIH or those APS I patients at risk of developing hepatitis. To investigate this further we have chosen to look for putative liver-specific antigenic targets in APS I patients.

IDIOPATHIC AUTOIMMUNE HEPATITIS

As in most other organ-specific autoimmune disorders, the aetiology of AIH is unknown. It is a rare disease with an estimated prevalence in Northern Europe of 170 cases per 1 million population. The Swedish physician Jan Waldenström was the first, in 1950, to recognise and describe patients suffering from what was later to be designated autoimmune hepatitis (AIH). He reported on a persistent hepatitis with female preponderance (female: male ratio 4:1), hypergammaglobulinaemia and an aggressive clinical course carrying a high mortality. This early description still holds true, but additional features have been discerned implying an autoimmune pathogenesis with a polygenic influence. The reported features, such as an increased incidence of additional autoimmune disorders both in patients and relatives, an HLA association, circulating liver-specific autoantibodies, and a good response to immunosuppressive treatment, are well in line with the indirect criteria of an autoimmune disease proposed by Rose and Mackay. Morphological characteristics are progressive destruction of hepatic parenchyma with an intense immunological activity mainly in the periportal area, and not seldom progressing to panlobular and multilobular necrosis and active cirrhosis. This picture is not specific for AIH, but may also be seen in hepatitis of viral origin and in other liver disorders such as primary biliary cirrhosis and Wilson's disease. Although AIH is seen in children and young adults, the majority of the patients are above the age of 50 years at onset.

AIH carries a high mortality, with a five-year survival rate of 50% and a 10-year survival rate of 10% if untreated. Immunosuppressive treatment with prednisone alone or in combination with azathioprine is the treatment of choice, resulting in 5- and 10-year life expectancies similar to those in age- and sex-matched healthy individuals. Unfortunately, relapses are common and sustained remission is only seen in about 30% of the patients. In end-stage cirrhosis, liver transplantation is the ultimate option and
Organ-specific autoimmune diseases

AIH is among the best indications for this, with a 5–year survival rate of over 90%, although there are recent reports of recurrence of AIH to various degrees.

There has been some controversy as to whether AIH is a disease entity or should be included in the group of conditions known as chronic active hepatitis (CAH) as idiopathic or cryptogenic CAH. In 1993 a general consensus was reached by an international panel concerning diagnostic criteria and a scoring system for diagnosis of AIH (Table 3). No particular signs, symptoms or liver test abnormalities are sufficiently specific to be considered diagnostic on their own. Based on the number of fulfilled criteria the diagnosis of AIH is regarded as definite or probable.

It is generally considered that AIH is the result of multiple factors. An immunogenetic background rendering the patient susceptible to autoreactivity, in combination with one or several triggering factors, is proposed and will be discussed further below.

Susceptibility

A family history of other autoimmune disorders such as thyroid disease (Hashimoto's thyroiditis and Grave's disease) and rheumatoid disease is often present. A strong correlation to the HLA A1-B8-DR3 haplotype and specifically the DR3 and DR4 allotypes is frequently seen. These markers are also common in other autoimmune disorders and appear to be associated with a heightened immune responsiveness. This may account for the observed hypergammaglobulinaemia in AIH patients. HLA DR3 and DR4 are seldom inherited in haplotype, and a difference in the clinical picture, with a younger onset of disease in DR3-positive than in DR4-positive patients, is seen. This is consistent with the higher age at onset in Japanese AIH patients; further, DR3 rarely exists in the population as a whole. Impaired suppressor T cell activity coupled with the earlier mentioned HLA A1-B8-DR3 haplotype is another suggested model of susceptibility. Reduced levels of CD8+ T lymphocytes with a proposed suppressor function is seen in children and young adults with AIH, and CD4+ cells capable of inducing autologous B lymphocytes against liver-specific antigenic targets have been reported. Another immunogenetic aberration observed is an isolated partial deficiency of the C4 complement factor, which is known to play a role in virus neutralisation, a feature also seen in a number of other autoimmune diseases.

Triggering factors

A proposed mechanism for induction of the autoimmune response is the occurrence of changes in hormonal regulation, as many of the patients are peri- or post-menopausal women. No evidence supporting this idea, however, has yet been produced.
Table 3. Scoring system for diagnosis of autoimmune hepatitis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Score</th>
<th>Parameter</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>+2</td>
<td>Positive</td>
<td>-2</td>
</tr>
<tr>
<td>Male</td>
<td>0</td>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td><strong>Serum biochemistry</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of elevation of serum alkaline phosphatase vs aminotransferase</td>
<td>-3</td>
<td>Anti-mitochondrial antibody</td>
<td>-2</td>
</tr>
<tr>
<td>&gt;3.0</td>
<td>-2</td>
<td>IgM anti-HAV, HBsAg or HBe positive</td>
<td>-3</td>
</tr>
<tr>
<td>&lt;3.0</td>
<td>+2</td>
<td>HCV RNA positive by PCR</td>
<td>-3</td>
</tr>
<tr>
<td><strong>Total serum globulin, γ-globulin or IgG</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Times upper normal limit</td>
<td>+3</td>
<td>Other aetiological factors</td>
<td></td>
</tr>
<tr>
<td>&gt;2.0</td>
<td>+3</td>
<td>Recent hepatotoxic drug usage or</td>
<td></td>
</tr>
<tr>
<td>1.5-2.0</td>
<td>+2</td>
<td>parenteral exposure to blood products</td>
<td></td>
</tr>
<tr>
<td>1.0-1.5</td>
<td>+1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.0</td>
<td>0</td>
<td>Yes</td>
<td>-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>+1</td>
</tr>
<tr>
<td><strong>Autoantibodies (titres by immunofluorescence)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA, SMA or LKM-1</td>
<td>+3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1:80</td>
<td>+3</td>
<td>Male 35-50; female 25-40</td>
<td>0</td>
</tr>
<tr>
<td>1:80</td>
<td>+3</td>
<td>Male 50-80; female 40-60</td>
<td>-1</td>
</tr>
<tr>
<td>1:40</td>
<td>+2</td>
<td>Male&gt;80; female &gt;60</td>
<td>-2</td>
</tr>
<tr>
<td>&lt;1:40</td>
<td>+1</td>
<td>HLA DR3 or DR4</td>
<td>+1</td>
</tr>
<tr>
<td>Children</td>
<td>0</td>
<td>Other autoimmune diseases in patient or first degree relative</td>
<td>+1</td>
</tr>
<tr>
<td>ANA or LKM-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1:20</td>
<td>+3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:10 or 1:20</td>
<td>+3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>or SMA</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;1:20</td>
<td>+3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:20</td>
<td>+2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1:20</td>
<td>+2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Interpretation of aggregate scores:** Definite AIH, >15 before treatment and >17 after treatment; probable AIH, 10-15 before treatment and 12-17 after treatment.


According to Johnson & McFarlane, 1993.
Infection is commonly proposed as a triggering factor of autoimmunity and there are several well-documented cases of AIH developing in susceptible individuals after infection with hepatotropic viruses.\(^\text{106, 244}\) One possible mechanism is molecular mimicry, and cross-reactivity with shared epitopes between known liver-specific autoantigenic target proteins and HBV and HCV polyproteins has been described.\(^\text{115, 150}\) Drugs or other chemicals, e.g. dihydralazine, tienilic acid and disulfiram, are capable of inducing AIH.\(^\text{133}\) The proposed mechanism of action is a covalent binding of a reactive metabolite of the drug to the metabolising enzyme, thus generating an immunogenic "neoantigen".\(^\text{92}\) In favour of this theory, autoreactivity against the metabolising enzyme, namely cytochrome P450 (CYP) isoenzyme CYP1A2 in the case of disulfiram and dihydralazine\(^\text{37, 75}\) and CYP2C9 for tienilic acid,\(^\text{25}\) has been found. This form of AIH usually resolves following withdrawal of the drug, but may persist in susceptible individuals.

**Mechanisms of autoimmune liver damage**

The pathway generally considered to lead to liver damage in AIH is that orchestrated by CD4-positive T cells, predominantly Th2 cells, with subsequent antibody-dependent cellular cytotoxic (ADCC) reactions against hepatocytes (Fig. 2). In favour of this model are the findings of aberrant HLA class II expression on hepatocytes,\(^\text{134}\) surface expression of known antigenic targets in hepatocytes, antibody-covered hepatocytes in vivo\(^\text{245}\) and the fact that some disease-correlated liver-specific autoantibody titres correlate with disease activity.\(^\text{110, 153}\) Furthermore, cloning and sub-typing of T cells from peripheral blood and liver biopsies revealed a majority of CD4+ cells with MHC class II-restricted reactivity against known liver-specific autoantigens such as liver-specific membrane lipoprotein\(^\text{232}\), asialoglycoprotein receptor,\(^\text{136}\) and liver kidney microsomal type 1 antigen (LKM 1).\(^\text{138}\) These cells have also been shown to stimulate autoantibody production in autologous B cells. To date there is no evidence of involvement of direct T-cell cytotoxicity in AIH. Another observation favouring the importance of ADCC reactions in AIH is the striking response to prednisolone and azathioprine. These drugs exert their action by suppressing immunoglobulin production\(^\text{12, 36}\) and reducing the number of natural killer cells,\(^\text{198, 220}\) which are the two main components of ADCC mechanisms.

**Autoantibodies in AIH**

An abundance of autoantibodies have been identified in sera from patients with AIH (Table 4). They have mainly been characterised by their subcellular localisation in immunohistochemical staining and named thereafter, but lately some of the target autoantigens have also been identified. Immunoreactivity against nuclear, cytoskeletal, cytoplasmic and hepatocellular membrane antigens has been demonstrated.\(^\text{155, 160}\) There is no single autoantibody, however, that is sufficiently specific or diagnostic for AIH, as is
the case with the anti-mitochondrial antibody, directed against the E2 subunit of pyruvate dehydrogenase and present in almost 90% of patients with primary biliary cirrhosis.  

Autoantibody screening in AIH patients is mainly used as one of the diagnostic tools mentioned earlier. There is, as yet, no direct evidence of a direct pathogenetic role of these autoantibodies. One of the identified target proteins is cytochrome P4502D6 (CYP2D6), the major target of LKM-1 autoantibodies, which are serological markers of AIH type 2. Anti-CYP2D6 autoantibodies are present in 95-100% of patients with type 2 AIH. CYP2D6 is involved in detoxification of several drugs and is located in the endoplasmic reticulum. There are reports, though contradictory, of expression of several CYP450 isoenzymes on the surface of hepatocytes, indicating that autoantibodies may participate in the destructive process. Recently, the targets for soluble liver antigen (SLA) antibodies and liver-pancreas antigen (LP) antibodies were found to be identical and identified as a 50 kDa protein, with an as yet unknown function. These antibodies are specific for AIH and they are present in approximately 30% of the patients.

### Table 4. Autoantibodies and identified autoantigens in AIH

<table>
<thead>
<tr>
<th>Subcellular localisation</th>
<th>Antibody nomenclature</th>
<th>Identified autoantigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nuclear</td>
<td>ANA</td>
<td>ss-DNA, ds-DNA, sn-RNP, t-RNA, lamin A and C, cyclin A</td>
</tr>
<tr>
<td>Cytoskeletal</td>
<td>SMA</td>
<td>F-actin</td>
</tr>
<tr>
<td>Cytoplasmic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsomal</td>
<td>LKM1, LKM2, LKM3, LM</td>
<td>CYP2D6, CYP2C9, UGT-1, CYP1A2</td>
</tr>
<tr>
<td>Soluble</td>
<td>SLA/LP, LC1, LC2</td>
<td>50 kDa protein*, formiminotransferase cytochrome oxidase, -</td>
</tr>
<tr>
<td>Hepatocellular membrane</td>
<td>LSP</td>
<td>Asialoglycoprotein receptor</td>
</tr>
</tbody>
</table>

**Abbreviations:** ANA, anti-nuclear antibody; SMA, smooth muscle antibody; LKM, liver-kidney microsomal antibody; LM, liver microsomal antibody; SLA, soluble liver antigen antibody; LP, liver-pancreas antigen antibody; LC, liver cytosolic protein antibody; LSP, liver-specific membrane lipoprotein antibody; ss, single stranded; ds, double stranded; UGT, UDP-glucuronosyltransferases. *Recently identified antigen with unknown function, Wies et al, Lancet, 2000.
ADDISON'S DISEASE

Primary adrenal failure, Addison's disease, is nowadays mainly caused by organ-specific autoimmune destruction of the adrenal cortex. Other less common causes of the disease include tuberculosis, HIV, sarcoidosis, amyloidosis, adrenoleucodystrophy, haemorrhage and a rare form of hereditary congenital hypoplasia due to DAX-1 mutations.

The estimated prevalence of Addison's disease is about 1 in 25,000 individuals and there is a marked female preponderance. The age at clinical onset is most commonly between 20 and 40 years. In about 50 % of the patients other autoimmune diseases such as vitiligo, thyroid disease, gonadal insufficiency, type 1 diabetes mellitus, hypoparathyroidism and pernicious anaemia are present, usually as part of one of the two autoimmune polyendocrine syndromes APS I and II. The autoimmune destruction, with the major target autoantigen identified as the steroid synthesising enzyme 21-hydroxylase, is a slow process with overt clinical symptoms that appear when about 90 % of the adrenal cortex has been irreversibly destroyed. This destruction results in inadequate secretion of glucocorticoids, mineralocorticoids, dehydroepiandrosterone (DHEA) and DHEA-derived androgens (Fig. 4). The aetiology of the autoimmune process is still unknown, and as the adrenal cortex is irreversibly destroyed at the time of diagnosis, there is no curative treatment. Patients with Addison's disease therefore have to rely on life-long replacement of adrenal hormones.

Clinical findings
The clinical manifestation of Addison's disease is often insidious, which is well in line with the gradual destructive process and concomitant hormonal insufficiency. Owing to the unspecific nature of the symptoms, adrenal insufficiency may go undetected for some time until an acute illness or stress provokes an adrenal crisis.

Common signs at presentation are generalised weakness, fatigue, weight loss due to anorexia, nausea and disturbed intestinal function, with both diarrhoea and constipation. Postural dizziness and salt craving are other common symptoms, which are attributed to the mineralocorticoid deficiency. Another typical finding in these patients is hyperpigmentation of the skin, especially on sun-exposed areas and areas often subjected to chronic mild trauma such as the knees, elbows and knuckles, scars and the buccal mucosa. The explanation for this is the lack of cortisol-induced negative feedback to the corticotropes of the pituitary, resulting in increased secretion of compounds (ACTH and proprionelanocortin-related peptides) stimulating pigment synthesis in the melanocytes. Effects of androgen deficiency in women are decreased or absent pubic and axillary hair, and some patients suffer decreased libido.
Psychiatric symptoms are frequently described, especially in patients with long-standing adrenal insufficiency. The main findings are cognitive impairment with memory disturbances, which occurs in 5-20% of the patients, and depression, which has been reported in 20-40%.

Treatment and prognosis
Before treatment with adrenocortical extracts was introduced by Swingle and Pfiffner in 1931, more than 80% of the patients died within 2 years after diagnosis. The addition of salt solutions improved the treatment slightly, as did deoxycorticosterone, which was synthesised in 1937, but it was the introduction of synthetic cortisone in 1949 that finally made successful treatment possible. With a combined glucocorticoid and mineralocorticoid replacement regime, patients with Addison's disease have an unaltered lifespan.
Patients receiving standard replacement therapy still experience reduced general well-being, however, and complain of mood disturbances, weakness, reduced physical activity, and cognitive dysfunction. Since the 1960s, treatment of adrenal insufficiency has changed little. At the start of this study, most textbooks recommended administration of hydrocortisone twice daily. During the last few years, however, reports in favour of a 3- to 4-dose regime mimicking the normal cortisol secretion curve have been published; this treatment has produced improvement, though not complete, of the above-mentioned symptoms. Bearing in mind the unsatisfactory symptom alleviation in patients with Addison's disease, it is interesting to note that no measures have been taken to correct the adrenal androgen insufficiency. This question was raised in an early report in 1943. A young girl with Addison's disease was given testosterone in addition to desoxycorticosterone acetate and was found to "benefit from the testosterone, as evidenced by gain in weight, growth in stature, and an apparent increase in strength and endurance".

**DEHYDROEPIANDROSTERONE**

Dehydroepiandrosterone and its sulphate ester DHEA-S are steroid hormones that are synthesised in the adrenal cortex in response to ACTH. Although identified in the 1930s, their physiological role and biological actions are not well known, apart from being important precursors of peripheral androgen and oestrogen biosynthesis. Lately, interest has focused on indications that DHEA and DHEA-S (DHEA(S)) possess anti-ageing properties and may be possible markers for physiological ageing. This has resulted in a large number of studies in both animals and humans concerning serum concentrations of DHEA(S) in the presence of different disease states and in association with different metabolic and physiological conditions, some of which will be summarised below.

**Metabolism and physiology**

DHEA is synthesised from pregnenolone, a reaction catalysed by 17 $\alpha$-hydroxylase. A hydrosteroid sulphatase, sulfotransferase, converts DHEA to DHEA-S, a reaction that can be reversed and that may take place both in the adrenals and in peripheral tissues. The adrenal cortex is the primary source of DHEA and accounts for 100% of the production in women and 90-95% in men, with the remaining 5-10% deriving from the testes. DHEA serves as a precursor for both androgens and oestrogens (Fig. 4). DHEA(S) serve as precursors to almost 100% of the androgen production in women and account for ~75% and 100% of active oestrogen synthesis in pre- and post-menopausal women, respectively. DHEA in itself has a predominance of androgenic over...
estrogenic activity, although the possibility that it exerts both oestrogen- and androgen-like effects depending on the hormonal milieu has been proposed.71, 126

DHEA-S circulates in concentrations 250-500 times higher than those of DHEA, partly due to differences in metabolic clearance rates.139 Both hormones are secreted in response to ACTH, with DHEA exhibiting a diurnal variation.217 DHEA concentrations show an age and sex dependence, one of the reasons for the speculations on possible anti-ageing activity. The concentration is high in the foetus and at birth, but declines from the first month and then increases at the beginning of adrenarche to peak between 20 and 30 years of age. A decline follows, and at the age of 70-80 years approximately 20% of the peak values are observed.123 An age-dependent decline in the response to ACTH is also seen.191

DHEA is designated a neurosteroid, as de novo synthesis takes place in the central nervous system.54, 210 It has been found to act both as a GABA-A antagonist and as an N-methyl-D-aspartate receptor agonist,59 resulting in an enhanced excitatory effect in affected neurones, suggesting that DHEA(S) has a potential to exert clinically relevant effects in the central nervous system.

**DHEA in health and disease**

To date there is no disease that is known to be caused by an isolated DHEA(S) dysfunction. The age-related fall in the circulating levels of DHEA(S), which has not been observed for any other steroid, has been implicated in some of the degenerative processes and in effects of ageing such as osteoporosis,64, 82 increased cardiovascular mortality6, 19 and decreased glucose tolerance.22, 170 Administration of DHEA to elderly persons, re-establishing steroid values to levels observed in young adults, has been found to be associated with a subjectively improved sense of psychological and physical well-being,160, 259 decreased insulin resistance,22, 65 an enhanced lean body mass65, 159 and improved bone mineral density.129

Recent studies in adults and adolescents with major depression have revealed a blunted circadian variation of DHEA concentrations and a decreased DHEA/cortisol ratio compared to those in healthy controls.90, 184 Upon treatment with DHEA, a significant improvement in symptoms was noted in relation to increased levels of DHEA(S) and increased DHEA/cortisol.260, 261 Furthermore, reduced levels of DHEA(S) were significantly associated with a depressed mood in post-menopausal women.20

Data from animal studies indicate that DHEA enhances immune function.186 The findings in humans are less impressive, but DHEA replacement in elderly subjects has been shown to skew the immune response from a humoral to a cytotoxic one.24 It has
Organ-specific autoimmune diseases

been proposed that the low DHEA(S) levels seen in SLE patients\textsuperscript{226} may play a role in
the pathogenesis of SLE. DHEA treatment have proven beneficial\textsuperscript{21, 242} A decrease in
disease activity and prednisone requirement, and an increase in IL-2 production, have
been noted, especially in women.\textsuperscript{223}
CURRENT INVESTIGATION

RESULTS

Identification of cytochrome P4501A2 and aromatic L-amino acid decarboxylase as hepatic autoantigens in APS I (Paper I)

Autoimmune hepatitis is the most feared disease manifestation in patients with APS I among whom it occurs in 10-20%, with an onset at a young age and carrying a high mortality. The autoimmune nature has been assumed rather than proven, and no known disease-specific marker existed at the start of this study. We set out to identify possible hepatic autoantigens. Candidate target autoantigens were liver-specific cytochrome P450 enzymes, based on the earlier observations that steroidogenic cytochrome P450 enzymes are autoantigens in APS I, and the recently identified APS I-associated pancreatic β-cell autoantigen, aromatic L-amino acid decarboxylase, with a known liver distribution.

Sera from eight APS I patients, of whom three had biopsy-verified chronic active hepatitis, and ten healthy blood donors, specific CYP1A2, CYP2D6, and AADC antisera were used for indirect immunofluorescence staining of unfixed cryo-sections of rat and human liver. All eight patient sera showed a panlobular cytoplasmic staining pattern and three sera (3/3 with CAH) also markedly stained peri-venous hepatocytes, similarly to the staining of a specific anti-CYP1A2 antiserum. In Western blots, using cytosolic and microsome fractions of human and rat liver, seven of the sera (2/3 with and 5/5 without CAH) identified a 55 kDa protein corresponding in mobility to AADC and three sera (3/3 with and 0/5 without CAH) identified a 60 kDa protein corresponding to that identified by a specific CYP1A2 antiserum. The latter was clearly distinguishable from the 52 kDa protein identified by the CYP2D6 antiserum. None of the control sera identified either of the proteins. Immunoprecipitation of radioactively labelled in vitro transcribed and translated (ITT) CYP1A2 and AADC, generated the same results with the one exception that all eight sera precipitated AADC. Finally, all CYP1A2-positive sera inhibited CYP1A2-specific 7-methoxyresorufin O-demethylase (MROD) activity in vitro.
Organ-specific autoimmune diseases

**AADC antibodies and disease correlation in APS I (Paper II)**

AADC was identified as a hepatic autoantigen in study I, but no clear association with AIH was observed in the limited number of patients investigated. In contrast to the liver-restricted CYP1A2, AADC is present in a wide variety of tissues and we aimed to further examine AADC immunoreactivity and correlate it with the presence of different disease manifestations in APS I patients. Since AADC was initially identified as an autoantigen that is present in the β-cell, its role as an autoantigen in isolated type 1 diabetes mellitus was also investigated.

Sera from 69 APS I patients, 138 type 1 diabetes mellitus patients and 91 healthy controls were screened for the presence of AADC antibodies, using radioactively labelled protein and immunoprecipitation. To investigate the disease-association, the two-sided Fisher’s exact test was used to assess differences in proportions between groups. AADC antibodies were found in ~50% (35/69) of APS I patients and these antibodies significantly correlated to the presence of AIH and vitiligo. Approximately 92% (11/12) of APS I patients with AIH were AADC-positive, compared to 42% (24/57) of the patients without the disorder, and similarly, 80% (12/15) of the APS I patients with vitiligo were positive, compared to 43% (23/54) without. There was no significant correlation between AADC antibodies and type 1 diabetes mellitus in the APS I patients and none of the 138 patients with isolated type 1 diabetes mellitus were AADC-positive.

**APS I-associated hepatic autoantigens in other autoimmune liver disorders (Paper III)**

To investigate the presence of CYP1A2 and AADC autoantibodies in autoimmune liver disorders, other than APS I-associated AIH, sera from a large patient population (n=302) with a variety of liver diseases were screened for the presence of autoreactivity against the ITT enzymes. The recently identified intestinal autoantigen tryptophan hydroxylase (TPH) was included in the study, as a statistically significant association with AIH had been demonstrated.

Sera from 62 patients with primary biliary cirrhosis, 57 with primary sclerosing cholangitis, 67 with autoimmune hepatitis, 9 with drug-induced hepatitis and 107 patients with viral hepatitis, and 120 healthy blood donors were investigated. Apart from one AADC-positive patient with type 2 AIH, no other patients tested positive for any of the autoantigens. The AADC-positive patient was found on re-examination to fulfil the criteria for APS I.
DHEA replacement in women with Addison's disease (Paper IV)

Women with Addison's disease suffer from chronic glucocorticoid and mineralocorticoid deficiency, which requires life-long replacement therapy. Furthermore, there is an associated failure of adrenal DHEA synthesis and, in women, a consequent androgen insufficiency, neither of which are corrected by replacement. Despite attempts to optimise glucocorticoid and mineralocorticosteroid therapy, these women report a reduced quality of life, often complaining of fatigue and reduced well-being. We believe that these symptoms may partly be caused by the DHEA and androgen deficiencies and that women with Addison's disease may thus benefit from DHEA replacement.

To define a suitable dose, DHEA was given to nine women with Addison's disease in either of two doses, 50 mg or 200 mg, during a three-month period. An oral daily dose of 50 mg restored the DHEA(S) levels to normal, whereas in those patients receiving 200 mg, the DHEA-S levels were slightly above the normal reference value. The circulating levels of androgens were restored in all patients to values within the lower reference range. A slight rise in insulin growth factor (IGF)-1 was noted, and also a decrease in low density lipoprotein (LDL) and high density lipoprotein (HDL) although the LDL/HDL ratio remained unaltered. There was no effect on insulin sensitivity, as measured with the euglycaemic hyperinsulinaemic clamp technique, or on the body composition as assessed by dual energy x-ray absorptiometry. A number of side effects, including acne and increased apocrine sweat secretion, were reported, all of which were reversed when DHEA was discontinued. A majority of the women (5/9) experienced a marked increase in psychological and general well-being, as reported in personal interviews, whereas one woman noted increased irritability and aggressiveness.
**DISCUSSION**

**Identification of hepatic autoantigens in APS I (Papers I-III)**

In this study autoantibodies directed against two hepatic autoantigens were identified, namely CYP1A2 and AADC. As is the case in many organ-specific autoimmune diseases, these autoantigens are intracellularly located enzymes involved in major biosynthetic pathways. These results strengthen the assumed autoimmune nature of the chronic active hepatitis seen in APS I patients.

CYP1A2-positive sera inhibited enzymatic activity *in vitro*, indicating that autoantibodies react with the active site of the enzyme. This has been observed earlier for autoantibodies against CYP2D6 in type 2 AIH patient sera, but no evidence for *in vivo* inhibition has been produced. On the contrary, Manns and co-workers found that of a large population of CYP2D6-positive patients, all were extensive metabolizers, expressing a functionally intact enzyme. This implies that autoantibodies do not affect intact hepatocytes, an assumption in line with the sparse reports of an ability of autoantibodies to exert intracellular effects. There have been recent observations, however, of surface expression of different CYPs on hepatocytes, including CYP1A2, indicating that antibodies may participate in the destructive process. There are also reports favouring the idea that Th1- and Th2-type responses with consequent ADCC mechanisms are responsible for the liver destruction in CYP2D6-positive AIH type patients, but this question has not yet been addressed in APS I patients.

CYP1A2 is known to be exclusively present in the liver, although one recent report also suggests expression in human lung tissue. In the liver, CYP1A2 is located predominantly in peri-venous hepatocytes, which is well compatible with the immunostaining pattern obtained with the APS I sera (Paper I), which has not been previously described in AIH. CYP1A2 has been shown to be the target autoantigen in drug-induced hepatitis caused by dihydralazine and disulfiram, and a possible pathogenetic role for drug-metabolising CYPs in complex with their metabolites in autoantibody formation has been proposed. Although none of these drugs are regularly used in the treatment of APS I patients, the mechanism as such should not be ruled out. CYP1A2 is one of the major drug-metabolising enzymes in the liver, and APS I patients are not infrequently subjected to rather extensive pharmacological treatment. Worthy of note in this context is that ketoconazole, a known inhibitor of several CYPs, albeit not CYP1A2, is frequently used in APS I patients and one may speculate on the possibility of an altered hepatic CYP expression pattern as a result of this treatment, with unopposed CYPs being compensatorily induced.
AADC autoreactivity has not been reported on in AIH. The physiological role of AADC in the liver is unknown, but a role in general decarboxylation and detoxification of L-amino acids has been proposed. In a case report featuring a rare hereditary AADC deficiency syndrome, monozygotic twins were described as presenting with signs of CNS and peripheral serotonin and catecholamine deficiency, i.e. hypotonia, defective temperature regulation, dysphoria and postural hypotension. Furthermore, no activity of AADC was found in a liver biopsy specimen, but the gastrointestinal function was said to appear normal. None of the above-mentioned symptoms have been reported in APS I patients. Possible retention of L-amino acids in the liver with subsequent toxic destruction has not been reported and the question has probably not been addressed in APS I patients.

AADC and CYP1A2, intracellularly located enzymes involved in important tissue-specific functions, fit well into the group of previously identified autoantigens involved in organ-specific autoimmune diseases. They also contribute to the two major groups of antigenic targets seen in APS I, namely cytochrome P450 enzymes and enzymes involved in the synthesis of neurotransmitters. What is the cause of this uniform pattern? Could it be that these enzyme systems are up regulated in cells subjected to stress (endogenous or exogenous) and expressing danger signals (see ”The Danger hypothesis”, described earlier), activating an immune response? In such an up regulated state, the degradation and presentation of endogenous proteins may be altered, resulting in loss of tolerance. In a normal situation immunoregulatory mechanisms are sufficient to down-regulate such mechanisms, but in susceptible individuals the autoimmune reaction may be sustained. The reports that AIRE protein may have a potential role in the generation of self-tolerance and that the mutations seen in APS I might lead to loss of this tolerance are of course of great interest in this context.

Although no direct evidence of pathogeneticity of either AADC or CYP1A2 antibodies has been produced in the present study, a disease association with AIH is demonstrated (Papers I and II). Regarding the CYP1A2 association, one may argue that the eight APS I patients studied are too few to allow any conclusions to be drawn, but our results have since been confirmed in studies performed in the Sardinian and Finnish APS I populations. An additional hepatic autoantigen has been identified in these two patient groups, namely CYP2A6, but no association with AIH was found. In a recent study in which the prevalence and clinical association of the major antigens were assessed in sera from 90 APS I patients, in addition to AADC and CYP1A2, the intestinal autoantigen tryptophan hydroxylase was highly associated with AIH. TPH has not been reported to occur in the liver, but cannot be ruled out as a possible autoantigen until further studies have been performed.
Organ-specific autoimmune diseases

The identification of AIRE has made mutational analysis a possible diagnostic tool, identifying approximately 90% of APS I patients, but does not contribute any clinical information, as no genotype/phenotype correlation has yet been described. Analysis of a combination of known antibodies results in the same sensitivity, with the addition of valuable clinical information.

The absence of CYP1A2, AADC and TPH antibodies in other autoimmune liver diseases strengthens the specificity of these antibodies as markers of APS I-associated AIH (paper IV). In about 5% of APS I patients, AIH is the first manifestation, with a very young onset, usually before the age of 2 years. Antibody-testing for CYP1A2 and AADC may thus be valuable in very young patients, but also in AIH patients negative for established serological markers. A correct diagnosis is important since it facilitates early diagnosis and treatment of other manifestations of this syndrome.

DHEA replacement in women with Addison's disease (Paper IV)

We carried out a dose-finding study in nine women with Addison's disease and found that in these patients a daily dose of 50 mg is sufficient to restore DHEA(S) and androgen levels to those seen in young adults. These results are well in accordance with those found in studies on DHEA replacement in patients with panhypopituitarism and in young women after transient inhibition of adrenal function by dexamethasone suppression. We find that women with Addison's disease offer a unique opportunity to study the effects of DHEA, since there is a true deficit relative to age. Two of the women in our study suffered from gonadal insufficiency on an autoimmune basis and it was interesting to note that the androgen levels were restored just as well in these women as in the women with intact gonadal function. These results demonstrate that extra-adrenal and gonadal transformation of DHEA is sufficient to generate potent androgens, in support of earlier findings.

In reports demonstrating that women, but not men, with Addison's disease have decreased bone mineral density compared to controls, it has been suggested that androgens may be important in maintaining bone mass. Our patients did have abnormally low bone mineral density at baseline (mean z-score at the lumbar spine -0.9 and femoral neck -0.7), but no effect on bone metabolism was seen during the trial. This was not unexpected, in view of the short treatment period. However, in recent studies DHEA replacement for 12 months has been shown to increase bone mineral density and reduce markers of bone turnover in postmenopausal women, suggesting that long-term assessment of DHEA treatment in women with Addison’s disease is indicated. It is
tempting to speculate whether one of the beneficial effects of DHEA on bone formation may be mediated by IGF-1, since, in accordance with other studies, a slight rise in circulating IGF-1 level was found. The short treatment period is also likely to explain the lack of effect on body composition - in contrast to that seen in older women and young men in previous studies.

We found no effect of DHEA on insulin sensitivity or the serum lipid profile, and thus we have no indication that DHEA replacement to near physiological levels predisposes to decreased insulin sensitivity or an unfavourable lipid profile. Long-term assessment of DHEA replacement is required, however, for further evaluation.

An increase in psychological and general well-being was experienced by a majority of the women, whereas one woman perceived increased irritability and aggressiveness. There were no apparent differences in DHEA(S) or sex-hormone levels in this woman compared to the other women, indicating that there might be individual differences in responses to DHEA(S) or androgens. This in turn emphasises the need for individual dose adjustments and suggests lower doses than 50 mg may sometimes be preferable. Apart from mild androgenic effects such as acne and increased apocrine sweat secretion, no serious adverse effects were observed and five of the women wanted to continue DHEA treatment at the end of the study. We are well aware that our reports on well-being are self-reported subjective values and therefore not conclusive and we are presently about to complete a double-blind, placebo-controlled DHEA replacement study to further investigate long-term effects of the treatment in this patient group. However, our findings are in line with recently published reports that DHEA was found to improve sexuality in women and to improve overall well-being, mood and fatigue in both men and women with Addison's disease.
Organ-specific autoimmune diseases

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

We report original findings of CYP1A2 and AADC as hepatic autoantigens associated with AIH in APS I. Furthermore, antibodies directed against these antigens were found to be specific for APS I. As such, they are valuable diagnostic markers for APS I in patients with AIH and possibly also for those APS I patients at risk of developing AIH. The latter is particularly important, since fulminant hepatitis without previous warning may occur in APS I patients, and immunosuppressive treatment early in the course of the disease might prove beneficial. To further evaluate the role of these autoantigens in the pathogenesis of AIH, it would be interesting to investigate CYP1A2 and AADC reactivity in consecutive serum samples from APS I patients and to correlate this reactivity with disease activity. Studies of T cells in liver biopsies from APS I patients, and their reactivity against CYP1A2 and AADC, are another approach that might add useful information, since seemingly little research has been done on T cells and their role in APS I.

AADC antibodies are present in a good half of APS I patient sera, making them one of the major serological markers of this disease. The role of these antibodies is not easy to interpret as, in contrast to other APS I associated antigens, AADC show a wide tissue distribution. The strong correlation of these antibodies to AIH is intriguing, especially since the physiological role of AADC in the liver is largely unknown. Current knowledge of autoantigens as enzymes with important functions in the affected tissue points to the need for further research on the physiological role of AADC in the liver.

With our finding that CYP1A2 is an immunological target in the liver, yet another cytochrome has been identified as an autoantigen in APS I. Why this preference of the immune response in APS I? The enzyme-inhibiting properties of the CYP1A2 antibodies that we have demonstrated indicate that at least some of the epitopes recognised are located within the active site of the enzyme. Epitope mapping of APS I-associated CYP antigens and studies on patterns of CYP expression in the affected organs may contribute interesting information regarding the pathogenesis of APS I.

The identification of the gene responsible for APS I is a promising break-through in the search for aetiological and pathogenetic mechanisms of autoimmunity, and studies performed in mice indicate that Aire may play a role in the generation of self-tolerance. Provided that it develops autoimmune disease, the Aire knock-out mouse may make it possible to study the course of events leading to disease, hopefully relevant not only to APS I, but also to organ-specific autoimmune diseases in general.
We have shown that a daily replacement dose of DHEA of 50 mg sufficiently restores the levels of DHEA(S) and androgens in women with Addison's disease without severe side-effects. In addition, a subjectively increased sense of general well-being was reported. In the past year, several larger-scale, placebo-controlled studies have been undertaken and have shown similar beneficial effects of DHEA replacement in this patient group, but also in the elderly and in patients with major depression. It is therefore reasonable to suggest that in addition to the existing life-saving steroid replacement, DHEA replacement will be a valuable complement to restore psychological and physical well-being in patients with Addison's disease. To further evaluate the effects on the bone mineral content and blood lipid profile, among other things, long-term studies of DHEA replacement are needed. Patients with Addison's disease serve as a useful group to study effects of DHEA(S), since they exhibit a true deficit relative to age. Interesting additional information on the physiological role of DHEA may thus result from such studies.
ACKNOWLEDGEMENTS

These investigations were carried out at the Department of Medical Sciences, Uppsala University, Sweden. Many people have contributed to this thesis and I would like to take the opportunity to thank them all, and especially:

**Sverker Ljunghall**, former head of the department, who holds a genuine interest in clinical and experimental research and was the first one to establish full-time PhD student positions at the department. I am also grateful for the valuable access to the osteoporosis unit, created by him.

**Kjell Öberg**, the present head of the department, for providing excellent working conditions and for financial support during the completion of this thesis.

**Olle Kämpe**, my supervisor, for welcoming me into your team with my only merit being the mother of three boys, for guiding me into clinical and experimental research in the field of autoimmunity interspersed with, among other things, endless lectures on the noble art of cottage foundation (torpargrunder) construction, exquisite meals and wonderful skiing, for struggling to ensure secure financial support and last, but not least, for establishing and maintaining a wonderful research-group.

**Fredrik Rorsman**, my second supervisor, for being such a straight forward and pleasant person to work with, for sharing your knowledge of molecular biology, for never hesitating to spend your valuable spare time, in spite long working-hours at the clinic, at the lab bench helping out with strange looking (smelling) agar-plates or answering never-ending queries on everything there is to inquire about and for your ability to show me my abilities to see this work through.

**Eystein Husebye**, my co-worker and co-author, for your valuable contribution and for useful discussions in the preparation of manuscripts. For being a superb travel companion by paving the way through end-less check-in lines and your calming influence during dangerous flights, for a hearty welcome in your home in Bergen and for being such a dear friend.

**Ola Winqvist**, who introduced me to the wondrous world of experimental research by giving me a first "mission impossible": The identification of 25-OH as an hepatic something…and during that period teaching me all there was to know about useful lab techniques, for a warm welcome in your home in La Jolla and for numerous inspiring and useful discussions. "Give me five!"
My colleagues in the "Kämpe-Rorsman" Group, **Annika Söderbergh**, for sharing a rudimentary room, the women's half of the lab, a box in the freezer, stodgy food in Oxford but above all warm friendship, **Håkan Hedstrand**, for sharing a "skrubb", your wonderful sense of humour, for heartening discussions on life and science and for becoming a very dear friend, **Olov Ekwall**, for sharing a Rolls Royce desk and letting me inherit the "wide screen", for travel companionship and your patient attitude towards my insufficiency with computers and for great friendship, **Filip Sköldberg**, for occupying the men's half of the lab, for your sound scepticism and for being such a truly smart and sympathetic guy, **Eva Landgren**, for great friendship and your know-how in basic science, for bringing my native Skåne into the lab and for straightening my priorities when needed, **Thomas Nilsson**, for your profound clinical, experimental, "Jamte" and what-not know-how, and your general "no problem" attitude, **Gunnel Nordmark**, for being an excellent teacher in clinical skills, and **Åsa Hallgren** and **Katrin Österlund** for genuine friendship and for excellent technical assistance whenever needed.

The initial extended group with **Anders Karlsson**, for your profound knowledge in experimental and clinical endocrinology and for many discussions from which I have benefited greatly, **Margareta Ericsson**, **Majstin Wik** and **Anna-Carin Englund** for giving me a wonderful start and for helping out with immunohistochemical stainings, Western blots and cell culturing, **Therese Dahlman** and **Kerstin Westermark** for contributing to the inspiring atmosphere.

The Monday Seminar Club with, **Lars Rönnblom**, **Anders Rönnblom**, **Sophie Bensing**, **Per Marits** and **Patrik Larsson** for interesting seminars and numerous useful discussions.

All my co-authors, and especially **Jan Gustafsson**, for supplying patient sera and for fruitful discussions, **Christian Berne**, **Lottie Helström** and **Hans Mallmin** for expert clinical advise and **Hans**, also for your mastery of File Maker Pro and DXA-measurements, **Jaakko Perheentupa** and **Stefan Lindgren**, for generously sharing your valuable patient sera and clinical knowledge and **Mikael Manns** and **Petra Obermayer-Straub** for supplying patient sera and the invaluable CYP1A2 vector.

To all the friends in the Melhus group, **Håkan Melhus**, **Andreas Kindmark**, **Birgitta Wilén**, **Sara Johansson**, **Lisa Kurland**, **Fredrik Stiger**, **Maria Branting** and **Julia Karlsson** for pipettes, primers, enzymes, tea, friendship and lateral thinking.
Organ-specific autoimmune diseases

Past and present fellow PhD students, Anna Schölin, for friendship and sharing the ups and downs of a PhD student's everyday life, Eva Bornefalk, Peter Stålberg, Mikael Kullin, Kenneth Jonsson, Jan Melin and all other friends at the Centre for Clinical Research for small talk and a helping hand whenever needed.

Colleagues Brita Winsa, Britt Skogseid and Elisabeth Björk for serving as inspiring models in research and clinical work and for being such good friends.

Elisabet Dew, for patiently guiding me through university and hospital bureaucracy, especially at times when dead-lines were long passed and Anna Carlbom Härd, Elizabeth Dehlin and Lena Hultström for help with all kinds of practical matters.

Maud Marsden and Cindy Wong, for painstakingly revising the English text.

Lena Milton, Anna Engström and Gudrun Andersson, for being the best of friends with an unfailing supportive attitude, no matter what.

The best of parents, my mother and father Susanne and Mehari, and my brothers Samuel, with his family and Jonas, all of whom in different overt and subtle ways, have helped me in the efforts of writing this thesis.

David, Andreas, Jonatan and Simon my incredible sons for bringing endless amounts of joy and hockey, football, skateboarding, fighter piloting...well, distraction into my life.

Per, my beloved husband and companion in life, and inspiring colleague in life sciences.

Financial support was generously provided by the Swedish Medical Research Council, The Swedish Society of Medicine, the Swedish Society for Medical Research, the Swedish Cancer Society, the Torsten and Ragnar Söderberg Fund, the Bergwall Fund, the Åke Wiberg Fund, the Lars Johan Hierta fund, the Lennander Fund, the Ernfors Family Fund, the Förenade Liv Mutual Group Life Insurance Company, the Erland Wessler Fund, the Agnes and Mac Rudberg Fund, and the Foundation of Queen Silvia’s Jubilee Fund which are hereby fully acknowledged.
REFERENCES


**Organ-specific autoimmune diseases**


Organ-specific autoimmune diseases


Czaja AJ. Drug therapy in the management of type 1 autoimmune hepatitis. Drugs 1999;57:49-68.


Organ-specific autoimmune diseases


Organ-specific autoimmune diseases


Organ-specific autoimmune diseases


**Organ-specific autoimmune diseases**


Organ-specific autoimmune diseases

189. Parker DR, Kingham JG. Type I autoimmune hepatitis is primarily a disease of later life. Qjm 1997;90:289-96.


Organ-specific autoimmune diseases


231. Taurog JD, Maika SD, Simmons WA, Breban M, Hammer RE. Susceptibility to inflammatory disease in HLA-B27 transgenic rat lines correlates with the level of B27 expression. J Immunol 1993;150:4168-78.


Organ-specific autoimmune diseases


Organ-specific autoimmune diseases
