Clinical and Experimental Studies in Primary Sjögren’s Syndrome and Systemic Lupus Erythematosus

GUNNEL NORDMARK
Dissertation presented at Uppsala University to be publicly examined in Rudbeckssalen, Rudbecklaboratoriet, Uppsala, Friday, October 28, 2005 at 13:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

Autoimmune mechanisms and genetic susceptibility contribute to the pathogenesis of primary Sjögren’s syndrome and SLE. These chronic systemic autoimmune diseases have many serological and clinical features in common and have an impact on daily life. The studies in this thesis aim to elucidate their autoimmune mechanisms, define susceptibility genes and evaluate effects of androgen supplement on health-related quality of life.

Autoantibodies against α-fodrin, a widely distributed cytoskeletal protein, were detected at similar frequencies in sera from patients with primary and secondary Sjögren’s syndrome and SLE. Consequently, testing for antibodies against α-fodrin would not add diagnostic value compared to conventional serological analysis and does not discriminate between these diseases.

The type I interferon (IFN) system was found to be activated in primary Sjögren’s syndrome. IFN-α containing cells were detected in minor salivary gland biopsies, while sera from patients with primary Sjögren’s syndrome induced IFN-α production in the presence of apoptotic and necrotic cell material. This ability of sera correlated with the presence of antibodies against RNA-binding proteins and IFN-α production was dependent on RNA in immune complexes. The natural interferon producing cells/plasmacytoid dendritic cells (NIPC/PDC) were the IFN-α producers and blocking of FcγRIIIa inhibited the production. Single nucleotide polymorphisms (SNPs) in two genes in the type I IFN signalling pathway, those for tyrosine kinase 2 and interferon regulatory factor 5, were strongly associated with SLE in a Swedish, Finnish and Icelandic population. The minor allele frequencies were lower in SLE patients than in healthy controls. These SNPs may decrease the function of the type I IFN system, thereby conferring protection against SLE.

Supplementation with dehydroepiandrosterone (DHEA) in glucocorticoid treated women with SLE led to mild improvements in health-related quality of life in respect of mental well-being and sexuality, whereas physical well-being was unaffected.

Keywords: Sjögren’s syndrome, SLE, α-fodrin, interferon-α, single nucleotide polymorphism, dehydroepiandrosterone

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ISSN 1651-6206
ISBN 91-554-6349-5
urn:nbn:se:uu:diva-5943 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-5943)
To Lucy & Mike
List of papers

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


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<tr>
<td>ACR</td>
<td>American College of Rheumatology</td>
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<td>AECC</td>
<td>American-European consensus criteria</td>
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<tr>
<td>ANA</td>
<td>antinuclear antibody</td>
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<td>APC</td>
<td>antigen presenting cell</td>
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<tr>
<td>BDCA</td>
<td>blood dendritic cell antigen</td>
</tr>
<tr>
<td>CD</td>
<td>cluster of differentiation</td>
</tr>
<tr>
<td>CpG</td>
<td>cytosine-phosphate-guanine</td>
</tr>
<tr>
<td>DC</td>
<td>dendritic cell</td>
</tr>
<tr>
<td>DHEA</td>
<td>dehydroepiandosterone</td>
</tr>
<tr>
<td>DHEAS</td>
<td>dehydroepiandosterone-sulphate</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<tr>
<td>dsDNA</td>
<td>double-stranded DNA</td>
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<tr>
<td>FcR</td>
<td>Fc receptor</td>
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<td>GC</td>
<td>germinal centre</td>
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<tr>
<td>GVHD</td>
<td>graft versus host disease</td>
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<td>HCV</td>
<td>hepatitis C virus</td>
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<td>HIV</td>
<td>human immunodeficiency virus</td>
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<td>HLA</td>
<td>human leukocyte antigen</td>
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<tr>
<td>HRQOL</td>
<td>health-related quality of life</td>
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<tr>
<td>HSCL-56</td>
<td>Hopkins Symptom Check List-56</td>
</tr>
<tr>
<td>IC</td>
<td>immune complex</td>
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<tr>
<td>IDDM</td>
<td>insulin dependent diabetes mellitus</td>
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<tr>
<td>IFN</td>
<td>interferon</td>
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<tr>
<td>IFNAR</td>
<td>interferon α/β receptor</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>IRF</td>
<td>interferon regulatory factor</td>
</tr>
<tr>
<td>ISGF3</td>
<td>interferon stimulating gene factor 3</td>
</tr>
<tr>
<td>ISRE</td>
<td>interferon stimulated response element</td>
</tr>
<tr>
<td>ITT</td>
<td>in vitro transcription and translation</td>
</tr>
<tr>
<td>LD</td>
<td>linkage disequilibrium</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>MSG</td>
<td>minor salivary gland</td>
</tr>
<tr>
<td>NHL</td>
<td>non-Hodgkin lymphoma</td>
</tr>
<tr>
<td>NIPC</td>
<td>natural interferon-producing cells</td>
</tr>
<tr>
<td>ODN</td>
<td>oligodeoxynucleotide</td>
</tr>
<tr>
<td>PBMC</td>
<td>peripheral blood mononuclear cells</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>PDC</td>
<td>plasmacytoid dendritic cell</td>
</tr>
<tr>
<td>PGWB</td>
<td>Psychological General Well-Being</td>
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<tr>
<td>pSS</td>
<td>primary SS</td>
</tr>
<tr>
<td>RF</td>
<td>rheumatoid factor</td>
</tr>
<tr>
<td>RNA</td>
<td>ribonucleic acid</td>
</tr>
<tr>
<td>RNP</td>
<td>ribonucleoprotein</td>
</tr>
<tr>
<td>SF-36</td>
<td>Short Form -36</td>
</tr>
<tr>
<td>SLE</td>
<td>systemic lupus erythematosus</td>
</tr>
<tr>
<td>SLEDAI</td>
<td>SLE disease activity index</td>
</tr>
<tr>
<td>SLE-IIF</td>
<td>SLE-interferon inducing factor</td>
</tr>
<tr>
<td>SLICC</td>
<td>Systemic Lupus International Collaborating Clinics</td>
</tr>
<tr>
<td>Sm</td>
<td>Smith antigen</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
<tr>
<td>SS</td>
<td>Sjögren’s syndrome</td>
</tr>
<tr>
<td>SS-A/Ro</td>
<td>Sjögren’s syndrome antigen A</td>
</tr>
<tr>
<td>SS-B/La</td>
<td>Sjögren’s syndrome antigen B</td>
</tr>
<tr>
<td>sSS</td>
<td>secondary SS</td>
</tr>
<tr>
<td>Th</td>
<td>T helper cell</td>
</tr>
<tr>
<td>TLR</td>
<td>toll like receptor</td>
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</table>
Introduction

Sjögrens’s syndrome (SS) and systemic lupus erythematosus (SLE) are two systemic chronic autoimmune inflammatory diseases with several similarities regarding the clinical picture and immunological abnormalities, but also with some important differences (1). Both SS and SLE occur mainly in females with a 9:1 gender ratio. SS can occur either alone as primary SS (pSS), or as secondary SS (sSS) in association with other connective tissue diseases, most commonly SLE (2).

Some extraglandular manifestations in pSS are similar to the systemic features seen in SLE; e.g. Raynaud’s phenomenon, non-erosive arthritis, leucopenia, thrombocytopenia and dermal vasculitis. Other organ involvements differ; e.g. the relatively rare interstitial nephritis in pSS contrasts with the immune complex (IC) mediated glomerulonephritis seen in nearly half of the SLE patients (3). Antinuclear antibodies (ANA) are present in sera from approximately 75-85% of SS and 95-99% of SLE patients, and antibodies to SS antigen A and B (SS-A/Ro and SS-B/La) are seen in varying frequencies in both diseases (4).

Hypergammaglobulinemia is present in both SS and SLE as a sign of increased B cell activity but IC formation and activation of the complement system is usually not seen in SS except in cases of extraglandular manifestations such as dermal vasculitis (5). SLE, on the other hand, is characterised by IC formation, and complement consumption is a sensitive sign of disease activity.

General systemic features shared by both SS and SLE are chronic fatigue not relieved by sleep, myalgia, arthralgia and a low grade fever, although the latter is seen more often in SLE. SLE is also characterised by flares and remissions, whereas SS usually has a stable disease course (6). Patients with an initial diagnosis of SS may fulfil the ACR criteria for SLE over the years (7). SLE with sSS also has clinical, serological and genetic features similar to SS (1). This suggests a common etiopathological background.
Background

Sjögren’s syndrome

History and epidemiology

In 1933 the Swedish ophthalmologist Henrik Sjögren presented his thesis ‘Zur kenntnis der Keratoconjunctivitis Sicca’ where he described 19 cases of keratoconjunctivitis sicca, many of which also displayed dry mouth and arthritis, probably representing rheumatoid arthritis (RA) with sSS (8). The first case reports of xerostomia had already appeared in 1888 by Hadden and Hutchinson (8, 9) and in 1892 Mikulicz described a patient with bilateral swelling of the lacrimal and salivary glands and the entity was called Mikulicz disease (MD) (10). Gougerot also described a few cases in 1926 and the eponym ‘Gougerot-Sjögren’s syndrome’ is sometimes used in the French literature. In 1953 a study confirmed the same histopathology in MD and SS, with MD considered part of SS, which became the eponym used thereafter (9).

Disease onset is around 40-50 years of age, although SS has been described in children (11, 12). Prevalence studies have shown figures between 0.5% and 3.3% depending on the selection of patients and classification criteria used (13-15). A recent estimate using the American-European consensus criteria (AECC) (16) is around 0.5% of the female Caucasian population aged 35-74 years (17).

Clinical features

Glandular manifestations

SS is an autoimmune disease characterised by lymphocytic infiltration of the exocrine glands, primarily the lacrimal and salivary glands, resulting in keratoconjunctivitis sicca and xerostomia (18). Sicca symptoms also affect other exocrine glands resulting in tracheitis sicca with bronchial hyperreactivity, vaginitis sicca and dry skin (19). Chronic swelling of the parotid glands is seen in pSS, and SLE with sSS, sometimes called SLE-SS overlap, but rarely in sSS associated with RA (1, 20). General accompanying symptoms are arthralgia, myalgia and fatigue, present in about half of the patients.
Extraglandular manifestations
Extraglandular manifestations occur in nearly 50% of SS patients, most commonly Raynaud’s phenomenon and/or a mild non erosive arthritis. Leucopenia (< 4.0 x 10^9/L) is seen in about 40% of the patients, whereas thrombocytopenia and haemolytic anemia are rare (1). About 20% have pulmonary involvement, mainly bronchial hyperreactivity, or less frequently interstitial lung disease progressing to fibrosis. Pleuritis in pSS is less common (21). An asymptomatic renal tubular acidosis is common (35%) but an overt interstitial nephritis is only seen in a few percent. Glomerulonephritis is rare (3). Gastrointestinal manifestations include dysphagia due to dryness and oesophageal dysmotility, atrophic gastritis, pancreatic insufficiency, and a mild elevation of liver enzymes. Vasculitis occurs in 10% of patients, mainly manifested in the skin as a palpable purpura or ulcers. Pure sensory neuropathy is seen in about 20%, myositis in < 5% and CNS involvement is rare (11, 22).

SS is also associated with organ-specific autoimmune diseases, most commonly autoimmune thyroiditis where more than 30% of SS patients have antibodies against thyroperoxidase (TPO) and 10-15% develop hypothyroidism (23). Primary biliary cirrhosis and celiac disease are also associated with SS (24). There are no well defined disease activity parameters in SS, but a core set of outcome measures for long term follow up have been proposed (25).

Lymphoma
A 44-fold increased risk of malignant non-Hodgkin lymphoma (NHL) has been reported in SS, which is the highest incidence of a lymphoproliferative disease among autoimmune diseases (26). The prevalence of lymphoma among SS patients is around 5% (27). The vast majority of NHL are of B cell type and arise in the mucosa-associated lymphoid tissues, so called MALT lymphoma, i.e. the major salivary glands, tonsils, gastrointestinal tract or lungs (28). Lymphoma in the major salivary glands arises from benign lymphoepithelial lesions/myoepithelial sialoadenitis, where the infiltrating B cells undergo clonal proliferation and malignant transformation (29). NHL in other locations has occasionally been shown to originate from a benign clone in the major salivary glands (30). Predictors for lymphoma development are parotid swelling, lymphadenopathy, palpable purpura and low complement C4 levels (27, 31). There is no increased overall mortality in SS but a nearly 8-fold increased mortality due to lymphoproliferative disease, which is predicted by low C3 and/or C4 levels (27, 32).
Diagnosis and classification

The diagnosis of primary SS has been based on different classification criteria of which the San Diego criteria (18), the European criteria (33) and the Copenhagen criteria (34) have been most commonly employed. Independent Greek (35) and Japanese criteria (36) have been used as well. This has created problems in comparing prevalence figures and results from different studies, since there has been little overlap between the different sets of criteria and not all sets have required autoimmunity for diagnosis. The San Diego criteria have been the strictest in requiring either a positive minor salivary gland (MSG) biopsy and/or positive autoimmune serology.

In 2002, new AECC was formed for the classification of primary and secondary SS, as well as exclusion criteria (16). Future validation will reveal if these criteria hold and possibly will be accepted as American College of Rheumatology (ACR) criteria (Table 1). For the diagnosis of primary SS at least 4/6 items are required, with items IV and/or VI being mandatory. These criteria yield a sensitivity of around 90% and a specificity of 95%. For the diagnosis of sSS a decreased lacrimal and unstimulated whole salivary flow together with sicca symptoms are sufficient if drugs can be excluded. Further exclusion criteria are listed in Table 1.

Sicca can be due to a variety of causes, most commonly drugs, stress, smoking and diabetes. Classification criteria are not intended for diagnosis but are often used as an aid in the diagnostic procedure. These revised AECC may help in finding those patients with an autoimmune cause of their sicca problems who need to be followed for the development of extraglandular manifestations, other autoimmune diseases or lymphoma (27).

Etiology

The etiology of SS is largely unknown, but a combination of a genetic predisposition, infectious agents, and hormones has been postulated to be of importance (41).

Genetics

Family members of patients with SS have a higher incidence of SS and SLE, other autoimmune diseases and immunological abnormalities than unrelated individuals (20). The highly polymorphic major histocompatibility complex (MHC) class II antigens are the strongest genetic risk factors for developing autoimmune diseases (42). Different histocompatibility leukocyte antigen (HLA) haplotypes are associated with SS in different ethnic groups. In Caucasians HLA-DRB1*0301, DQA1*0501 and DQB1*0201 are associated with SS (43). These haplotypes have a stronger association to the presence of anti-SS-A and/or anti-SS-B antibodies than to the disease itself (44).
Table 1. *Revised international classification criteria for Sjögren’s syndrome (16)*

<table>
<thead>
<tr>
<th>Item</th>
<th>Parameter</th>
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<tbody>
<tr>
<td>I.</td>
<td><strong>Ocular symptoms:</strong> a positive response to at least one of the following questions:</td>
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<tr>
<td></td>
<td>1. Have you had daily, persistent, troublesome dry eyes for more than 3 months?</td>
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<tr>
<td></td>
<td>2. Do you have a recurrent sensation of sand or gravel in the eyes?</td>
</tr>
<tr>
<td></td>
<td>3. Do you use tear substitutes more than 3 times a day?</td>
</tr>
<tr>
<td>II.</td>
<td><strong>Oral symptoms:</strong> a positive response to at least one of the following questions:</td>
</tr>
<tr>
<td></td>
<td>1. Have you had a daily feeling of dry mouth for more than 3 months?</td>
</tr>
<tr>
<td></td>
<td>2. Have you had recurrently or persistently swollen salivary glands as an adult?</td>
</tr>
<tr>
<td></td>
<td>3. Do you frequently drink liquids to aid in swallowing dry food?</td>
</tr>
<tr>
<td>III.</td>
<td><strong>Ocular signs</strong> – that is, objective evidence of ocular involvement defined as a positive result for at least one of the following two tests:</td>
</tr>
<tr>
<td></td>
<td>1. Schirmer’s I test, performed without anaesthesia (≤ 5 mm in 5 minutes)</td>
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<tr>
<td></td>
<td>2. Rose bengal score or other ocular dye score (≥ 4 according to van Bijsterveld’s scoring system) (37).</td>
</tr>
<tr>
<td>IV.</td>
<td><strong>Histopathology:</strong> In minor salivary glands (obtained through normal-appearing mucosa) focal lymphocytic sialoadenitis, evaluated by an expert histopathologist, with a focus score ≥ 1, defined as a number of lymphocytic foci (which are adjacent to normal-appearing mucous acini and contain more than 50 lymphocytes) per 4 mm² of glandular tissue (38).</td>
</tr>
<tr>
<td>V.</td>
<td><strong>Salivary gland involvement:</strong> objective evidence of salivary gland involvement defined by a positive result for at least one of the following diagnostic tests:</td>
</tr>
<tr>
<td></td>
<td>1. Unstimulated whole salivary flow (≤ 1.5 ml in 15 min)</td>
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<td></td>
<td>2. Parotid sialography showing the presence of diffuse sialectasis (punctate, cavitative or destructive pattern), without evidence of obstruction in the major ducts (39).</td>
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<tr>
<td></td>
<td>3. Salivary scintigraphy showing delayed uptake, reduced concentration and/or delayed excretion of tracer (40).</td>
</tr>
<tr>
<td>VI.</td>
<td><strong>Autoantibodies:</strong> presence in the serum of the following autoantibodies:</td>
</tr>
<tr>
<td></td>
<td>1. Antibodies to Ro (SSA) or La (SSB) antigens, or both</td>
</tr>
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*Exclusion criteria:* past head and neck radiation treatment, hepatitis C infection, acquired immunodeficiency syndrome (AIDS), pre-existing lymphoma, sarcoidosis, graft versus host disease, use of anticholinergic drugs (since a time shorter than 4-fold the half-life of the drug).
Polymorphisms in cytokine genes have been investigated and an association between SS and IL-6 and IL-10 promoter polymorphisms have been noted in a Finnish population (45, 46). There are no obvious candidate genes in SS, but polymorphisms in the Fas-receptor (CD95) and Fas-ligand (FasL) genes have been found (42). A polymorphism in the Ro 52 gene has been associated with the presence of anti-Ro52 antibodies in SS (47).

A recent gene expression array study revealed several genes involved in inflammation, antigen presentation and virus defence including interferon inducible proteins, which were up-regulated in MSG from 10 patients with SS compared to 10 controls (48). The genes encoding the anti-apoptotic protein Bcl-2, and carbonic anhydrase II, involved in secretion, were instead downregulated.

**Viruses**

Several viruses e.g. Epstein-Barr virus (EBV), HCV and retroviruses such as human T-cell leukaemia virus-1 (HTLV-1) and human immunodeficiency virus (HIV) have been discussed in SS pathogenesis, but no single specific virus has been shown to be closely involved (41). Viruses could cause autoimmune disease by triggering an immune response that becomes prolonged or even perpetuating. HCV and HIV are known to cause inflammatory infiltrates in the MSG (49). During HCV infection the virus replicates in the salivary glands and anti-HCV antibodies are secreted in the saliva. However, sicca symptoms are rarely seen in HCV-infection and the serology is negative (50). The EBV genome can be detected in 5% of SS MSG, but with no correlation to NHL or EBV-associated lymphoma (49).

The relationship between non-HIV retroviruses and autoimmune disease has been investigated, and retroviral particles and gene fragments have been isolated from SS MSG. HTLV-1, which is endemic in Japan and causes myopathy, has been associated with SS in this population (49).

**Hormonal influence**

The role of sex hormones is unclear despite the female gender predisposition. SS is usually manifested in peri- and postmenopausal age groups, when both estrogen and androgen levels decrease. Low levels of androgens are reported in SS, most commonly low levels of dehydroepiandrosterone-sulphate (DHEAS), although normal DHEAS levels have also been reported (51, 52).

Increased prolactin is a constant finding in nearly 20% of patients, whereas estrogen and progesterone levels are normal (52, 53). There are no restrictions in using hormone replacement therapy in SS, nor oral contraceptives, although in young women with SS and a positive serology one may be cautious due to the risk of precipitating SLE.
Alpha-fodrin

Protein structure
Fodrin (non-erythroid spectrin or brain spectrin) is a major cytoskeletal protein present in most eukaryotic cells (54). Originally the cytoskeletal protein spectrin was described in erythrocyte membranes but later also in neuronal cells and was designated non-erythroid spectrin, brain spectrin or fodrin (55). Fodrin is abundant in most tissues including skeletal muscle, uterus, kidney, liver, adrenal glands and intestinal epithelium as well as salivary glands. The distribution in these cells forms a lining of the cell membrane, therefore the name fodrin (from Greek: fodoros, lining) (55, 56).

Fodrin consists of two polypeptides, α-fodrin with a molecular weight (mw) of 240 kDa, and β-fodrin (mw 235 kDa), which together form a rod-shaped heterodimer in a head-to-tail fashion where the amino (N)-terminal end of the α-subunit associates with the carboxy (C)-terminal end of the β-subunit. These heterodimers can associate in a further head-to-tail alignment to form tetramers, which are attached to the cell membrane. The β-subunit is responsible for membrane attachment whereas the α-subunit is responsible for binding to actin, calmodulin and microtubules, and fodrin is considered part of the cytoskeleton (57). Protein sequences of the α- and β-subunits reveal high homology to each other and both subunits consist of repeated sequences of 106 amino acid residues, the so called spectrin repeat, which is also present in related proteins such as α-actinin and dystrophin (58).

The gene
Alpha-fodrin is highly conserved among species, displaying 96% homology between chicken and human, compared to only 58% homology between human α-fodrin and human erythroid spectrin (59). The gene encoding human α-fodrin, (SPTAN1) has been mapped to 9q33-->q34 (60) and consists of 7787 nucleotides encoding 2472 aminoacids. The complementary DNA (cDNA) clone covering the first 5’ coding region, JS-1, was isolated from a human liver cDNA library and includes basepairs 1-1784 encoding the first 561 aminoacids of the N-terminal end of α-fodrin. The JS-1 clone has subsequently been used in α-fodrin assays in Sjögren’s syndrome and is the one used in our present study (61).

Effects in immune cells and secretory cells
Alpha-fodrin binds to the cytoplasmic domain of CD45 in leukocytes, where it is involved in the regulation of tyrosine phosphatase activity and subsequently of B- and T-cell activity (62). When B- and T-cell receptors are ligated they are redistributed and clustered on the cell surface forming ‘caps’. Simultaneously, fodrin is redistributed to form corresponding intracellular ‘subcaps’, demonstrating that it can move within cells (63).
A redistribution of α-fodrin during endocrine and exocrine secretion has been shown in chromaffin adrenal and parotid acinar cells, respectively (56, 57), where the redistribution from the plasma membrane to the cytosol is reversible, indicating that α-fodrin is not degraded during secretion. Antibodies to α-fodrin inhibit the Ca^{2+}-dependent chatecholamine release in endocrine adrenal cells (64).

Redistribution of α-fodrin during apoptosis
The term apoptosis (from Greek; falling), describes the morphological changes seen during programmed cell death. Apoptosis is characterised by an energy dependent cell death that does not cause any inflammation in the surrounding tissues, as is seen in the other type of cell death, necrosis, which causes extensive inflammation (65). The cytoplasm and nucleus condense, nuclear chromatin is cleaved by endonucleases into smaller DNA fragments and cytoskeletal proteins are cleaved by proteolytic enzymes, caspases. The cell membrane forms blebs, which finally disrupt into small apoptotic bodies. These apoptotic bodies expose phosphatidylserine, a phospholipid in the cell membrane, which is recognised by macrophages that subsequently ingest the apoptotic cell bodies, simultaneously producing TNFβ, thereby inhibiting inflammation.

Alpha-fodrin is cleaved by proteolytic enzymes during apoptosis to 120 kDa, 150 kDa or 155 kDa fragments depending on the protease (54, 66). Most commonly α-fodrin is cleaved by caspase 3 or calpain to 120 kDa and 150 kDa fragments (54). During apoptosis, α-fodrin is relocated from the cytoplasm to the apoptotic blebs of human salivary gland cells and fodrin disruption may be partly responsible for the membrane blebbing seen in apoptosis (67).

Alpha-fodrin as autoantigen
Alpha-fodrin was described as a candidate autoantigen in pSS and sSS in a mouse model of SS (61, 68). The NFS/sld mouse thymectomised 3 days after birth developed spontaneous inflammatory lesions in the salivary and lacrimal glands, and sera from these mice were reported to contain IgG antibodies that bound to salivary duct epithelial cells. The antigen was purified and protein sequencing revealed identity to the N-terminal part of human α-fodrin. Sera from these mice recognised a 120 kDa fragment of α-fodrin and splenic T-cells proliferated against this fragment and produced Th1-type proinflammatory cytokines. Antibodies against the 120 kDa fragment and a recombinant N-terminal fusion protein, JS-1-glutathione-S-transferase, were detected by immunoblot in sera from 41/43 (95%) of pSS and 5/8 (63%) of sSS patients, but not in SLE, RA or control sera (61).
Minor salivary gland biopsy

Histopathology
The histopathology seen in SS consists of periductal lymphocyte foci of ≥ 50 cells/focus, with normal appearing glandular tissue adjacent to the infiltrates (38). Perivascular foci can be seen and have been associated with SLE with sSS (1) whereas atrophy or fibrosis is not a feature of SS. There are several scoring systems for the number of foci/4 mm² gland (38, 69). Most commonly the number of foci per 4 mm² of glandular tissue is counted to a maximum of 10 independent foci. Glands with conflated foci are assigned a score of 12 (38). According to AECC ≥ 1 focus/4 mm² is considered pathological (16). This gives a diagnostic sensitivity and specificity of about 80% (70). The lack of sensitivity may be due to sampling errors if insufficient numbers of glands are obtained or possibly to a varying degree of inflammation in early stages (18). A lower focus score in smokers has been reported, as well as a reversal of the inflammation with high dose steroids (71, 72).

Inflammatory and epithelial cells
The infiltrating cells are predominantly activated CD4⁺ Th cells (75%), with some CD8⁺ T cells. In addition, macrophages, dendritic cells (DC) and approximately 20% B cells with a few plasma cells are present (20, 73). In 17%-65% of SS MSG, germinal centers (GC) can form with B cells and DC, characteristic of a secondary lymphoid follicle (73, 74). GC cells express CD20 (B cells) and markers for follicular DC. The presence of follicular DC may indicate that antigen presentation takes place, but whether the antigen is originating locally in the gland, or transported by inflammatory cells and trapped in the MSG GC is not known.

The inflammatory cells express MHC class I and II, the adhesion molecules intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and their ligands lymphocyte function associated antigen 1 (LFA-1) and very late activation antigen 4 (VLA-4) (74-76). The co-stimulatory molecules CD40 and CD40L (CD154) are also expressed (77) and a minor proportion of the lymphocytes express the IL-2 receptor (78).

Epithelial acinar and ductal cells also express MHC class I and II, adhesion molecules and costimulatory molecules and have the properties to act as antigen presenting cells (APC) (77, 79).
Figure 1. Minor salivary gland biopsy from a patient with Sjögren’s syndrome. The lymphocyte foci are prominent and interspersed in the normal glandular tissue. Haematoxylin-eosin staining. Magnification x 50.

Figure 2. A closer view of the same gland reveals dense lymphocytic infiltration and an area of macrophages (arrow) within this large periductal focus. Perivascular inflammation is also seen. Haematoxylin-eosin staining. Magnification x 250.
Cytokines and chemokines
Infiltrating T cells produce proinflammatory cytokines IL-1, IL-2, IL-6, IL-12, TNF-α and IFN-γ, the latter upregulating MHC I and II expression on both inflammatory and epithelial cells (75, 80). Cytokine mRNA expression in SS MSG biopsies, have also shown up-regulation of mRNA gene transcription for proinflammatory cytokines in both inflammatory and epithelial cells (80-82). The anti-inflammatory cytokine TGFβ is also produced, presumably as a regulator of inflammation (80). IFN-α production in SS MSG has occasionally been described (75). A clear Th-1 cytokine profile is therefore present in the glands.

Chemokines play an important role in recruiting inflammatory cells. The B cell-attracting chemokine 1 (BCA-1, CXCL13) is expressed by acinar and ductal epithelial and endothelial cells and its receptor CXCR5 is expressed on the infiltrating B cells (83, 84). The CXCL13 – CXCR5 interaction is crucial for attracting B cells and is absent in salivary glands from normal individuals (83). Other chemokines expressed on ductal epithelial cells and high endothelial venules (CXCL12, CCL21) attract T cells and DC (74, 83).

Taken together, the expression of these chemokines by epithelial and endothelial cells in the SS MSG shows that the acinar and ductal cells are not merely innocent bystanders, but are actively participating in the recruitment of inflammatory cells.

B cells and clonal proliferation
B cells in the salivary glands produce autoantibodies including rheumatoid factors (RF), anti SS-A/Ro and anti SS-B/La (85). The local production of autoantibodies to the SS-A subunits Ro52 and Ro60 and to the SS-B protein La48, correlates with the presence of these antibodies in sera, and it is hypothesised that a proportion of the circulating anti-SS-A and anti-SS-B antibodies are synthesised in the glands (85, 86). Anti-Ro52, Ro60 and La48 antibodies preferentially of IgA and IgM isotypes, have been demonstrated in saliva from SS patients, were the levels in saliva correlated both with the MSG focus score and plasma levels of the corresponding antibody (87).

Studies of the rearranged Ig V genes expressed by B cells isolated from SS MSG show independent clones of hypermutated B cells, indicating an antigen-driven clonal proliferation (88). An initially polyclonal B cell activation can progress to become monoclonal and eventually transform into a malignant NHL (89).

Salivary gland B cells are stimulated by B-lymphocyte stimulator (BlyS)/B cell activating factor (BAFF), which is expressed on monocytes/macrophages and DC, and is up-regulated in SS glands (90). The total number of B cells in blood is normal in SS, but there is a depletion of CD27⁺, CD5⁺ memory B cells in blood and instead an accumulation of these cells in the affected glands (80).
Apoptosis

Apoptosis in salivary epithelial cells from SS patients has been extensively studied (91). An increased apoptosis in both acinar and ductal salivary gland cells has been reported by some (92), but not by others (93). In normal human salivary gland cells, on the other hand, apoptosis is only detected in ductal cells, presumably as a result of normal cell turnover. The pro-apoptotic protein Bax is expressed in epithelial cells whereas the anti-apoptotic proteins Bcl-2 and Bcl-XL are expressed in the infiltrating lymphocytes which render them resistant to apoptotic cell death (blocked apoptosis) (77, 92).

Apoptosis in acinar and ductal epithelial cells can be stimulated by the proinflammatory cytokines synthesised in the MSG, which up-regulate the Fas-receptor on the cells (91). In addition, tumor necrosis factor receptor-1 and the mannose-6-phosphate receptor are expressed. These apoptotic receptors on the epithelial cells are ligated by FasL, TNF-α and granzyme B, respectively (94), produced by T cells in the MSG.

Autoantibodies

Antinuclear antibodies, anti-SS-A/Ro and anti-SS-B/La

ANA is present in sera from approximately 80% of SS patients, usually with a speckled pattern (7, 95). Sera from SS patients often display autoantibodies against the ribonucleoproteins SS-A/Ro and SS-B/La. The frequency in SS varies with the method used for detection and the SS criteria used (Table 2) (96, 97). Anti-SS-A antibodies are also seen in SLE and SLE-SS overlap, in RA with sSS (26%) and in RA without sSS, systemic sclerosis and polymyositis (3-15%) (1, 96, 97). Anti-SS-B antibodies are more SS specific and are seen in low frequencies in SLE and rarely in RA with sSS (1-7%) (96).

Table 2. Comparison of the frequencies of antinuclear antibodies in SS and SLE (1, 95-98)

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>Sjögren’s syndrome</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td>75-89 %</td>
<td>95-99%</td>
</tr>
<tr>
<td>anti-dsDNA</td>
<td>0-5 %</td>
<td>20-70%</td>
</tr>
<tr>
<td>anti-SS-A</td>
<td>60-100%</td>
<td>36-68%</td>
</tr>
<tr>
<td>anti-SS-B</td>
<td>40-98%</td>
<td>6-14%</td>
</tr>
<tr>
<td>anti-RNP</td>
<td>1-15%</td>
<td>30%</td>
</tr>
<tr>
<td>anti-Sm</td>
<td>0%</td>
<td>5-30%*</td>
</tr>
</tbody>
</table>

* Anti-Sm antibodies are detected in sera from 5% of European SLE patients and in sera from 30% of mixed North American SLE patients (98).

SS-A consists of two subunits, Ro52 and Ro60, referring to their molecular weight, while SS-B contains one 48 kDa protein, La. Ro52, Ro60 and La are
associated with small RNA molecules, the human cytoplasmatic RNAs, hYRNA. Ro52, Ro60 and La are distributed both within the nucleus and cytoplasm (97, 99). Ro60 and La bind directly to the hYRNAs whereas Ro52 binds to Ro60. The functions of the Ro and La proteins are not fully understood, but La binds different RNA transcripts and is thought to be a transcription termination factor for RNA polymerase III (97). Ro60 is thought to be involved in small RNA quality control and cell repair after UV exposure (100). Both Ro and La are translocated to the cell membrane during apoptosis where they presumably are exposed for the immune system (67).

Anti-SS-B/La antibodies are always seen together with anti-SS-A/Ro. (97). There is an intra- and intermolecular epitope spreading of the immune response where mice immunised with a specific La epitope, develop antibodies against several La and Ro epitopes, which may explain the coexistence of these antibodies (101).

The presence of anti-SS-A and anti-SS-B antibodies correlates with specific clinical manifestations in SS: earlier disease onset, parotid swelling, increased focus score on MSG biopsy, lymphadenopathy, peripheral neuropathy and cutaneous vasculitis (20, 96). IgG anti-SS-A and anti-SS-B antibodies are transferred across the placenta and are associated with neonatal lupus and congenital heartblock in the newborn. The risk for a SS-A/SS-B positive mother to have a child with congenital heartblock is around 2% (102). The risk has been attributed mainly to Ro52 antibodies, and recent epitope mapping has defined antibodies against a Ro52 region (aminoacids 200-239; p200) which bind to cardiomyocytes and affect Ca\(^{2+}\) homeostasis (103).

**Anti-RNP and anti-Sm antibodies**

Other autoantibodies with specificity for RNA-binding proteins are the RNP and Sm antibodies, which target small nuclear RNA (snRNA) and their attached proteins, the ribonucleoprotein (RNP) or Smith (Sm) proteins. There are 5 different snRNAs, designed U1, U3-6. U1 binds three RNP subunits, recognised by anti-RNP antibodies, namely A, C and 70kDa, which together form a U1snRNP complex, called a spliceosome. The spliceosome is involved in splicing off pre-mRNA introns. The Sm proteins consist of several subunits attached to all U RNAs, and anti-Sm antibodies are directed mainly against the B, B’ or D subunits (98). Anti-RNP and anti-Sm antibodies are seen more frequently in SLE than in SS (Table 2).

**Anti-type 3 muscarinic acetylcholinereceptor antibodies**

Salivary gland cells express muscarinic acetylcholine receptors, which respond to acetylcholine, and increase secretion from the glands. Antibodies against the M3 receptor subtype (M3R) have been described in sera from 56%-82% of patients with SS (104). These antibodies of preferably IgG iso-
type, inhibit cholinergic transmission in smooth muscle and cultured human salivary gland cells, and bind to a recombinant human M3R. Reports have shown that conventional immunological methods fail to detect M3R antibodies in SS sera, despite the ability of the same sera to inhibit cholinergic transmission in salivary gland cells (105), and M3R antibody assays have not yet been developed for clinical use.

Treatment

Local treatment

Symptomatic treatment for the sicca symptoms includes topical application of eye-drops and local treatment with saliva substitution or saliva stimulating oral spray, lozenges or chewing gum with the addition of fluoride. Topical treatment with cyclosporine A eye drops has been shown to increase tear production and decrease the inflammatory cells in the conjunctiva, but are today not available (106). Trials have evaluated the effect of IFN-α in lozenges, where an increase in unstimulated whole salivary flow has been noted as well as a reduction in the lymphocyte MSG infiltrates (107, 108). Local IFN-α up-regulates the gene expression for aquaporin 5 (AQP5), i.e. water-channels, in human parotid gland cells (109).

Systemic treatment

Salivary and lacrimal flow can be stimulated by cholinergic drugs such as pilocarpine and cevimeline. These drugs increase the unstimulated whole salivary flow as well as lacrimal flow (110). Concomitant asthma, heart disease, treatment with beta-blockers or glaucoma limits its use. Common side effects are profuse sweating, diarrhoea or problems with accommodation.

Antimalarials i.e. chloroquin or hydroxychloroquin are used for mild arthritis or arthralgia/myalgia, but the effect on sicca symptoms or fatigue is not convincing (111). There is seldom need for glucocorticoid or immunosuppressive treatment and it is important to limit the use of immunosuppressive drugs since SS per se has an increased risk for lymphoma, which is also the case for azathioprin (112). In cases of severe extra-glandular engagement of lungs with pleuritis or alveolitis, or kidneys with interstitial nephritis, glucocorticoid treatment and or cytotoxic drugs, preferably azathioprin or cyclophosphamide is used.

Supplementation with DHEA has been studied but the results to date have not shown any improvement in lacrimal or salivary flow (113). TNF-α inhibitors have been used in a few trials in SS with varying results (114, 115). The rationale for this is the presence of TNF-α and TNFR in infiltrating mononuclear cells and ductal epithelial cells in the glands and the normalisation of AQP5 by TNF-α inhibitors (115). There are anecdotal reports of patients with SS being treated with high dose glucocorticoids or cyclophos-
phamide where the sicca symptoms have resolved (72, 116). Rituximab (anti-CD20 monoclonal antibody) has been used successfully to treat SS parotid lymphoma with concomitant improvement of the sicca symptoms (117).

Systemic lupus erythematosus

History and epidemiology
SLE was first described as early as the 10th century, when the term ‘lupus’ was used medically to describe cutaneous lesions resembling a wolf bite (118). Initially lupus was associated with leg ulcers but after the 16th century considered primarily a facial lesion. The term ‘lupus erythemateux’ was introduced by Cazenave in 1856 where it was also distinguished from lupus vulgaris, i.e. tuberculosis. Soon thereafter Kaposi associated disseminated cutaneous lesions extending beyond the face with systemic symptoms and in the late 19th century Osler gave a more precise description of organ manifestations such as arthritis and nephritis. Immunological abnormalities were connected to the disease in 1909 noting a false positive test for syphilis and the LE cell was discovered in 1948, a few years before the ANA were detected.

The annual incidence has been estimated to 4.8/100 000 and the prevalence to 68/100 000 in a southern Swedish population (119). The prevalence in a white US population has been estimated to 40/100 000, while the incidence and prevalence in an Afro-American US population is three-to four fold higher (120). An increase in incidence has been reported while the mortality has decreased. The 10 year survival rate is around 90%, with disease severity, morbidity and mortality being higher in patients of Afro-Carribean descent.

Clinical features
SLE is the prototype for a systemic autoimmune disease characterised by multiple organ involvement and an array of autoantibodies and immunological disturbances, see below. A mild disease presents with dermal involvement, photosensitivity and arthralgia or arthritis. Of moderate disease severity are pleuritis, pericarditis or haematological involvement, whereas severe manifestations are those of the CNS or kidneys (121). SLE can have different disease patterns, relapsing-remitting, chronic active and long quiescent, the last seen in long-standing disease (122). The chronic active pattern is the most common, showing that despite glucocorticoid and immunosuppressive treatment the disease is persistently active in a number of patients, causing
long term morbidity (123). A debilitating feature for the patient is a low grade fever, chronic fatigue and decreased health-related quality of life (124).

**Diagnosis and classification**

A clinical diagnosis of SLE is usually made when the patient has two SLE-typical organ manifestations, together with an SLE serology (125). For classification purposes the 1982 ACR criteria are used, where a diagnosis of SLE requires at least 4/11 criteria fulfilled (Table 3). These criteria have 96% sensitivity and an equal 96% specificity (126). An attempt to revise these criteria was made in 1997 when it was suggested to change item 10 by deleting 10a, positive LE preparation, and change item 10d to include a positive finding of antiphospholipid antibodies and/or lupus anticoagulans (127). This revision, however, has not yet received ACR criteria status.

**Etiology**

SLE is a complex disease caused by interaction of genetic, hormonal and environmental factors. The genetic and hormonal aspects will be discussed elsewhere. The most prominent environmental factor is UV light, which can trigger not only a skin rash but also a severe systemic flare (128). UV exposure causes apoptosis of keratinocytes, exposing intracellular autoantigens such as DNA- and RNA-binding proteins against which SLE patients have autoantibodies (129).

Similar to SS, viruses have been implicated in the disease etiopathogenesis (49). EBV has been most closely linked to pediatric and adult SLE where anti-EBV antibodies have been reported in significantly higher frequencies in SLE patients compared to normal controls. Persistent EBV infections detected by PCR are also more common in SLE than in healthy individuals. Other viruses that have been studied include CMV, parvovirus B19 and retroviruses, none of which have been consistently linked to SLE (130).

Other exogenous agents contributing to the etiology are certain drugs such as procainamide, hydralazine and sulfasalazine, alfalfa sprouts and tobacco use whereas alcohol may be protective (131, 132).
<table>
<thead>
<tr>
<th>Criterion</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Malar rash</td>
<td>Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds</td>
</tr>
<tr>
<td>2. Discoid rash</td>
<td>Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions</td>
</tr>
<tr>
<td>3. Photosensitivity</td>
<td>Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation</td>
</tr>
<tr>
<td>4. Oral ulcers</td>
<td>Oral or nasopharyngeal ulceration, usually painless, observed by a physician</td>
</tr>
<tr>
<td>5. Arthritis</td>
<td>Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion</td>
</tr>
<tr>
<td>6. Serositis</td>
<td>a) Pleuritis - convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion</td>
</tr>
<tr>
<td></td>
<td>b) Pericarditis - documented by ECG or rub or evidence of pericardial effusion</td>
</tr>
<tr>
<td>7. Renal disorder</td>
<td>a) Persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantification is not performed</td>
</tr>
<tr>
<td></td>
<td>b) Cellular casts - may be red cell, haemoglobin, granular, tubular, or mixed</td>
</tr>
<tr>
<td>8. Neurologic disorder</td>
<td>a) Seizures - in the absence of offending drugs or known metabolic derangements; e.g. uremia, ketoacidosis, or electrolyte imbalance</td>
</tr>
<tr>
<td></td>
<td>b) Psychosis - in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance</td>
</tr>
<tr>
<td>9. Hematologic disorder</td>
<td>a) Hemolytic anemia - with reticulocytosis OR</td>
</tr>
<tr>
<td></td>
<td>b) Leukopenia - less than 4,000/mm³ total on 2 or more occasions OR</td>
</tr>
<tr>
<td></td>
<td>c) Lymphopenia - less than 1,500/mm³ total on 2 or more occasions OR</td>
</tr>
<tr>
<td></td>
<td>d) Thrombocytopenia - less than 100,000/mm³ in the absence of offending drugs</td>
</tr>
<tr>
<td>10. Immunologic disorder</td>
<td>a) Positive LE cell preparation OR</td>
</tr>
<tr>
<td></td>
<td>b) Anti-DNA: antibody to native DNA in abnormal titer OR</td>
</tr>
<tr>
<td></td>
<td>c) Anti-Sm: presence of antibody to Sm nuclear antigen OR</td>
</tr>
<tr>
<td></td>
<td>d) False positive serologic test for syphilis known to be positive for at least 6 months and confirmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test</td>
</tr>
<tr>
<td>11. Antinuclear antibody</td>
<td>An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with 'drug-induced lupus' syndrome</td>
</tr>
</tbody>
</table>
The immune system

Immune cells in lupus

SLE patients have several abnormalities of the immune system, including the immune cells, autoantibody production, IC formation and clearance by the complement system. B cells in SLE show increased proliferation and production of high affinity autoantibodies that have undergone somatic hypermutation as a sign of an antigen-driven immune response. This B cell activation is T cell dependent. The ability of Th cells to recognise self antigens is due to a loss of tolerance (133). When Th cells recognise self peptides presented on MHC class II proteins, cytokines are produced that stimulate B cells to undergo class-switching followed by hypermutation, producing autoantibodies of high affinity. SLE patients have a decreased number of T cells with varying CD4+/CD8+ ratios and an increased apoptosis of T cells is seen in SLE (133, 134).

Dendritic cells are central in autoimmunity, being professional APC. Studies in SLE have reported a reduced proportion of DC among the peripheral blood mononuclear cells (PBMC), with reduced expression of adhesion molecules and a diminished T cell stimulatory capacity (135). This could be explained by a lack of immature DC progenitors due to a biased differentiation from stem cells, recruitment of DC to inflamed tissues or an increased apoptosis of these cells. The natural interferon producing cell/plasmacytoid dendritic cell (NIPC/PDC) is described in the chapter on the type I IFN system.

Autoantibodies

ANA are detected in sera from 95-99% of patients with SLE, with a homogenous or speckled pattern, and antibodies against dsDNA, SS-A, SS-B, RNP and Sm antigens are also seen (Table 2). The occurrence of anti-SS-A antibodies correlate to photosensitivity and rash seen in the ANA negative subacute cutaneous lupus erythematosus (136). Sm antibodies are rare but almost pathognomonic for SLE and their presence is part of the immunological criterium for SLE (126). Recently anti-RNP and anti-Sm antibodies have been associated with an activation of the type I interferon signalling pathway in SLE (137). Anti-Sm antibodies are always seen together with anti-RNP antibodies, whereas the latter can be seen alone.

Antibodies against double stranded DNA (dsDNA) are present in 20-70% of SLE patients depending on disease activity and have 95% specificity for SLE (138). IC consisting of DNA and anti-dsDNA antibodies can be found in the kidneys and a rising anti-dsDNA level seems to be associated with a renal flare, although not all anti-dsDNA antibodies are pathogenic (139). Anti-dsDNA antibodies represent a heterogeneous population binding to different parts and types of DNA, including nucleosomes and hypomethylated CpG-rich DNA normally seen in bacteria but also in increased levels in
SLE (140). Other antibodies against nucleic acid binding proteins are the ribosomal P and histone antibodies seen in neuropsychiatric or drug-induced lupus, respectively.

Antibodies against the first complement component C1q have also been associated with nephritis and are seen in the hypocomplementaemic urticarial vasculitis syndrome (141). Antiphospholipid and anti-beta-2-glycoprotein antibodies are also directly pathogenic causing arterial and venous thrombosis, and are the basis of the false positive test for syphilis. Other antibodies seen in SLE are directed against blood cells or endothelium (142).

**Immune complexes and the complement system**

Antigens and antibodies may form IC. IC are cleared by the complement system by initially binding to C1q in the classical pathway. An increased IC binding activates the complement system which is reflected in a decreased function of the classical pathway with decreased levels of C3 and C4, and increased levels of C3d, being a split product of C3. A defect in clearance of IC is seen in SLE due to complement deficiencies and during active disease where complement factors are consumed (143). IC are also cleared by binding to Fc gamma receptors (FcγR) on macrophages. Functional polymorphisms of FcγR are seen in SLE, where low affinity receptors affect the IC clearance, see below (144).

**Genetics**

**SLE – a complex disease**

SLE is a genetically complex disease, i.e. not caused by a single gene in a simple Mendelian inheritance pattern, but rather by several susceptibility genes acting together on a genetic background (145, 146). There is a well documented familial aggregation of SLE with 10% of first-degree relatives also affected, and a positive test for ANA is more common among SLE relatives than in the normal population (147). The concordance is 2-5% in dizygotic twins and 24-48% in monozygotic twins (148). The risk for siblings of SLE patients to develop the disease is 2% (1/50), which compared to the population incidence risk of 1/1000-1/2000 comprises a 20-40 fold increased risk. These numbers are similar to the genetic component of other autoimmune diseases such as RA and IDDM (145).

Genetic studies of complex diseases can be performed by two main methods: linkage analysis or association studies (149). In linkage analysis families with more than one affected individual, multiplex families, are analysed. Genetic markers called DNA microsatellites are commonly used in linkage studies as they are highly informative. The theory is that genetic markers inherited with a disease phenotype are likely to be close to the disease sus-
ceptibility loci on the chromosome. This method defines large genetic distances containing possibly hundreds of genes and the exact disease-associated gene can be difficult to define (145, 146). Linkage analysis using single nucleotide polymorphisms (SNPs) as markers, see below, are less informative than microsatellites, but are more abundant in the genome and can therefore be used to map disease loci with higher resolution (150).

Association studies are case-control studies of affected individuals compared to unrelated healthy controls matched for ethnicity, sex, age and preferably area of residence. This is used for analysing candidate genes, i.e. when there is a hypothesis of which genes may be involved in the disease pathogenesis. Comparing two chromosomes of two human individuals, on average 1:1000 base pairs differ, so called single nucleotide polymorphisms (SNPs) (151). SNPs are genotyped and the allele frequencies in affected individuals are compared to those in healthy controls. The SNP itself may not be involved in the disease pathogenesis, but may be in close proximity to the disease associated gene. Closely located alleles have a low recombination fraction, are transmitted together and are in linkage disequilibrium (LD). Alleles in LD constitute haplotypes (150).

**Linkage susceptibility loci**

Several loci have been found to show linkage to SLE in different studies and different populations (152, 153). Some of the best confirmed loci are 1q23 where FCGR2A and FCGR3A are strong candidate genes, 6p11-21 harbouring the MHC haplotypes and C4 genes, and 2q37 where the the programmed cell death-1 (PDCD-1) gene is a likely candidate gene (152, 154, 155). Other regions have also been defined including 19p13.2 where the Tyk2 gene is located (154, 156).

**Genes in the MHC loci**

The strongest genetic association with autoimmune diseases is shown by the highly polymorphic MHC genes on chromosome 6. In SLE, associations with MHC class II and III genes have been made where HLA-DR2 and 3 confer a 2-3 times increased risk (145). In Caucasians HLA-DRB1*0301 (DR3) and HLA-DRB1*1501 (DR2) as well as the extended haplotypes DRB1*1501/DQB1*0602 and DRB1*0301/DQB1*0201 are associated with SLE (157). The latter is also seen in SS and is associated with the presence of anti-SS-A/SS-B antibodies (44).

Complement components C4 and C2 are located among the MHC class III genes and the C4 deficiency C4A null (C4A*Q0) allele is associated with a 75% risk of developing SLE, while the risk in C2 deficiency is about 20% (143). The C4A*Q0 allele is in LD with several SLE susceptibility MHC II alleles and the exact contribution of C4A*Q0 is difficult to estimate (145, 158).
Genes in non-MHC loci

C1q (chromosome 1p36) deficiency is extremely rare, but when present in homozygous form gives a risk for SLE of > 90% (143). FcγRII (CD32) and FcγRIII (CD16) bind to IC and functional polymorphisms in the genes have been demonstrated. In FCGR2A, codon 131 translates to either a histidine (H) or an arginine (R), where homozygous R131/R131 have low affinity for IgG2 and an increased risk for lupus nephritis (144). FcγRIIa is also required for IFN-α production by NIPC/PDC after stimulation with interferogenic IC (159). Polymorphisms in the genes for IL-10, complement receptor 1 and mannose-binding lectin have also been associated with SLE (145).

A regulatory polymorphism in the PDCD1 gene encoding the immunoreceptor PD-1, which is thought to control peripheral tolerance, has been identified and its ligand PD-L1 is IFN-α regulated (155).

Hormonal influence

The onset and development of SLE is strongly influenced by sex hormones, with female gender as one of the greatest risk factors for developing the disease (160). The female:male ratio of 9:1 is observed during the fertile years, whereas there is less gender difference in incidence among older patients. The disease has a tendency to flare during pregnancy (161) and when using oral contraceptives (162). Hormone replacement therapy can precipitate SLE (163), but appears to be safe in stable patients in remission (164).

In a meta-analysis of several studies on sex hormone levels in women and men with SLE, most patients had levels within normal limits but still significantly different from healthy controls (160). This was particularly true for female patients who had increased levels of estradiol and prolactin and decreased levels of progesterone and androgens, including DHEA and its sulphated form DHEAS and testosterone. The reason for this is not clear, but alterations in the metabolism of estrogens and androgens have been described (165, 166) and androgen levels in female SLE patients are particularly low when the disease is active (167). There are fewer studies on men, but meta-analysis only revealed increased prolactin levels, whereas the estrogen and testosterone levels were normal. There were not enough studies to assess the DHEA/DHEAS levels in male SLE patients (160).

Dehydroepiandrosterone

DHEA is the main adrenal steroid and is sulphated into DHEAS in the adrenal gland and in the peripheral tissues, the serum levels of DHEAS being a thousand-fold higher than DHEA (Figure 3). DHEA is also further metabolised to more active steroids such as androstenedione, testosterone and estrogens (168) and the effects of DHEA are probably partly mediated through
this conversion. No specific nuclear receptor for DHEA has yet been identified.

![Figure 3](image_url)

**Figure 3.** DHEA is synthesized in the adrenal cortex in several steps from cholesterol. DHEA is sulphated into DHEAS in the adrenals and peripheral tissues in a reversible reaction. DHEA is also converted into androstenedione, testosterone and estrogens.

DHEAS levels are depressed in Addison’s disease (169, 170), hypopituitarism (171) and during glucocorticoid treatment. Ongoing glucocorticoid treatment is part of the explanation for the low levels seen in females with SLE but serum levels are reduced even in untreated SLE patients (172).

**Quality of life**

Patients with SLE have a worse perception of health and well-being compared to controls, measured by the widely used quality of life instrument Short-Form 36 (SF-36) (124, 173). In a study using SF-36 where SLE patients were compared to patients with RA, the quality of life scores were similar in the two disease groups, except for physical function, where the RA patients scored significantly lower, indicating more physical disability (124). The SF-36 scores in SLE have been correlated to disease activity measured by the SLE disease activity index (SLEDAI) (174), and to permanent organ damage measured by the Systemic Lupus International Collaborating Clinics/American College of Rheumatology (SLICC/ACR) damage index. (175, 176). Furthermore, the SF-36 domain ‘mental health’ score predicts depression in patients with SLE (177).
Treatment

Glucocorticosteroids are frequently used in low doses for skin and joint manifestations and as long term maintenance therapy. Moderate doses are used for serositis and cytopenias and high doses for short periods in nephritis or CNS involvement. The side effects of glucocorticoids are numerous and SLICC organ damage is correlated to cumulative glucocorticoid use (123).

Antimalarials are beneficial for skin and joint manifestations. One mechanism for action is inhibition of the acidification of the endosomal compartment, interfering with antigen loading on MHC. Immunosuppressive and cytotoxic drugs are used when needed, most commonly azathioprin and cyclophosphamide. In recent years mycophenolate mofetil has shown efficacy as maintenance therapy in lupus nephritis (178). Methotrexate or cyclosporine A are also sometimes used.

B cell therapy with anti-CD20 monoclonal antibodies (Rituximab) is emerging as a promising treatment for hematologic manifestations and nephritis (179). The B cell tolerogen LJP 394 decreases anti-dsDNA titers but the clinical benefit in reducing renal flares is limited (180). T cells are targeted by CTLA-4Ig which binds to CD80 on APCs, blocking the costimulatory CD80/CD28 interaction. This inhibits the activation of CD4+ Th cells, a central mechanism in immune activation. CTLA-4Ig has not yet been used in SLE. Anti-cytokine therapy with IL-10 monoclonal antibodies is also being investigated (179). TNF-α blockers are generally contraindicated due to their ability to induce ANA and anti-dsDNA antibodies but single trials have been performed showing that TNF-α blockers reduce proteinuria and arthritis in SLE patients without causing a disease flare (181). Autologous stem cell transplantation has been performed in severe cases and small trials. The remission rate has been around two thirds but procedure related mortality is not negligible (179).

The type I interferon system

General overview

The type I interferons

Interferons (IFNs) are cytokines that by definition interfere with virus infections. The IFNs are devided into types I, II and the recently described type III (182, 183). Type I IFNs consists of IFN-α, -β, -ε, -ω and -κ, which bind to the same receptor, the type I IFN receptor (IFNAR). There are 13 IFN-α genes with high homology on chromosome 9, all without introns, and single genes for the other type I IFNs (182). Type II consists only of IFN-γ and type III of IFN λ 1-3, none of which bind to the IRNAR (183).
The type I IFN system consists of the type I IFNs described above, the IFN-producing cells, the stimuli for IFN production, the IFNAR with its signalling pathways and the target cells for IFN action.

**The interferon-α producing cell**
Many cells can produce IFN-α/β in response to viral infections, including monocytes and myeloid DCs but the major IFN-α producing cell is the NIPC/PDC (184, 185). The NIPC/PDC represent only 0.1% of the PBMC, but is 10 times as effective as the monocyte in producing IFN-α (186). NIPC/PDC also produce low levels of IL-12, IL-6 and TNF-α, with a clear Th1 profile and the chemokines IL-8, IP10, CCL3 and CCL4 (185, 187). Rather than circulating, the NIPC/PDC mainly reside in primary (bone marrow and thymus) and secondary (lymph nodes, spleen, tonsils) lymphoid organs and can be found in inflamed tissues such as SLE or psoriatic skin rash (188, 189).

The origin of the NIPC/PDC, as to whether it is of myeloid or lymphoid lineage is unclear and NIPC/PDC lack common T- and B-cell markers and are negative for the myeloid markers CD11b, CD11c and the costimulatory molecules CD80/86 (190). Surface molecules on NIPC/PDC include MHC II, CD4, CD36, CD40, CD44, CD72, CD83, IL-3R (CD123), FcγRIIa and the chemokine receptor CXCR3, whereas toll like receptors (TLR) 7 and 9 are expressed in endosomes (159, 190, 191). The surface molecules blood dendritic cell antigen-2 (BDCA-2) and BDCA-4 are expressed exclusively on NIPC/PDC and can be used to identify this cell population (192).

**Stimuli for IFN-α production and its receptors**
Both exogenous and endogenous inducers can activate IFN-α production. Exogenous inducers are most viruses, bacteria including Mycobacterium tuberculosis and some parasites (185, 193). Nucleic acids are strong inducers of IFN-α/β production in NIPC/PDC, notably viral dsRNA, the synthetic dsRNA analogue poly I:C and bacterial unmethylated CpG DNA motifs. Unmethylated 5’CpG3’ dinucleotides form short oligodeoxynucleotides (ODN) with immunostimulatory (is) properties, is-DNA. Unmethylated 5’CpG3’ motifs are a property of bacterial DNA, whereas eukaryotic DNA contains less CpG motifs and the cytosine is often methylated. Synthetic drugs such as imiquimod used to treat condylomas caused by human papilloma virus, induces IFN-α production through binding to TLR 7 (194).

TLR 1-11 recognise a wide variety of microbial antigens and molecules, the so called Pathogens Associated Molecular Patterns (PAMP), and initiate the innate immune response against the invading microbes. TLR 7 and 9 are the main TLRs expressed in NIPC/PDC, confined to the endosomal compartment (195). TLR signal via different intracellular pathways, where phos-
phorylation of IFN stimulatory factors (IRF) -3, -5 and -7 takes place, ultimately leading to IFN-α gene transcription and protein synthesis (Figure 4).

Endogenous IFN-α inducers are IC consisting of nucleic acids and antibodies. IC bind to FcγRIIa on NIPC/PDC, are internalised in endosomes, bind to TRL and initiate cell signalling that leads to IFN-α production as described above (Figure 4). NIPC/PDC only express FcγRIIa, which recognise IC, and is also expressed on DC, monocytes, macrophages, B cells and granulocytes. Polymorphisms in the FcγRII genes have been described in patients with SLE (144).

**Figure 4.** Activation of NIPC/PDC by viral RNA/DNA or CpG-rich DNA binding to TLR 7 and 9, or by immune complexes containing endogenous nucleic acids/nucleic acid binding proteins as autoantigens and autoantibodies against dsDNA or RNA/RNA-binding proteins, which bind to FcγRIIa. This complex is internalised into endosomes where it binds to TRL 7/9. Subsequently IRF -3, -5, and -7 are phosphorylated and promote type I IFN gene transcription and protein synthesis.

**IFNAR and the Jak-Stat signalling pathway**

IFN-α and -β act on the same receptor, the IFNAR, which consists of 2 subunits, IFNAR-1 and IFNAR-2 (182, 196). Binding of IFN-α/β causes the receptor subunits to form a heterodimer. The main signalling pathway through the IFNAR is the Jak-Stat pathway. This involves tyrosine kinase 2 (Tyk2) which interacts with IFNAR-1 and Janus kinase 1 (Jak1) which inter-
acts with IFNAR-2. Binding of IFN-α/β activates Tyk2 and Jak1, which phosphorylate signal transducer and activator of transcription (Stat) 1 and 2. Activated Stat1 and Stat2 associate with IRF9 to form the complex ISGF3 (interferon stimulated gene factor 3), which translocates to the nucleus and bind to interferon-stimulated response elements (ISRE). ISRE induce transcription of a wide variety of genes encoding for proteins with anti-viral and immunoregulatory properties. In addition, ISRE induces transcription of IRF-3, -5 and -7, which all also act in transcription of IFN-α/β induced genes, and IRF induced genes, in a positive feedback manner (Figure 5).

IRF-5 is constitutively expressed in NIPC/PDC, DC, monocytes and B cells, and forms a complex in the cytoplasm with IRF-3 and -7. Upon viral infections IRF-5 is rapidly phosphorylated and translocated to the nucleus where, together with IRF-3 and -7, it promotes transcription of IFN-α genes resulting in IFN-α production, as well as promoting transcription of IFN- and IRF-induced genes (197). IRF-5 also induces transcription of a number of genes coding for proteins involved in cell signalling, apoptosis and cell cycle regulation. IRF-5 can be upregulated by the tumor suppressor gene p53 and consequently is in many ways involved in cell proliferation. Lack of IRF-5 is also seen in primary lymphocytic malignancies (197).

**Figure 5.** IFN-α/β bind to the IFNAR which activates Tyk2 and Jak1 that phosphorylate the transcription factors Stat1 and Stat2. Stat1 and 2 associate with IRF9 to form a complex, interferon stimulated gene factor 3, ISGF3, that interacts with IFN stimulated response element, ISRE, and initiates gene transcription for IFN-induced proteins and IRF induced proteins. IRF-3, -5 and -7 also promote these gene transcriptions.
Effects on the immune system
IFN-α acts on many cells and has a pleiotropic effect on both the innate and adaptive immune systems (191). An important effect is antiviral, by inhibiting viral replication, inducing anti-viral proteins and inducing apoptosis in virus-infected cells (182). Proliferation of tumour cells is inhibited and IFN-α treatment is being used for certain malignancies such as hairy cell leukaemia and carcinoids, in addition to treatment of HCV (182, 198).

IFN-α has several immunomodulatory effects. DC are matured with increased expression of MHC I and II, CD80/86, chemokines, chemokine-receptors, BlyS and IL-12 production. NIPC/PDC are matured with increased IFN-α production, so called priming. Th cells are stimulated into a Th1 response with upregulation of the IL-12 receptor and Tc have increased cytotoxic activity. B cells undergo an Ig isotype switch and both T and B cells have prolonged survival. NK cell and macrophage activity is also stimulated (195). Taken together IFN-α enhances antigen presentation, a Th1 response and B cell activation and serves as a link between innate and adaptive immunity (191). The IFN-α production by NIPC/PDC can be inhibited by TNF-α and IL-10 (199).

Role in autoimmunity
IFN-α in SLE
The type I IFN system has been extensively studied in SLE (200). Increased serum levels of IFN-α have been noted in SLE patients and correlate with disease activity and number of organs involved. A correlation with specific clinical (fever and skin rash) and serological (high anti-dsDNA titer, low complement and leukopenia) manifestations has been reported (201). Gene expression profiling from SLE PBMC has revealed an upregulation of interferon stimulated genes, the IFN signature, as a sign of an in vivo relevance of the ongoing IFN-α production (202, 203). This signature is most prominent in patients with severe disease manifestations: renal, CNS and haematologic, and in pediatric SLE of recent onset (202).

Sera from SLE patients, but not from controls, have the capacity to stimulate PBMC from healthy blood donors to produce IFN-α. The IFN-α stimulating factor consists of DNA and anti-dsDNA antibodies in complex and has been denoted the SLE-interferon inducing factor (SLE-IIF) (204, 205). Interferogenic IC bind to FcγRIIa on NIPC/PDC and blocking of these receptors inhibits the IFN-α producing ability of the SLE-IIF (159, 206).

Increased apoptosis and reduced clearance of apoptotic cells is a feature of SLE (134). The IFN-α production in healthy PBMC, induced by SLE sera in combination with apoptotic U937 cells, showed that in vitro, neither DNA nor anti-dsDNA antibodies were necessary. Instead there was a correlation with the presence of anti-RNP antibodies (206).
It was later confirmed that apoptotic and necrotic U 937 cells and necrotic PBMC, released material that together with SLE-IgG could induce IFN-α production in NIPC/PDC. Enzymatic DNase treatment inhibited the IFN-α production induced in combination with apoptotic U937 cells, whereas RNase treatment inhibited the induced IFN-α from both apoptotic and necrotic U937 cells and necrotic PBMC. Also, only sera containing antibodies to RNA-binding proteins (anti-SS-A, anti-SS-B, anti-RNP and anti-Sm antibodies) had the IFN-α inducing capacity in combination with apoptotic or necrotic U937 cells (207). The nature of the RNA/RNA-binding proteins as the antigen component in the IC is being investigated (Lövgren et al, unpublished data).

The cells responsible for the IFN-α production in response to IC have consistently proven to be NIPC/PDC, confirmed by their expression of the markers BDCA-2 and -4 and ligation of BDCA-2 inhibits the IFN-α-production (208). Patients with SLE have reduced numbers of circulating NIPC/PDC but with an intact IFN-α producing capacity (209). Elevated serum IFN-α is probably derived from NIPC/PDC recruited to tissues, mainly lymph nodes and skin where these cells have been detected (189, 210). The capacity for normal PBMC to produce IFN-α after stimulation with SLE-IIF is greatly enhanced by an initial stimulation of the cells with IFN-α2b and GM-CSF, so called priming, as above (199, 204).

**IFN-α in other autoimmune diseases**

Activation of the type I IFN system is likely to be a general autoimmune mechanism where tolerance is broken and autoimmunity promoted. Evidence for this is the number of autoimmune conditions that can be seen after IFN-α treatment, most commonly thyroid antibodies and thyroiditis, but also autoimmune haemolytic anemia, RA, IDDM and polymyositis (198, 211).

Signs of ongoing IFN-α production are seen in IDDM, where pancreatic islet cells express IFN-α and increased serum levels of IFN-α have been reported (212). Psoriatric skin lesions have infiltration of NIPC/PDC with an ongoing IFN-α production (188). Muscle biopsies from dermatomyositis patients show an increase in the IFN-α induced gene expression and a positive immunohistochemical staining for BDCA-2 and IFN-α, indicating that the IFN-α producing cell is the NIPC/PDC (213). RA synovia and liver biopsies from patients with primary biliary cirrhosis (PBC) have also shown an activated type I IFN system (214, 215).
Present investigation

Aims of the project

The general aim of the thesis was to study autoimmune processes in SS with regard to a novel autoantibody as well as the type I IFN system, linking SS to SLE autoimmune processes, and to study the genetics of the type I IFN system in SLE. In addition, the important health-related quality of life was studied in SLE.

The specific aims were:

- To evaluate the significant value of analysing α-fodrin autoantibodies in the diagnosis of primary SS and its possible clinical correlates (paper I).

- To study the type I IFN system in primary SS and its possible activation both systemically and locally in the affected salivary glands (paper II).

- To identify candidate genes related to the type I IFN system, which may be associated to disease susceptibility of SLE (paper III).

- To evaluate the effects on health-related quality of life by androgen supplementation in women with SLE (paper IV).
Materials and methods

Patients

Primary Sjögren’s syndrome patients

A total of 62 patients with primary Sjögren’s syndrome were included from the rheumatology clinic in Uppsala, Sweden, and participated in paper I (n = 56) and paper II (n = 38). 32 patients participated in both studies I and II. All patients fulfilled the European criteria from 1993 (33). In paper I 49 patients also fulfilled the San Diego criteria (216). For the purpose of paper II, the 38 patients included who all fulfilled the San Diego criteria, were reclassified according to the new AECC, and 36/38 fulfilled these criteria (16). The whole cohort consisted of 57/62 females (92%) and 5/62 (8%) males, with a mean age of 57.9 ± 12.4 years and a disease duration of 11.3 ± 8.8 years. Autoantibody frequencies were detected as follows: ANA 66%, anti-SS-A 56%, anti-SS-B 32%, RF 54%. A minor salivary gland biopsy was performed in 39/62 (63%) and 74% had a focus score ≥ 1 (38). Extraglandular disease was seen in 73%, most commonly Raynaud’s phenomenon and arthritis (30%). 7/62 (11%) had an associated thyroiditis and 2/62 (3%) had a previous lymphoma, both in the parotid gland.

SLE patients

A total of 714 patients with SLE were included in the studies, where 95 patients were included from the rheumatology clinic in Uppsala and participated in studies I-IV (Table 4). All patients fulfilled the ACR criteria (126). In the Uppsala cohort there were 82/95 (86%) females and 13/95 (14%) males.

Table 4. SLE patients participating in the studies

<table>
<thead>
<tr>
<th>Center</th>
<th>paper I</th>
<th>paper II</th>
<th>paper III</th>
<th>paper IV</th>
<th>total</th>
</tr>
</thead>
<tbody>
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<td>78</td>
<td>23</td>
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</tr>
<tr>
<td>Lund</td>
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<td>-</td>
<td>161</td>
<td>18</td>
<td>179</td>
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<td>Umeå</td>
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<td>-</td>
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<td>Finland</td>
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</tr>
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<td>Iceland</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>-</td>
<td>30</td>
</tr>
<tr>
<td>TOTAL</td>
<td>67</td>
<td>20</td>
<td>679</td>
<td>41</td>
<td>714</td>
</tr>
</tbody>
</table>

* Five patients were included in all 4 studies, 22 in 3 studies, 34 in 2 and 34 patients in 1 study.

In vitro transcription and translation assay

In paper I, serum reactivity against α-fodrin was evaluated using an in vitro transcription and translation assay (ITT). The cDNA clone (base pairs 1-1784), encoding JS-1, corresponding to the amino-terminal 561 aminoacids of human α-fodrin, was provided in the vector pGEM4 (59). The JS-
1/pGEM4 plasmid was propagated in E.Coli after electroporation, the JS-1 insert cleaved with restriction enzymes and subcloned into the cloning vector pCR Script™ SK(+), and finally ligated into the ITT vector pSP64 poly A. The insert was sequenced to verify the JS-1 sequence.

An ITT was performed in a single tube reaction with the JS-1/pSP64 poly A plasmid as template, RNA polymerase, RNase inhibitors, aminoacids except methionine which was added as (35S)-methionine, and a reticulocyte lysate. The size of the radioactive (35S)-methionine JS-1 protein was analysed by sodium dodecyl sulphate polyacrylamide gel electrophoresis, and had the expected size of 64 kDa.

Immunoprecipitation with patient sera and the (35S)-methionine JS-1 antigen was performed, bound antibodies precipitated by protein A Sepharose and unbound JS-1 washed away. Samples were transferred to a microtiter plate and radioactivity counted in a MicroBeta counter. A JS-1 antibody index was calculated as: (cpm sample-cpm negative control)/(cpm positive control/cpm negative control) x 100 (217). An SS patient with high anti-JS-1 antibody titers served as a positive control, and bovine serum albumin as a negative control. A cutoff index value was set as the mean index value for healthy blood donors +3 S.D. Since not all sera were analysed in the same reaction, the majority of sera being analysed at least twice, and the cutoff index value varied between assays, the results are given as S.D. A serum with an S.D. >3 is considered positive for anti-JS-1 (anti-α-fodrin antibodies).

![Diagram of in vitro transcription and translation](image)

Figure 6. In vitro transcription and translation of the JS-1 cDNA clone, in a single reaction, where the (35S)-methionine labelled JS-1 protein is synthesised, then mixed with patient sera, precipitated on protein A Sepharose, and the radioactivity measured in a beta-counter, as a measure of bound anti-JS-1 antibody (217, 218).

Interferon-α immunoassays and induction

Serum levels of IFN-α were measured using a dissociation-enhanced lanthanide fluorimunoassay (DELFIA). The solid phase was coated with the monoclonal antibodies (mAb) LT27:273 and LT27:293, which recognise...
most IFN-α subtypes but not IFN-α2b used in the priming of PBMC cultures (205). Patient sera were added and a secondary europium-labelled anti-IFN-α mAb LT27:297 used for detection.

In IFN-α induction, blood donor PBMCs were cultured in 96-well plates together with the priming cytokines IFN-α2b and GM-CSF. IFN-α inducers (patient serum/patient IgG/synthetic dsRNA poly I:C/ dsDNA plasmid pcDNA3/HSV/ODN 2216) were added and IFN-α produced in the cultures were measured as described above, simplified by only using mAb LT27:293 as capture Ab (206). In some experiments, monoclonal antibodies against the FcγRIIa or RNase A/DNase 1 were added, respectively.

A bovine polyclonal anti-IFN-α Ab was used for immunohistochemistry of MSG, with a biotinylated goat anti-bovine secondary Ab for detection. In the FACS analysis, a biotinylated anti-IFN-α mAb was used, LT27:295, together with a FITC-conjugated anti-BDCA-2 antibody.

Genotyping on microarrays

Genotyping in paper III was performed using an ‘in-house’ four colour fluorescent tag-array minisequencing method (219, 220). DNA from SLE patients, relatives and controls was extracted from whole blood. The DNA sequence flanking the SNP of interest was amplified with multiplex PCR in 384 well plates. Prior to the minisequencing PCR reagents (dNTPs and PCR primers) are removed using exonuclease and phosphatase. Detection primers complementary to the template but terminating on the nucleotide 5’ to the SNPs are designed. Fluorescent dideoxynucleotides (ddNTP), each with a different fluorophore, and DNA polymerase were added. The ddNTP complementary to the SNP allele will extend the primer at the 3’end and base pair with the SNP nucleotide on the template. Further extension is prohibited by the dideoxy state of the nucleotides.

A 20-base SNP-specific sequence ‘tag’ on the 5’end of the detection primer is complementary to an anti-tag attached to a glass surface and is used to capture the extended detection primers by hybridisation (221, 222). The hybridisation takes place in an ‘array of arrays’ format allowing up to 80 samples and 200 oligonucleotides to hybridise on each microarray slide. Fluorescence is detected using a microarray laser scanner, data interpreted and the genotypes determined (223).
Health-related quality of life questionnaires

The Medical outcomes survey (MOS) SF-36 is a generic instrument i.e. can be used in patients and healthy populations, regardless of age, sex and disease, for assessing HRQOL (173, 224). SF-36 is the MOS recommended for use in SLE (225). It consists of 36 questions divided into 8 different domains, and one question on health transition (improvement/deterioration) over the last year, not included in the final score. Domains are as follows: physical function (PF), role limitation due to physical problems (RP), bodily pain (BP), general health (GH), vitality (VT), social function (SF), role limitation due to emotional problems (RE) and mental health (MH). The scores are transformed to a 0-100 scale, where a higher score indicates better HRQOL. Normative data for different populations, including the Swedish, is available. PF, RP, BP and GH constitute the physical component summary score (PCS) and VT, SF, RE and MH the mental component summary score (MCS) (226). Calculating the PCS and MCS in its original, involves adjusting to the US 1998 general population norm, where the mean is 50 and the S.D. is 10.

The Psychological General Well-being index (PGWB) is a validated questionnaire comprising 22 items covering essentially the same aspects of HRQOL as SF-36 (227). The score range is 32-122, with a higher score for better well-being. Hopkins Symptom Check List 56 (HSCL-56) covers psychological and somatic problems related to mental distress, where each item is scored on a 0-4 scale (228). A lower score indicates better mental health. McCoys sex scale questionnaire is adjusted to a Swedish version, where 9
questions on a 1-7 scale, are divided into 3 domains. Normative data for Swedish women are available for both PGWB, HSCL-56 and McCoy (229-231) The partner questionnaire comprising 12 questions, was developed to assess changes in mood and behaviour in men and women with growth hormone (GH) deficiency (232).

SLE disease activity and damage indices

SLEDAI score
There are several scoring systems for evaluating disease activity in SLE, where the SLE Disease Activity Index (SLEDAI), Systemic Lupus Activity Measure (SLAM) and the British Isles Lupus Assessment Group (BILAG) are the best validated and most commonly used (233). In the original SLEDAI, disease activity was defined as reversible manifestations of the disease with new onset or recurrence within the last 10 days (174). A later version, SLEDAI-2K allowed persistent activity to be scored (234). In the current studies (papers I, IV) a modified SLEDAI (mSLEDAI) score has been used in which complement levels and anti-dsDNA antibodies are excluded (122). In short, the mSLEDAI score gives weighted values for different organ system disease activities at the time of assessment or during the previous 10 days. The maximum mSLEDAI score is 101 but in practice a score of >20 is seldom seen (Table 5). A mild increase in disease activity may not be captured with the mSLEDAI score.

Table 5. Weighted mSLEDAI scores

<table>
<thead>
<tr>
<th>activity parameter</th>
<th>score</th>
</tr>
</thead>
<tbody>
<tr>
<td>seizures, psychosis, organic brain syndrome, visual disturbances, cranial nerve disorder, lupus headache, cerebrovascular accident, vasculitis</td>
<td>8</td>
</tr>
<tr>
<td>arthritis, myositis, urinary casts, hematuria, proteinuria, pyuria</td>
<td>4</td>
</tr>
<tr>
<td>new rash, alopecia, mucosal ulcers, pleurisy, pericarditis</td>
<td>2</td>
</tr>
<tr>
<td>fever, thrombocytopenia, leukopenia</td>
<td>1</td>
</tr>
</tbody>
</table>

SLICC/ACR damage index
Irreversible organ damage, after the onset of SLE, regardless of its cause and present for at least 6 months, is assessed in the Systemic Lupus International Collaborating Clinics/ACR (SLICC/ACR) damage index, and used in paper IV (175). The accumulated damage is correlated to disease duration, cumulative corticosteroid dose, high disease activity at diagnosis and disease activity over the years (123, 235).

Statistical analysis
Parametric tests for comparison between groups were used in paper I (Student’s unpaired t-test) whereas non-parametric tests were used in papers
II, III, and IV. Comparison between groups was done by Mann-Whitney U test (papers II, III, IV) and for comparison within groups Wilcoxon’s signed rank test was used (paper IV). Comparison of frequencies was done by Fisher’s exact test in all papers, and correlations were sought by Spearman’s rank correlation (papers II, IV). In paper IV, Friedman’s test for repeated measurements was used to analyse overall within-group effect over time. $P < 0.05$ was considered significant for all analysis above.

In paper III the Pseudomarker program was used for joint linkage and association analysis (236). $P < 10^{-4}$ was considered significant according to this program. A Bonferroni correction for multiple comparisons was performed. Logistic regression analysis was used for correlation of genotypes with clinical data.
Results and discussion

Autoantibodies to α-fodrin are detected in primary Sjögren’s syndrome and SLE in similar frequencies and are of limited value (Paper I)

The initiative for this work came in 1997 after Haneji et al. published their results describing α-fodrin as an organ-specific candidate autoantigen in SS (61). A concept in autoimmunity is that organ-specific autoantigens are tissue-specific, and often enzymes of importance for the specific metabolism in the affected organ, such as antibodies against 21-hydroxylase in Addison’s disease (237), thyroid peroxidase (TPO) in Hashimoto thyroiditis (238) and tryptophan hydroxylase in autoimmune polyendocrine syndrome type 1 (APS1) (218). Alpha-fodrin, being almost ubiquitous, was therefore not a likely candidate as an organ-specific autoantigen and the aim was to test that hypothesis.

Patients with SS, SLE and SLE with sSS were included in the study. Fifty-six SS patients fulfilling the European criteria of 1993 were included (33), 43 of whom also fulfilled the San Diego criteria confirming an autoimmune disease (18). SLE patients were classified as having sSS if both the unstimulated whole salivary flow and the Schirmer-1 test were pathological. SLE patients classified as not having sSS had normal results in both tests. The patient characteristics and autoantibody profiles of the SS, SLE and SLE-sSS patients were similar to other studies, revealing that SLE-sSS constitutes an intermediate group (1).

We chose to use the ITT assay for autoantibody detection, described in the methods section (217). The reason for this choice was that several organ-specific autoantibodies have been detected with ITT (218, 239, 240) and the assay has proven to be robust in workshop comparisons with ELISA and immunoblotting (241). With protein A Sepharose precipitation we detected anti-α-fodrin antibodies of the IgG isotype and the results should preferably be compared with IgG ELISA (Table 6).

We found anti-α-fodrin antibodies in 16/56 (29%) of SS sera, 25/53 (47%) of SLE sera and 3/14 (21%) of SLE-sSS sera, none of the different frequencies being significant. None of the 16 blood donors showed α-fodrin reactivity. One of 30 (3%) of RA sera tested was positive (unpublished data).
Table 6. Frequencies (%) of α-fodrin autoantibodies in pSS, sSS, SLE, RA and healthy controls, in different studies

<table>
<thead>
<tr>
<th>Study</th>
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<th>RA</th>
<th>controls</th>
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<tr>
<td>Haneji 1997 (61)</td>
<td>41/43 (95)</td>
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<td>0/14</td>
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<td>9/15 (60)</td>
<td>3/28 (11)</td>
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<td>nd</td>
<td>IB</td>
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<td>Witte 2000 (243)</td>
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<td>13/22 (60)</td>
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<td>2/12 (17)</td>
<td>1/160 (0.6)</td>
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<td></td>
<td>47/85 (55)</td>
<td>9/22 (41)</td>
<td>1/50 (2)</td>
<td>5/12 (42)</td>
<td>3/160 (2)</td>
<td>ELISA IgG</td>
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<td><strong>16/56 (29)</strong></td>
<td><strong>3/14 (21)</strong></td>
<td><strong>25/53 (47)</strong></td>
<td><strong>1/30 (3)</strong></td>
<td><strong>0/16 (0)</strong></td>
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<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>IB</td>
</tr>
<tr>
<td></td>
<td>4/21 (19)</td>
<td>3/6 (50)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>IP</td>
</tr>
<tr>
<td>Ruffatti 2004 (245)</td>
<td>26/80 (32)</td>
<td>nd</td>
<td>16/50 (32)</td>
<td>14/30 (47)</td>
<td>1/60 (1.7)</td>
<td>ELISA IgA</td>
</tr>
<tr>
<td></td>
<td>17/80 (21)</td>
<td>nd</td>
<td>13/50 (26)</td>
<td>4/30 (13)</td>
<td>5/60 (8)</td>
<td>ELISA IgG</td>
</tr>
<tr>
<td>Bizarro 2004 (246)</td>
<td>17/174 (23)</td>
<td>nd</td>
<td>na</td>
<td>na</td>
<td>na/40</td>
<td>ELISA IgA</td>
</tr>
<tr>
<td></td>
<td>27/174 (16)</td>
<td>nd</td>
<td>na</td>
<td>na</td>
<td>na/40</td>
<td>ELISA IgG</td>
</tr>
<tr>
<td>Szanto 2005 (247)</td>
<td>8/46 (17)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>na</td>
<td>ELISA IgA</td>
</tr>
<tr>
<td>Sordet 2005 (248)</td>
<td>13/46 (28)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>na</td>
<td>ELISA IgG</td>
</tr>
<tr>
<td></td>
<td>19/107 (18)</td>
<td>nd</td>
<td>8/32 (25)</td>
<td>10/43 (23)</td>
<td>6/48 (12)</td>
<td>ELISA IgA</td>
</tr>
<tr>
<td></td>
<td>6/107 (6)</td>
<td>nd</td>
<td>7/32 (22)</td>
<td>1/43 (2)</td>
<td>0/48</td>
<td>ELISA IgG</td>
</tr>
</tbody>
</table>

*unpublished data, IB; immunoblot, ELISA; enzyme linked immunosorbent assay, ITT; in vitro transcription and translation assay, IP; immunoprecipitation, n.d; not done, n.a; not available

Our anti-α-fodrin antibody frequency in SS was considerably lower than the results published at that time (61, 242, 243). Further studies have also not been able to reproduce the high frequency and specificity of α-fodrin autoantibodies for SS originally found by the Japanese (61). Several subsequent studies have confirmed an anti-α-fodrin antibody frequency of around 30% in pSS, and the occurrence of anti-α-fodrin antibodies also in SLE, RA and in a few percent of normal controls (242-248). A 100% frequency has been reported in juvenile SS and α-fodrin autoantibodies may be an early disease
marker (249). Different methods for detection have been employed, as well as different ELISA kits, which may explain some of the discrepancies in results. Recently, α-fodrin antibodies have also been found in other autoimmune diseases: multiple sclerosis, thyroiditis, Grave’s ophthalmopathy and Crohn’s disease (250, 251).

The presence of α-fodrin autoantibodies correlated weakly to a positive test for RF in SS and anti-dsDNA antibodies in SLE, but more strongly to a positive mSLEDAI score in SLE, indicating an active disease (122). Both α-fodrin and SS-A/SS-B are present in most or all cells and are therefore systemic rather than organ specific autoantigens. Contrary to the majority of autoantigens in SS and SLE, α-fodrin is not a nucleic acid binding protein. The sensitivity in SS for anti-SS-A (60-100%) and anti-SS-B antibodies (40-98%) (1) is higher than the approximate sensitivity for α-fodrin antibodies (30%) and testing for the latter cannot replace conventional diagnostic serology.

We conclude that anti-α-fodrin autoantibodies are present in sera from patients with SS, SLE and SLE-sSS in similar frequencies and represent another systemic autoantibody found in these related diseases. An organ specific autoantigen in the SS MSG is still to be discovered.

The type I interferon system is activated in primary Sjögren’s syndrome as a possible etiopathogenic mechanism (Paper II)

Sjögren’s syndrome and SLE have many similar clinical and serological features as indicated in the introduction, and activation of the type I IFN system is of importance in SLE (200). Studies have also shown that an activated type I IFN system is not specific for SLE, but also seen in other autoimmune diseases (252). In addition, there are occasional case reports of SS precipitated by IFN-α treatment (253). Earlier studies in SLE indicated that the presence of antibodies against RNA-binding proteins was part of the IFN-α inducing IC when healthy PBMC were stimulated by sera in combination with apoptotic or necrotic cells (206, 207). SS patients have a high frequency of antibodies to the RNA-binding proteins SS-A/Ro and SS-B/La and it was therefore of interest to study the type I IFN system in SS.

Elevated serum levels of IFN-α were detected in only 3/38 (8%) of SS patients, all in the low range (2.3-3.1 U/ml) in comparison with 9/20 (45%) SLE patients (2.1-29 U/ml). Anti-dsDNA antibodies are only seen in 0-5% of SS patients (Table 2) and in this study 2/38 (5%) were anti-dsDNA antibody positive. One of them had increased serum IFN-α levels. The other two with elevated serum IFN-α did not have anti-dsDNA antibodies, and the general conclusion is that SS patients lack the endogenous IIF seen in SLE.
An ongoing viral infection as a cause for the slightly elevated IFN-α levels cannot be excluded.

In autoimmune conditions, the NIPC/PDC are recruited to inflamed tissues such as the skin in SLE and muscles in dermatomyositis (189, 213). We therefore looked for IFN-α production in the MSG since this is the main target tissue in SS. Seven of 7 biopsies from SS patients stained positive for IFN-α, mainly in inflammatory foci and only rarely in acinar epithelium. IFN-α has previously only occasionally been stained for in SS MSG, where it has been detected in acinar epithelial cells (75). The cellular identity of the IFN-α producing cell in the MSG was not determined in this study.

Sera from SS patients together with apoptotic (30/38 patients, 79%) and necrotic (32/38, 84%) U937 cell material, induced IFN-α production in normal PBMC at a higher frequency than sera from SLE patients (apoptotic = necrotic 10/20, 50%). This ability of sera has previously been shown to correlate with the presence of antibodies to RNA-binding proteins (199, 207). In these patient cohorts, antibodies to the RNA-binding proteins SS-A, SS-B, RNP and Sm, were more frequent in the SS patients, which may explain the difference in IFN-α inducing capacity of sera (Table 7).

Table 7. Frequency (%) of antibodies against RNA-binding proteins in patients with SS (n=38) and SLE (n=20)

<table>
<thead>
<tr>
<th>RNA-binding protein</th>
<th>Sjögren’s syndrome</th>
<th>SLE</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS-A</td>
<td>27/38 (71)</td>
<td>5/20 (25)</td>
</tr>
<tr>
<td>SS-B</td>
<td>20/38 (53)</td>
<td>3/20 (15)</td>
</tr>
<tr>
<td>RNP</td>
<td>1/38 (2.6)</td>
<td>3/20 (15)</td>
</tr>
<tr>
<td>Sm</td>
<td>2/38 (5.3)</td>
<td>2/20 (10)</td>
</tr>
</tbody>
</table>

Antibodies were analysed with a sensitive InnoLia immunoblot. With clinical immunology antibody analysis used routinely none of the SS patients had anti-Sm antibodies. The RNP positive patient is positive also with routine methods.

The IFN-α inducing capacity of sera also correlated with the presence of antibodies to either of the RNA-binding proteins. For the separate antibodies there was a correlation to the presence of SS-A (with apoptotic and necrotic cell material) and SS-B (only with necrotic cell material). The InnoLia immunoblot detected Ro52 and Ro60 separately but most patients had reactivity against both subunits and correlations were seen against both Ro52 and Ro60 and they are therefore treated together in the paper. The only SS patient with anti-RNP antibodies had a very high IFN-α capacity which, because of only one patient, did not reach significance compared to RNP negative SS patients.

We found evidence that the IFN-α inducer was IC consisting of RNA/RNA-binding proteins and corresponding antibodies, since RNase A treatment and blocking of FcγRIIIa both inhibited the IFN-α production, as did IgG depletion of sera. A clear dose-dependent relation between serum
concentration of IgG, and induced IFN-\(\alpha\) was found, typical of an IC-dependent reaction. We also found that all IFN-\(\alpha\) producing cells belonged to the NIPC/PDC population, by double staining for IFN-\(\alpha\) and BDCA-2. On the other hand, not all BDCA-2 positive cells produced IFN-\(\alpha\).

Clinically there was a correlation between induced IFN-\(\alpha\), together with apoptotic cell material, and a positive MSG, i.e. a focus score \(\geq 1\) and a tendency for a correlation between the actual focus score (range 0-12) and the level of IFN-\(\alpha\) induced. This seems logical if the autoimmune reaction takes place in the MSG. A correlation was also seen with dermatologic, hematologic and pulmonary manifestations. A correlation between serum IFN-\(\alpha\) levels and skin rash has been noted in SLE patients (201), and the presence of SS-A antibodies has been correlated to cutaneous vasculitis in SS (96). In conclusion, we found an activation of the type I IFN system in SS previously not described.

Polymorphisms in the tyrosine kinase 2 and interferon regulatory factor 5 genes are associated with systemic lupus erythematosus (Paper III)

Activation of the type I IFN system in SLE has been shown in several studies (200) and gene expression profiling in SLE PBMC has revealed an upregulation of IFN-induced genes (202, 203, 254). It was therefore of interest to investigate genes of the type I IFN system in SLE. We chose a candidate gene approach with the hypothesis that there would be a difference in susceptibility genes between SLE patients and controls. We investigated genes mainly in the IFN-signalling pathway, but also other genes in the type I IFN-system.

The 11 genes in the type I IFN system encode the following proteins: the 2 subunits of the IFNAR; IFNAR1, IFNAR2, Tyk2, Jak1, Stat1, Stat3, IRF5 (Figure 5), an IFN inducible protein IFI1 and three type I IFNs; IFNA21, IFNA6 and IFNB1. An additional two genes with association to the type I IFN system were included, FCGR2A and PDCD1. Initially 33 SNPs were randomly chosen from databases and validated (223). After SNPs in the genes for Tyk2 and IRF5 with highly significant associations to SLE were found, additional SNPs in these genes were genotyped, giving a total of 44 SNPs, including 11 in Tyk2 and 6 in IRF5.

We initially included 499 SLE patients from Sweden (Umeå, Uppsala and Lund) and 150 SLE patients from Finland. Since the genetic background may differ between these populations we analysed the Swedish and Finnish samples both separately and in combination. When the significant SNPs in Tyk2 and IRF5 were found, another 30 patients from Iceland were included, in total 679 SLE patients. The healthy unrelated controls were 256 Swedish
blood donors, including 60 age and sex matched, and 182 Finnish population controls.

Both joint linkage and association analysis using the Pseudomarker program (236) and association analysis between cases and healthy controls using Fisher’s exact test, gave strong signals for one SNP in Tyk2, (rs2304256) and one SNP in IRF5 (rs 2004640), for both the Swedish and Finnish populations separately, and in combination, with lower p-values for Tyk2 in the Swedish population (Table 8). An additional SNP in Tyk2 (rs 12720356) was significant in the Swedish population only and two more SNPs in IRF5 were nearly significant in the Finnish population. This indicates a difference in the genetics between these two populations. Adding the Icelandic samples further increased the significances to p < 10⁻⁸.

**Table 8. Minor allele frequencies of SNPs in Tyk2 and IRF5 in the Swedish and Finnish samples. P-values by joint linkage and association**

<table>
<thead>
<tr>
<th>Gene and SNP no.</th>
<th>Sweden</th>
<th>Finland</th>
<th>P values for Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SLE n=480 controls n=256</td>
<td>SLE n=109 controls n=121</td>
<td>Swe Fin</td>
</tr>
<tr>
<td>Tyk2 rs 2304256</td>
<td>0.24 0.32</td>
<td>0.21 0.27</td>
<td>1.00x10⁻⁶ 0.018</td>
</tr>
<tr>
<td>IRF5 rs2004640</td>
<td>0.38 0.48</td>
<td>0.37 0.52</td>
<td>8.5x10⁻⁵ 1.50x10⁻⁴</td>
</tr>
</tbody>
</table>

**Table 9. Correlations between genotypes for Tyk2 rs2304256 and IRF5 rs2004640 and ACR criteria using logistic regression analysis**

<table>
<thead>
<tr>
<th>SNP no./ACR criteria</th>
<th>Sweden n=464 genotypes and ACR frequencies (%)</th>
<th>Finland n=146 genotypes and ACR frequencies (%)</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tyk2 rs2304256 ACR 7 nephritis</td>
<td>AA n=17 AC n=177 CC n=270 1/17 47/177 87/270</td>
<td></td>
<td>0.026</td>
</tr>
<tr>
<td>IRF5 rs2004640 ACR 8 CNS</td>
<td>CC n=14 CA n=78 AA n=54 0/14 2/78 7/54</td>
<td></td>
<td>0.023</td>
</tr>
</tbody>
</table>

Correlation of genotypes with ACR criteria is shown in Table 9. The minor allele in Tyk2 is A (major allele C) and in IRF5 C (major allele A). In a logistic regression analysis in a co-dominant model assuming the two alleles are equally expressed, the genotypes were correlated to the ACR classification criteria and the presence of dsDNA antibodies. There was a tendency for a correlation between AA (the rare allele) homozygosity in Tyk2 (rs2304256) and a low frequency of nephritis in the Swedish patients
(p=0.026). In the Finnish patients there was a tendency for a correlation between IRF5 CC (the rare allele) homozygosity and reduced occurrence of CNS manifestations (p=0.023).

We found that the minor allele in the significant SNPs in Tyk2 and IRF5 was less frequent in SLE patients than controls. Conversely, the major alleles were more frequent in SLE compared to healthy individuals. Why would the common major alleles confer disease susceptibility?

The SNP in Tyk2 rs2304256 causes an amino acid substitution from valine to phenylalanine at position 362, which is characterised as ‘benign’ or ‘intolerant’ according to two different prediction programs. The other Tyk2 SNP rs12720356, which was significant in the Swedish patients, leads to a substitution of isoleucine to serine at position 684, predicted to be ‘damaging’ or ‘intolerant’. We speculate that these substitutions affect the function of Tyk2 resulting in decreased IFN-α signalling through the IFNAR and thus to decreased susceptibility to SLE. The IRF5 SNP rs 2004640 is the first nucleotide of intron 1, which may affect the splicing of exon 1 mRNA, thereby affecting the function of IRF5. These hypotheses remain to be proven. A survival benefit of the common major allele would obviously be a better defence against viruses, where the ‘side effect’ is increased autoimmunity.

Dehydroepiandrosterone supplement affects health-related quality of life in female patients with systemic lupus erythematosus (Paper IV)

This study was based on several observations, most importantly the decreased HRQOL that has been reported in SLE in several studies (124, 255). Secondly, patients with SLE have decreased serum levels of DHEAS, which is partly explained by ongoing glucocorticoid treatment, though lower than normal levels are seen even in untreated SLE (172). Thirdly, DHEA supplementation to women with Addison’s disease have shown varying results on HRQOL (170, 256) and lastly, high DHEA doses have shown to decrease disease activity (257, 258) which presumably would increase HRQOL.

The study design was a six months double-blinded placebo-controlled period followed by six months open treatment to both groups. The purpose of this design was to assess both the changes in the respective groups during the placebo-controlled period and also to evaluate changes in the placebo group as they started to receive DHEA after 6 months. As a possible alternative a 12 months placebo-controlled study could have been performed, but a crossover design is not recommended due to the long lasting effect of DHEA, where months of washout would have been necessary (256). In trials with
higher doses than the 20-30 mg daily used in our study, androgenic side effects might interfere with the blindness of the study.

Due to an age-dependent decrease in serum levels of DHEAS, we chose to give 30 mg daily for patients of ≤ 45 years, and 20 mg daily when ≥ 46 years. The serum levels of DHEAS at the time of inclusion were not known, but a pilot study had shown a negative correlation between prednisolone dose and serum levels of DHEAS, and we therefore chose to include only women on ≥ 5 mg prednisolone daily. Nevertheless, 10/37 (27%) women, all on 5 mg prednisolone daily, had normal serum levels of DHEAS in the lower range at study onset. In contrast, 95% and 62% had normal levels of testosterone and androstenedione, respectively, probably reflecting additional synthesis in the gonads, while in women DHEA is exclusively synthesized in the adrenal cortex (259). DHEA supplement raised the serum levels of DHEAS to mid-normal levels in all women except two, both on 30 mg daily, who had slightly elevated levels. We conclude that the doses given were adequate (Figure 8).

Figure 8. Serum levels of DHEAS at study onset were 0.95 ± 1.16 and 1.22 ± 1.10 µmol/L in the DHEA- and placebo-groups, respectively (reference value 1.8-10.3). DHEA-treatment raised serum DHEAS to 5.65 ± 1.90 µmol/L in the DHEA-group after 12 months. During open treatment serum DHEAS increased to 5.67 ± 2.97 in the former placebo-group.

The main finding was an improvement in mental well-being, assessed by SF-36 and HSCL-56, with concordant results in the PGWB and in addition, an improved sexual life measured by McCoy sex scale questionnaire. SF-36 is well validated in SLE, whereas PGWB, HSCL-56 and McCoy have to our knowledge not been used previously in SLE. These questionnaires were included to improve interindividual consistency and both PGWB and McCoy have been used in SS (260, 261). We confirmed a strong correlation between the improved SF-36 domains RE (DHEA-group 0-6 months) and MH (former placebo group 6-12 months), and HSCL-56 and PGWB total score, respectively. This emphasises that the results in SF-36 were not random.
Despite the randomisation of patients, which was performed by the pharmacy delivering the DHEA/placebo capsules, those in the DHEA-group tended to be older, with longer disease duration, higher prednisolone dose at study onset and slightly higher mSLEDAI and SLICC scores than the placebo group, although this was not significant. In addition, some patients in the DHEA-group experienced a disease flare between 6-12 months (nephritis recurrence, thrombocytopenia) with increased glucocorticosteroid doses, which might explain why the improvement in SF-36 RE and HSCL-56, seen between 0-6 months, was not sustained over 12 months. Obviously, this DHEA dose did not prevent disease flares.

Secondary endpoints were bone mineral density (BMD) and bone metabolism, body composition, serum lipids, disease activity and damage, side effects and safety. In brief, androgenic effects were detected as an increased waist-hip ratio and hormonal body hair score and biochemically as decreased high density lipoprotein (HDL) cholesterol and increased hematocrit. Only in 3/41 (7%) patients were these sufficient reasons for discontinuation of the study. The majority of patients did not experience any androgenic side effects and we therefore assume that the study was truly blinded over the first 6 months.

An increase in BMD during DHEA treatment has been observed in elderly women (262) and a protective role against glucocorticosteroid induced bone loss has been reported in SLE (263). Since the study time was a maximum 12 months in the DHEA group and the precision of the dual energy x-ray absorptiometry (DXA) does not detect small changes in BMD, a long term effect of DHEA on BMD can not be ruled out.

In conclusion, we found that supplementation with low doses of DHEA improved mental well-being and sexuality, whereas physical HRQOL was unaffected. Side effects were mild. DHEA supplement may be offered to glucocorticoid-treated women with SLE where mental distress or impaired sexuality is a problem, in an otherwise stable disease. Long term side effects on lipids and hormone dependent tissues may be a matter of concern.
General discussion

The studies in this thesis span the spectrum of autoimmunity, genetics and health-related quality of life in two predominantly female systemic autoimmune diseases, SS and SLE.

In paper I, the claim of an SS specific autoantibody was challenged as α-fodrin autoantibodies were initially reported to be SS-specific (61). SS is regarded as a systemic autoimmune disease and other systemic autoantibodies in SS (anti-SS-A/SS-B) are also seen in SLE. We hypothesised that this would be the case also with α-fodrin autoantibodies. Our results indeed confirmed similar frequencies (around 30%) of α-fodrin autoantibodies in sera from patients with pSS, sSS and SLE. All of the SS patients who tested positive for α-fodrin autoantibodies were also positive for at least one other autoantibody (RF, ANA, anti-SS-A/SS-B). Testing for α-fodrin autoantibodies seems not to have any discriminating value between SS and SLE nor any diagnostic value for SS.

As mentioned, α-fodrin is cleaved during apoptosis to its antigenic 120 kDa fragment and translocated to the apoptotic webs (67). Interestingly, a similar redistribution is seen for SS-A and SS-B during apoptosis and presumably these three autoantigens (α-fodrin, SS-A, SS-B) are then exposed for the immune system. An increased apoptosis has been reported in SS MSG epithelial cells (92) and an increased apoptosis and a defect clearance of apoptotic material is seen in SLE (134). Taken together this might suggest that autoantibodies against α-fodrin, SS-A and SS-B are a consequence of inappropriate presentation and regulation and that the primary antigen leading to the inflammation and dysfunction of the salivary and lacrimal glands remains to be identified.

The similarities in autoantibody profiles in sera from SS and SLE patients led us to the investigation of the type I IFN system in patients with SS (paper II). We found that sera from SS patients together with apoptotic or necrotic cell material had the capacity to induce IFN-α production in PBMC from healthy controls, which correlated to the presence of antibodies against RNA-binding proteins (SS-A, SS-B, RNP, Sm) in SS sera. Treatment with RNase abolished this IFN-α-inducing capacity of sera, indicating that RNA is important in these IC. A correlation between the presence of α-fodrin autoantibodies and IFN-α inducing capacity of SS sera was not made. Although the α-fodrin autoantigen is also expressed during apoptosis, it is not a
nucleic acid binding protein and not likely to be important for the IFN-α inducing capacity of sera in this system.

In contrast to results in SLE, we did not find increased serum levels of IFN-α in the majority of the SS patients and conclude that the SLE-IIF present in SLE sera is absent in SS sera. SLE-IIF consists of DNA and anti-dsDNA antibodies in complex, and anti-dsDNA antibodies are relatively rare in SS. We propose that in the interferogenic IC, DNA is the important nucleic acid in the circulation where RNA is rapidly degraded, whereas RNA is of greater importance in the tissues. Consequently we detected IFN-α containing cells in MSG of SS patients. The cellular identity of the IFN-α containing cells was not determined in this study. In a pilot study, RT-PCR of a MSG from one SS patients with IFN-α-containing cells showed IFN-α mRNA expression, confirming ongoing IFN-α gene transcription in the gland. To determine if the IFN-α producing cells in the MSG are NIPC/PDC is a future aim.

A pathogenic mechanism in the salivary gland is proposed whereby an initial viral infection may cause apoptosis of MSG acinar and epithelial cells and initiate the IFN-α production by NIPC/PDC, the initial priming event. Proinflammatory cytokines produced by the MSG infiltrating cells may then up-regulate FasR on acinar and epithelial cells, causing them to undergo apoptosis and expose RNA/RNA binding proteins for the immune system. B cells in the SS MSG produce anti-SS-A/SS-B antibodies which form IC with SS-A/SS-B protein binding to RNA. These IC are internalised via FcγRIIa on NIPC/PDC, stimulating further IFN-α production. IFN-α in the MSG would act on the IFNAR and activate the adaptive immune system, thus creating a perpetuating autoimmune process in the gland.

We hypothesised that candidate genes in the IFN-α signalling pathway may contribute to disease susceptibility in SLE and possibly SS. Since the type I IFN system has been more extensively studied in SLE we selected this patient group for the work in paper III. The selected 11 genes in the IFN-α signalling pathway represent only a fraction of the > 200 genes in this pathway. The SNPs in the 11 genes were selected in 2001 when the SNP databases were limited to a few SNPs per gene (223). Since then an increasing number of SNPs in the Tyk2 and IRF5 genes has been published. In the genes for Tyk2 and IRF5 we found SNPs with strong signals for joint linkage and association with SLE. This is remarkable considering the many genes and SNPs that were not investigated. Therefore several more genes and SNPs in the IFN-α signalling pathway may be associated with SLE and will be analysed in the future.

SLE patients had lower frequencies of Tyk2 and IRF5 minor alleles compared to healthy controls. The functional consequences of the Tyk2 SNP are under investigation. Apart from the hypothesis that the amino acid change in Tyk2 (rs2304256), V362F, interferes with the function of this kinase, is that
the SNP could be a marker in LD with another functional SNP. The func-
tional SNP could for example be in the promoter region affecting gene tran-
scription. Likewise the function of the SNP in IRF5 (rs2004640), located in
the splicing region of intron 1, needs to be investigated. Another future aim
is to look for disease susceptibility genes in the type I IFN system in patients
with SS.

Thus, we have shown an activation of the type I IFN system in SS and
disease susceptibility genes in the IFN-α signalling pathway in SLE. An
activation of the type I IFN system has also been reported to be of impor-
tance in other autoimmune diseases apart from SLE, such as psoriasis, der-
matomyositis and IDDM, and may represent a general mechanism for auto-
immunity when tolerance is lost (188, 212, 213). This implies potential
therapeutic targets in the type I IFN system, e.g. antibodies against FcγRIIa
or BDCA-2, preventing IFN-α-production from NIPC/PDC, soluble IFNAR
to prevent binding of IFN-α to the membrane-bound receptor or tyrosine
kinase inhibitors preventing the downstream IFN-α signalling through the
Jak-Stat pathway.

Apart from gene susceptibility and immunological disturbances, SS and
SLE are influenced by hormones. In paper IV we studied the effect of the
weakly androgenic hormone DHEA on health-related quality of life in
women with SLE. Serum levels of DHEAS are also lower than normal in
women with SS (51) and a trial with DHEA supplementation to women with
SS, assessing fatigue, is ongoing.

An improvement in mental well-being and sexuality was noted in this
study. DHEA has immunomodulatory properties with an up-regulation of
Th1 cytokines IFN-γ and IL-2, and a down regulation of Th2 cytokines IL-4,
-5, -6 and -10 (264). Theoretically this immune deviation could be beneficial
in SLE which is generally considered to be Th2 dominated. The reported
DHEA induced suppression of IL-6 in human PBMC in vitro is interesting
since increased IL-6 levels have been reported in cerebrospinal fluid from
SLE patients with CNS involvement (265, 266). IL-6 affects several brain
functions including impaired memory and learning (267). We speculate that
the improvement in mental well-being during DHEA treatment may partly
be through an effect on the CNS. A more specific investigation of the neuro-
psychological effects of DHEA in SLE might be valuable.

In conclusion, we found α-fodrin autoantibodies in similar frequencies in
SS and SLE, an activation of the type I IFN system in SS, novel disease sus-
cceptibility genes in SLE and a mild effect of DHEA supplement on health-
related quality of life in SLE.
Acknowledgements

This thesis work was carried out at the Department for Medical Sciences, Faculty of Medicine, Uppsala University. I would like to express my gratitude to all who have contributed to the work and made this thesis possible.

*Lars Rönnblom*, my supervisor, for introducing me to the magic world of interferon-α and SLE, for guiding me through medicine, rheumatology and research, for your enormous enthusiasm and energy, for being such an organised and reliable person and for long lasting friendship.

*Olle Kämpe*, my co-supervisor, for allowing me to start from scratch in your group, for your brilliant scientific skills and equally brilliant cooking skills and for creating such a pleasant atmosphere in your group.

*Ola Wingqvist*, my co-supervisor, for introducing me to lab work in La Jolla and believing that this was something a clinician could do, for your positive attitude and always having time to explain and for telling me that ‘now it’s close’ whenever I was in doubt.

*Ann-Christine Syvänen*, for supervising the genetics project with such enthusiasm and expertise, for introducing me to SNPs, genes, minisequencing and your nice and clever molecular medicine group and for believing that hard work is rewarded in the end.

*Fredrik Rorsman*, my actual supervisor in the first year, trying inexhaustibly to help me with PCR, ITT and all that and for always being helpful with a smile.

*Gunnar Alm*, for welcoming me into your interferon group, for your vast knowledge and kind personality and for your sense of the important details and excellent figure drawing skills.

*Ulla Lindqvist*, Head of the ‘Medsci’ Department and *Kjell Öberg*, Dean of the Medical Faculty, for creating a good research atmosphere and making this work possible.
All patients, who willingly participated in the studies. Without you this work could not have been done.

Snaevar, genotyping genius, for your enormous working capacity and endurance, for always answering my (silly?) questions and quickly replying to e-mails and for being such a nice Icelandic guy.

Ullvi, Maija-Leena, Tanja, my co-authors in the interferon paper, for explaining over and over again how things really are with these cells, for always being helpful and making time and for good friendship.

Johan Rönnelid, Stefan Cajander, Anders Karlsson, Anders Larsson, Christine Bengtsson, Gunnar Sturfelt, Andreas Jönsen, Katarina Lindroos, Ann-Cristin Wiman, Bozena Möller, Solbritt Rantapää-Dahlqvist and all other co-authors for valuable discussions and good co-operation. This thesis is a team effort.

Åsa, the centre around whom lab 21 revolves, for knowing where everything is, for helping me with peculiar pipettes, buffers and biopsies, for organising social activities and always being kind and reliable.

Eva, Gennet, Olov, Filip, Håkan, Annika, Mohammad, Mina, Magnus, Lillebil, Sophie, Katrin, Pernilla, Brita and all the old and new members of lab 21, and Signe, Per, Mona for scientific discussions and good laughter, Margareta for skillful lab analyses and Thomas for being an excellent nephrologist, for deep talks and not so deep talks, for emergency rescues of my old Honda and the occasional cannon ball firing.

Ann Knight, Head of the Rheumatology Department, for creating a nice working place in the clinic and for giving me this time when I needed it, and Roger Hällgren, our inspirational Professor, for never-ending enthusiasm and free thinking and for always being helpful and interested in difficult cases.

Karin, Ann S, Maria, Siddy, Stina, Dan, Johan, Hans, Hossein, Luis, for being such good rheumatology colleagues and friends, Eva B and Tomas W for being inspiring sparring partners in the thesis writing, Per Söderberg for excellent classification of dry eyes, Lotta, Inger, Anne, Helena, Britta, Sonja, Pia and all the staff at the Rheumatology Clinic who have helped and supported me in one way or another.
Håkan Forsberg, Göran Sundlöf, Fritz Huhtasaari, former Heads of the Medical Department in Luleå/Boden for giving me a solid clinical platform to stand on. ‘The optimal learning situation is the critically ill patient, one’s own anxiety and the book’.

Agneta, Bengt, Johanna, Anders R, Eija, Stig, Palle, Madde, Peter, Jörgen and all other friends at the Medical Department in former Luleå/Boden for sharing hardships and laughter and giving me only good memories from that time, Mats for your overall enthusiasm for everything, Lisbet and Yngve for introducing me to rheumatology and showing me how to inject joints blindly from a distance and always get it right and Eddy for collecting sera.

Anna, my ‘Uppsala-sister’, for opening your warm home and big family to Lucy and me, for always helping me with everything and for being the best of friends. Without you I would not have survived in Uppsala.

Susanne, Christina, Karin (again), Maria, Eva TJ, Elisabet, Marie, my student friends from Med-school, for lunches, coffees and dinners discussing the meaning of life and for just being there.

Eva R, Mimmi, Mona, my very best friends from long ago, for all good memories and for reminding me that there is a world outside the hospital. Is there?

Ingrid and John, my parents, for always supporting me and my ideas, no matter what, for being there whenever I need and for all the time Lucy has spent in Piteå. Without you this thesis would never have been written. Desireé and Urban, my dear sister and brother, for sharing your homes and families when I visit and for reminding me where my roots are.

Lucy, my little angel, for distracting me from work with your games, stories and laughter and for simply being the meaning of life.

Mike, my companion in life, for bumping into me at Tiananmen Square and never letting go, for scientific discussions, valuable comments and proof reading, for your relaxed attitude to life, emphasising the importance of winning and dining and holidays and for everything else.
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Acta Universitatis Upsaliensis

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Editor: The Dean of the Faculty of Medicine

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