The eye and mouth symptoms in Sjögren’s syndrome are caused by abnormalities in tear fluid and saliva. A criterion for diagnosis is usually inflammation found in the lip biopsy. So it goes without saying that this inflammation is found in virtually everyone with Sjögren’s syndrome. It therefore seems logical for the inflammation in the glands to be the cause of the symptoms. Later in this chapter another possible cause of the abnormalities in the tear fluid and saliva will be discussed.

Despite questions that have arisen concerning the role of inflammation in the glands, in many patients inflammation is present. The cause of this is unknown. Obvious causes such as bacterial or viral infection have not as yet been proven.

When discussing the cause of a disease, it is useful to draw a distinction between what causes someone to get the disease (aetiology) and how the symptoms of the disease come about (pathogenesis).

Nothing is known about the aetiology. We know that several hereditary characteristics, including gender and certain genes, have an influence on the risk of developing the disease.

More is known about the pathogenesis, but the first stages are unclear.

Sjögren’s syndrome is considered to be an autoimmune disease. The term autoimmune disease is explained in the box on the next page.

**Genetic factors**

The development of a genetic disease is entirely determined by genetic factors. Inheritance usually follows clear patterns and in the case of identical twins either both or neither has the disease. None of this applies to Sjögren’s syndrome. Sjögren’s syndrome is therefore not a genetic disease. However, the chance of developing the disease is nevertheless partially determined by genetic factors, while environmental factors probably also play a role. Although a few genetic factors are known, the environmental triggers are not.

**Female sex**

The largest genetic risk for Sjögren’s syndrome is the female sex as 90% of patients with Sjögren’s syndrome is female. This female preponderance is likely based on genes located in sex chromosome 23: having two X chromosomes and/or missing the Y chromosome.

**A parent has Sjögren’s syndrome**

The risk for autoimmune diseases is higher than normal for children of patients with Sjögren’s syndrome. The risk is higher if the parent is the mother who has the disease.

<table>
<thead>
<tr>
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<th>prevalence (%)</th>
<th>relative risk</th>
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<td></td>
</tr>
<tr>
<td>HLA B8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA DR3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA DR52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT4 SNP a rs7574865</td>
<td>20.8-23.7</td>
<td>1.3</td>
</tr>
<tr>
<td>HLA DR3-RCAa block epistatic interaction</td>
<td>8</td>
<td>48 b</td>
</tr>
<tr>
<td>immunoglobulin-like transcript 6</td>
<td>3</td>
<td>8</td>
</tr>
</tbody>
</table>

a SNP: single nucleotide polymorphism
b patients with antibodies to SSA/Ro and SSB/La
c the same haplotype has been found in chromosomes of patients with rheumatoid arthritis (24.7-28.9%) and systemic lupus erythematosus (31%) 47,48
Autoimmune diseases

The immune system normally only attacks foreign substances from outside the body. However, small changes on the outside of our own cells can be recognised as foreign by our immune system and subsequently killed. This is usually a good thing since abnormal cells can form a danger, e.g. malignant cells or cells containing a virus.

The immune response is sometimes directed against a microorganism and the antibodies and/or T lymphocytes may crossreact with the body’s own components. The background to this is a similarity between proteins or sugar compounds of microorganisms and components of our own body.

The question as to whether a specific disease is an autoimmune disease or not can only be answered after first establishing what we mean exactly by the term autoimmune disease. In the case of many so-called autoimmune diseases, there is no indication that the immune system is not in order. All that can be shown or assumed is that the immune system is involved in damage to cells and tissue.

In many infectious diseases, the immune system is responsible for the disease symptoms and not the microorganism itself. An example of this is hepatitis B in which the inflammation of the liver is mainly the result of the immune system’s response to the presence of the virus. Tuberculosis is an example of the same process in the case of diseases caused by bacteria. If, however, the pathogenic organism (bacterium or virus) causing the disease is known, the disease is no longer considered to be an autoimmune disease. Infectious diseases in which the damage is mainly caused by the response of the immune system (that can greatly vary from person to person) are particularly striking for the greatly varying, often relatively late consequences of the infection. Usually only a small percentage of the infected people develop the disease and with varying severity. People who are unlucky in this respect where certain micro-organisms are concerned may suffer no ill-effects from others. This is important for the survival of the species because when an epidemic occurs there are always some people who survive the disease or never catch it at all.

There are only a small number of diseases without a known pathogenic organism, where it has been proven that the symptoms are caused by the immune system. These are mainly diseases caused by antibodies. There are diseases that pregnant women with antibodies can transmit to the child in the womb via the placenta. Children retain antibodies from their mother for about 6 months after their birth. These antibodies protect them from infection for as long as they are unable to make the antibodies themselves.

However, if the mother has antibodies that could cause symptoms of disease, the children may also manifest these symptoms. Examples of these are certain thyroid disorders, myasthenia gravis and neonatal lupus that are caused by antibodies against the TSH-receptor on thyroid cells, antibodies against the acetylcholinereceptor on muscle cells and antibodies against SSA/Ro and/or SSB/La. When the mother’s antibodies disappear in the child, the disease symptoms in the child also disappear.

The fact that antibodies against the body’s own components are found in a specific disease does not mean that it is an autoimmune disease, not even if they precede the disease. For example: antibodies against the islets of Langerhans in the pancreas are the result of damage to these islets, usually many years before the number of islets has decreased to such an extent that diabetes mellitus manifests itself. Diabetes mellitus is in fact probably an autoimmune disease but caused by a different mechanism. A disease is usually considered to be an autoimmune disease if it complies with a number of criteria (see below).

Characteristic features considered to be an indication of the autoimmune nature of a specific disease (examples)

- no micro-organism or other cause has been found for the disease
- the disease occurs more often in certain families
- the disease occurs more often in people with certain genes of the HLA system
- the disease occurs more often in women than in men
- antibodies against the body’s own components occur in many or all of the patients with the disease
- the disease can sometimes be transmitted from mother to child in the womb
- the patient / relatives of the patient often also has / have another disease that is considered to be an autoimmune disease
- in the case of experimental animals, it is sometimes possible to stimulate the disease in other identical experimental animals by transferring antibodies or lymphocytes
- the disease can be “treated” with drugs that have an effect on the immune system or inflammatory response
HLA genes
Certain genes from the HLA or other systems increase the risk of developing the disease. Table 3.1 shows how often some of these occur in normal healthy people and how often in Sjögren’s syndrome patients. However, their influence on the chance of developing Sjögren’s syndrome is slight.

STAT 4 genes
Recent findings on STAT4 are interesting. An increased incidence of the haplotype rs7574865 has been found in patients with rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and Sjögren’s syndrome.\(^{44,47,48}\) Homozygosity is associated with a more than doubled risk of SLE and a 60 percent increased risk of RA.\(^{47}\)

The JAK/STAT pathway is the signaling target of a multitude of cytokines that are thought to play biologically significant roles in inflammation. STAT4 transmits signals induced by IL-12, IL-23, and type I interferons (IFNs). A major action of IL-12 through STAT4 signaling is to promote the differentiation of naive CD4\(^+\) T lymphocytes into T\(_{H1}\) cells, which produce IFN. These T\(_{H1}\) cells are thought to drive the chronic autoimmune response (figure 3.1).

STAT4 is also important for the development of the recently identified IL-17 secreting T helper cells, which are stimulated by IL-23. These T\(_{H17}\) cells play critical roles in autoimmune diseases through the production of IL-17. T\(_{H17}\) cells have been found to be the predominant infiltrating T cell in the salivary glands of patients with Sjögren’s syndrome.\(^{49}\)

Immunoglobulin-like transcript family
Immunoglobulin-like transcript (ILT) genes are located in the leukocyte receptor complex on chromosome 19 and consist of 13 homologous receptors with activating and inhibiting capacity. ILTs are mainly expressed on antigen-presenting cells (macrophages, dendritic cells and B lymphocytes. ILT2 is also expressed on NK cells and T-lymphocytes.

ILT6 is expressed only as a soluble molecule, but its presence is variable. Homozygous deficiency of ILT6 has been shown to be absent in 7.3% of multiple sclerosis patients but in only 3% of normal blood donors.

Kabalak \textit{et al} \(^{51}\) investigated 149 patients with Sjögren’s syndrome and 749 healthy blood donors for homozygous presence, homozygous deletion and heterozygous deletion of ILT6. Homozygous ILT6 deletion was found to be associated with Sjögren’s syndrome (8% in patients versus 3% in controls, p<0.01). The authors speculate on possible mechanisms. They suggest that absence of ILT6 may cause enhanced destruction of gland cells by CD8\(^+\) T cells. The authors realize, however, that the majority of infiltrating lymphocytes are CD4\(^+\) T cells and B cells. Exocrine glands, however, are not usually destroyed in Sjögren’s syndrome but mainly functionally defect. Gland destruction may occur in some patients but this is usually due to recurring secondary bacterial infection of the glands due to duct obstruction by viscous secretions (see further). A second explanation suggests that antiviral defense is impaired due to ILT6 deficiency. The authors cite data on the possible role of Epstein-Barr virus and coxsackievirus in triggering Sjögren’s syndrome.\(^{51}\) The possible role of these viruses, however, is not supported by clinical evidence.

Drugs
Many cases have been described on various drugs that can induce SLE, but reports on drug-induced Sjögren’s syndrome are very rare.

Darwaza \textit{et al} \(^{52}\) described a 60-year-old male patient who developed clinical features of Sjögren’s syndrome, with immunological features of drug-induced SLE, 4 years after initiation of therapy with hydralazine hydrochloride (for treatment of hypertension). The patient had reduced lacrimal and parotid salivary flow, but lacked the typical features of Sjögren’s syndrome on labial gland biopsy. One year after discontinuation of hydralazine therapy, the clinical parameters returned to normal.

Onishi \textit{et al} \(^{53}\) described a 57-year-old Japanese woman who developed skin eruption, pleuritis, pancytopenia, parotid gland swelling and glomerulonephritis after 7-month treatment with pegylated interferon-\(\alpha\) and ribavirin for chronic hepatitis C. Disease-specific autoantibodies such as...
anti-SSA, anti-SSB, anti-Sm and anti-dsDNA antibodies became positive. The diagnosis of SLE and Sjögren’s syndrome was made and treatment with glucocorticoid pulse followed by oral glucocorticoid was started. The authors conclude that it is highly probable that interferon-\(\alpha\) induced SLE and Sjögren’s syndrome in this case.

In both cases, Sjögren’s syndrome was diagnosed in addition to SLE, suggesting that the mechanisms of these drug-induced diseases are similar.

**Pathogenesis**

Table 3.2 shows a number of ways in which the disease symptoms may be caused.

**Regulatory T lymphocytes**

Regulatory T lymphocytes (\(T_{\text{reg}}\) cells) represent about 5% of CD4+CD25+ T cells. Treg cells play a critical role in the mediation of a suppressive function and in maintaining a broad range of T-cell antigen receptor specificities that prevent the development of autoimmune responses. \(T_{\text{reg}}\) cells thus are the main cells that maintain the immune homeostasis in the periphery and regulate autoimmunity. The percentage of \(T_{\text{reg}}\) cells has been found to be about three times lower in patients with SLE, systemic sclerosis, Sjögren’s syndrome and poly myositis/dermatomyositis than in healthy controls. The percentage of particular subsets (GITR+ \(T_{\text{reg}}\) cells) was higher in Sjögren’s patients with antibodies to SSA/Ro than in those without. Treatment was correlated with lower percentages of particular subsets of \(T_{\text{reg}}\) cells.

Future studies are needed to understand the relevance of these findings and to find out whether they play a role in the pathogenesis of these diseases.

**Table 3.2 Causes of disease symptoms in Sjögren’s syndrome**

- autoantibodies
- immune complexes
- focal lymphocyte infiltration
- malignant proliferation of lymphocytes (non-Hodgkin lymphoma)
- consequences of dry mucous membranes
- electrolyte imbalance (hypokalemia, acidosis)
Table 3.3 Classification of immunological responses according to the recognised antigen and significance

<table>
<thead>
<tr>
<th>antigen</th>
<th>significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>bacterium, virus</td>
<td>resistance</td>
</tr>
<tr>
<td>harmless external substances</td>
<td>allergy</td>
</tr>
<tr>
<td>body’s own components</td>
<td>autoimmune disease</td>
</tr>
</tbody>
</table>

Table 3.4 Possible autoantibodies in Sjögren’s syndrome and disease features

<table>
<thead>
<tr>
<th>autoantibodies to</th>
<th>disease feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSA/Ro and/or SSB/La</td>
<td>sensitive to sunlight</td>
</tr>
<tr>
<td>granulocytes</td>
<td>neonatal lupus in children</td>
</tr>
<tr>
<td>blood platelets</td>
<td>too few granulocytes</td>
</tr>
<tr>
<td>red blood cells</td>
<td>too few blood platelets</td>
</tr>
<tr>
<td>“phospholipids”</td>
<td>breakdown of red blood cells</td>
</tr>
<tr>
<td>thyroid TSH-receptor</td>
<td>antiphospholipid syndrome</td>
</tr>
</tbody>
</table>

Autoantibodies

Autoantibodies recognise the body’s own components. The function of antibodies is recognition and provoking a response from proteins or cells. Antibodies can be formed against virtually every substance and this substance is known as an antigen. If antibodies later encounter the antigen again (recognition), the consequences depend on the nature of the antigen (table 3.3). If the antibodies recognise components of a virus or bacterium, this creates resistance to the infectious disease concerned. If the antibodies are directed at harmless external substances (e.g. pollen), the response is called an allergy. If the antibodies damage the body’s own cells, we call this an autoimmune disease (T lymphocytes may also give similar responses).

Autoantibodies to SSA/Ro and SSB/La

The best known autoantibodies in Sjögren’s syndrome are those against SSA/Ro and SSB/La.1,4 These are proteins that occur in every cell of our body and play a role in the division of cells. Although it is not certain whether these antibodies can cause disease symptoms in Sjögren’s patients, they may possibly increase the skin’s sensitivity to sunlight.5 When women with these antibodies are pregnant, the antibodies also reach the foetus. There is then an approximately 10% risk of the baby developing neonatal lupus.6,7 The most common symptoms are skin disorders (that subside of their own accord) while the most serious complication that can occur is congenital heart block.8,9 Neonatal lupus is discussed in more detail in chapter 11.

Table 3.4 shows a number of consequences of the occurrence of specific autoantibodies in Sjögren’s syndrome. Granulocytes are certain types of white blood cells (see chapter 15, Clinical investigations). Antiphospholipid antibodies are discussed in the chapter 13 on pregnancy and lactation.

Autoantibodies against muscarinic M3 receptors

Based on a specific strain of mice (NOD mice) that develops diseases such as Sjögren’s syndrome, American scientists have developed a mouse without B-lymphocytes.29 B-lymphocytes are the white blood cells that make antibodies after maturing into plasma cells. In other words, these mutated mice make no antibodies. Like the normal NOD mice, they developed typical lymphocyte infiltrates in the salivary and lacrimal glands but made normal saliva and tears. This was striking since it was believed to be the infiltrates that caused the dryness. It was then seen that if antibodies from normal NOD mice or patients with Sjögren’s syndrome were injected into the mice without B-lymphocytes, they then developed symptoms of dryness. This therefore means that it was the antibodies that caused the reduction in fluid secretion and not the inflammation in the glands. It was shown to concern antibodies against muscarinic M3 receptors that are present in cells in the lacrimal and salivary glands. The glands normally secrete fluid when acetylcholine (ACh) is released from nerve ends and binds to the M3 receptor (see figures 3.2 and figure 7.2).30,31

Autoantibodies to the M3 receptor in the sera of patients with Sjögren’s syndrome recognise and bind to the M3 receptors of salivary glands in normal human people and patients with Sjögren’s syndrome in vivo.42 In recent years, various different researchers have attempted to demonstrate the presence of antibodies against M3 receptors in the blood of patients with Sjögren’s syndrome. This has only been successful for research purposes. No test is available that can be used in clinical practice to date.

Pilocarpine

Pilocarpine is a drug that binds to M3 receptors, causing the glands to be stimulated into producing fluid. This
probably also concerns M3 receptors that are not normally reached by acetylcholine from the nerves (see figure 3.3). Pilocarpine has been known for several hundred years and was discovered by South American Indians who chewed on the leaves of the Pilocarpus jaborandi shrub if they had a dry mouth. In 50-60% of Sjögren’s patients, use of pilocarpine leads to a decrease in dryness. Cevimeline is another new substance with the same effect, but is not available in Europe. Pilocarpine not only increases fluid production in lacrimal and salivary glands. Other exocrine glands and smooth muscle tissue may also respond to the drug. This is why some patients experience side effects such as sweating and a need to urinate more frequently (there are also M3 receptors in the bladder muscle). In some patients, pilocarpine helps the eyes but not the mouth, or vice versa. There are also people whose only response is to sweat.

It is possible that the different effects of pilocarpine may be connected with small differences in the M3 receptors of organs and between individual people. If this hypothesis is correct, it could also explain the different forms of Sjögren’s syndrome occurring in different people.

**Autoantibodies to 120 kD α-fodrin**

Japanese scientists have discovered that a certain strain of mice in which the thymus is removed 3 days after birth develops Sjögren’s syndrome. They also found that these mice have antibodies and T-lymphocytes that react with 120 kD α-fodrin, a fragment of 240 kD α-fodrin. 240 kD α-fodrin is a protein that particularly plays a role in fluid secretion by cells, but also occurs in the membrane of most cells in the body. The scientists then injected 120 kD α-fodrin into the mice without a thymus. The result was that these mice did not develop Sjögren’s syndrome.

120 kD α-fodrin is also found in the salivary glands of Sjögren’s patients, but not in healthy people. The antibodies and T-lymphocytes in the patients recognise the 120 kD α-fodrin, but not the 240 kD α-fodrin.

α--Fodrin also plays a role in apoptosis. Apoptosis is a process whereby a cell "switches itself off" in order to die. If a cell goes into apoptosis too soon, this is harmful because the cell is still young, not yet mature and not yet fully functional. Nor it is good if apoptosis takes place when the cell is too old, since damage can be caused by disruption to or loss of function. In other words, apoptosis needs to take place at exactly the right moment. In the case of apoptosis in patients with Sjögren’s syndrome, scientists suspect that too...
much 120 kD α-fodrin is being formed. Many Sjögren’s patients appear to have antibodies against 120 kD α-fodrin. More research is necessary to discover the exact significance of this.

**Immune complexes**
Immune complexes are complexes of antibodies, complement proteins and antigens. The formation of immune complexes is normal and is a way in which the body disposes of superfluous substances, e.g. bacterial residue. Depending on the composition of the immune complexes (type of antibody and antigen, size) they can be formed in the wall of blood vessels, resulting in inflammation of the blood capillaries (vasculitis). This is usually visible in the form of large or small haemorrhages in the skin (petechiae or purpura, respectively) on the lower legs.

**Focal lymphocytic infiltration**
Focal lymphocytic infiltration (localised clusters of lymphocytes) not only occurs in the lacrimal and salivary glands (figure 3.3), but may also occur in other organs such as the stomach, pancreas and kidneys. In the lip biopsy this is expressed as the focus score. A focus is a cluster of 50 or more lymphocytes and the focus score is the number of these foci in a 4 mm² section of tissue.\textsuperscript{10,11}

The contribution of the infiltrates to the functional impairment and damage to the glands is uncertain. It probably is far less the case than people think. Whereas 53% of the surface of a lip tissue specimen normally consists of gland cells, in the case of Sjögren’s patients this is 34%.\textsuperscript{12} Figure 3.2 shows the lip tissue specimen (with a focus score of 4) of a Sjögren’s patient with a very dry mouth. These data indicate that the infiltrates cannot be the only cause of the decrease in saliva formation. Experience with pilocarpine (see further) reinforces the idea that it may well be more complex than hitherto thought. On the other hand, focal lymphocytic infiltration may also be found in healthy people without complaints of dryness of the mouth. The frequency of focal lymphocytic infiltration in healthy volunteers has been found to be 15%. The positive focus score ranged from 2 to 6 and did not correlate with age, smoking, serologic findings or salivary flow in these persons.\textsuperscript{41}

In a recent examination of inter-rater reliability for a group of 5 board-certified pathologists interpreting the same series of labial salivary gland biopsies, the agreement was found to be uniformly poor for judgments of diagnostic status, focus scores, and histological characteristics of biopsy specimens. This lack of reliability is troubling.\textsuperscript{43}

**Lymphomas**
5-8% of Sjögren's patients develop a malignant non-Hodgkin’s lymphoma in places where focal lymphocytic infiltrates are present.\textsuperscript{13-17} This is about 44x higher than the normal risk. The lymphoma usually is a MALT lymphoma caused by lymphocytes in the mucous membranes.\textsuperscript{18} Treatment of lymphomas depends on the type of lymphoma. The prognosis for MALT lymphomas is relatively favourable in comparison with other forms of lymphoma.\textsuperscript{18}

**Consequences of dry mucous membranes**
Dry mucous membranes can themselves be the cause of disorders. The lack of adequate saliva can lead to cervical caries (gum line tooth decay)\textsuperscript{19-21} and oral infections with Candida albicans (a yeast or single-cell fungus).\textsuperscript{22,23}

The bacterium Streptococcus mutans, that plays a role in tooth decay, is found in greater numbers in the mouth of a Sjögren’s patient.\textsuperscript{14}

**Electrolyte imbalance**
The presence of lymphocyte infiltrates around the renal tubules (interstitial nephritis) may be accompanied by inadequate excretion of hydrogen. The urine
consequently becomes less acid than normal, while too much acid remains behind in the body (acidosis). As a result of the acidosis, the lungs automatically begin to hyperventilate and this in turn can cause symptoms such as tingling in the hands and feet, a tight feeling in the chest, palpitations, headache, light-headedness and frequent yawning and sighing. Instead of hydrogen, the kidneys excrete more potassium. This can lead to potassium depletion with temporary symptoms of paralysis.25–28

References

as a candidate autoantigen in primary Sjögren’s syndrome.
Science 1997; 276:604.

Latest additions or modifications (date: dd.mm.yyyy)

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