REVIEW

Parasympathetic nervous system dysfunction in primary Sjögren's syndrome

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In the past sicca syndromes were attributed to destruction of glandular tissue. It is now thought that cytokines, autoantibodies, and parasympathetic nervous system dysfunction all have an important role in the xerostomia and xerophthalmia in Sjögren's syndrome.

We recently followed the case of a middle aged woman who had sicca syndromes, verified by positive Schirmer's test, fluorescein staining of the cornea and conjunctiva, and abnormal salivary gland scintigraphy. She was anti-Ro and anti-La positive, but had no substantial lymphocytic infiltration on a small gland salivary biopsy. After an episode of viral (probably herpetic) encephalitis her sicca symptoms disappeared, both subjectively and according to clinical tests. This prompted us to review and summarise experimental and clinical data for the pathogenesis of xerostomia and xerophthalmia in Sjögren's syndrome.

Primary Sjögren's syndrome (primary SS) is a chronic inflammatory autoimmune disease characterised by lymphocytic infiltration of the exocrine glands, resulting in dry eyes and dry mouth. The aetiology is unknown and the prevalence uncertain.^{1,2} The diagnostic criteria, proposed by the American-European Consensus group, consist of six items (subjective dry eyes, subjective dry mouth, objective ocular involvement, abnormal focus score in lower lip biopsy, objective tests of oral involvement, and presence of anti-Ro or anti-La). At least four items must be positive to establish the diagnosis of primary SS, including the demonstration of anti-Ro or anti-La or a characteristic minor salivary gland biopsy.³ Apart from sicca symptoms due to the glandular affection, various extraglandular manifestations may develop, including central and peripheral nervous system lesions.⁴

Autonomic nervous system dysfunction may be an unrecognised feature of primary SS. The experimental findings and results of clinical studies pertinent to this issue are inconsistent. Andonopoulos *et al* found the symptoms of autonomic dysfunction in about one half of the investigated patients, while functional testing gave abnormal results in two thirds of them.⁵ Barendregt *et al* reported no differences in the sympathetic and parasympathetic control functions, comparing the cardiovascular regulation between patients with primary SS and healthy controls, and also no correlation between the results of clinical tests for parasympathetic dysfunction and decreased salivation or

tearing.^{6–8} A diminished or absent microvascular response to the intracutaneous application of the cholinergic agonist carbachol was demonstrated in half (11 of 22) of the patients with primary SS examined by Kovács *et al*. However, they did not detect any differences in the occurrence of specific organ manifestations, including autonomic, the severity of glandular impairment, and laboratory findings, between the patients responding and not responding to carbachol.⁹

Xerostomia and xerophthalmia have, until recently, been explained by progressive inflammatory infiltration and destruction of the exocrine glands, as histological changes of the glands were correlated with the salivary flow rates.¹⁰ In general, patients with the highest focus score in the minor salivary gland biopsy also had the most severe reduction in the salivary gland function.¹¹ Repeated biopsies also showed time dependent aggravation of sialoadenitis.¹²

However, in patients with primary SS the focus score did not always parallel the sialometric result.^{10,13} Furthermore, in patients with other connective tissue diseases no signs of a decreased salivary function were reported, despite inflammatory foci in the minor salivary glands.¹⁴ All these findings point to an additional mechanism implicated in the impaired salivary secretion in primary SS. Affection of the autonomic, mainly parasympathetic nervous system, which controls the exocrine secretion, may be responsible for the glandular dysfunction, resulting in diminished salivation and tears.^{15,16}

Lachrymal and salivary glands are richly innervated. Parasympathetic nerve endings, containing acetylcholine (ACh), vasoactive intestinal peptide, and other neurotransmitters, send signals predominantly to the glandular tissues. Their stimulation, mediated through the muscarinic cholinergic receptors (MACHR), results in the secretion of fluid and ions. Impulses originate in the autonomic nuclei in the medulla oblongata, where peripheral afferent signals from the mucous surfaces and information from the central autonomic network integrate.⁴ Vasoactive intestinal peptide, as a trophic stimulus, is believed to regulate the replication and growth of the acinar cells,¹⁷ while adrenergic nerves send signals to blood vessels and are therefore responsible for protein secretion. Among the five different types of muscarinic receptors, M3ACHR predominate in

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Abbreviations: ACh, acetylcholine; CAN, central autonomic network; MACHR, muscarinic cholinergic receptors; NO, nitric oxide; NOD, non-obese diabetic; SS, Sjögren's syndrome

the lacrimal glands, whereas equal amounts of M3AChR and M1AChR are present in the salivary glands.¹⁸ M3AChR are also found in blood vessels, contributing to vasodilatation via nitric oxide (NO), in sweat glands, intestinal and urinary tract smooth muscles, iris sphincter, and other organs under the autonomic nervous system control.¹⁶

“Regulation of salivation and tear formation can occur in the central autonomic nervous system or peripherally”

Theoretically, disturbances of the autonomic nervous function responsible for diminished salivation and tear formation can originate in the central autonomic nervous system, or peripherally, being localised either in peripheral autonomic nerve fibres, or synapses of nerve endings with glandular cells, or the effector cells themselves.

CENTRAL AUTONOMIC NETWORK

The central autonomic network (CAN) consists of functional units in multiple areas, which control autonomic functions in tonic, reflexive, and adaptive manners. They receive and integrate visceral, humoral, and environmental information.¹⁹ There is no firm evidence for the involvement of CAN in primary SS, either in animal models or in humans. However, certain drugs (namely, antidepressive drugs, the antihypertensive drug clonidine) decrease salivation, which is a side effect exerted on the central nervous system.²⁰ This suggests that CAN has an important role in parasympathetic dysfunction, including hyposalivation in primary SS. Furthermore, patients with Alzheimer's disease have decreased salivary flow rates without any focal mononuclear infiltrates in the salivary glands.²¹ Central autonomic dysfunction is also a proposed pathogenetic mechanism in patients with fibromyalgia, who have hyposalivation.⁴

PERIPHERAL NERVOUS SYSTEM

Peripheral nerve fibres

Parasympathetic nerves themselves may be the next possible site of disturbance. In the regions of focal lymphocytic infiltrates, only a few neural axons were detected by immunohistological methods, which identify neural axonal markers and neural peptides.²² In contrast, they were preserved in the areas with residual normal acini, but did not seem to compensate for the loss of salivation and tear formation.²³ Additionally, some reports favour the suggestion of unaltered innervation. Zoukhir *et al* demonstrated that the density and patterns of parasympathetic, sympathetic, and sensory innervation in the non-inflamed acinar tissue in the MRL/MP-Fas-lpr/lpr murine model of SS were indistinguishable from those in age matched control mice.²⁴ Furthermore, Kovács *et al* were unable to confirm the denervational aetiology of the autonomic nervous system disturbance at the level of peripheral nerve fibres by studying the effect of carbachol—an M3AChR agonist—introduced into the skin of 22 patients with primary SS. The extent of vasodilatation was significantly lower in patients with primary SS than in controls, supporting the hypothesis that the dysfunction existed at the receptor or postreceptor level.⁹

Defects in neural transmission in synapses

As already mentioned, ACh is the main neurotransmitter released from the parasympathetic nerve endings into the synaptic cleft. Its action is mediated through the binding to muscarinic receptors on the effector cells. In primary SS, an increased number of M3AChR were found in the patients' glands, which may be secondary to two possible mechanisms: (a) disturbed release of ACh, which might be inhibited by

cytokines, or (b) receptor blockade by specific antibodies. Both mechanisms have been proposed in the pathogenesis of primary SS.¹⁶

Lymphocytes and acinar cells synthesise various cytokines (tumour necrosis factor α , interleukin 1) which, among other effects, also inhibit ACh release from the nerve endings.^{25, 26}

The theory of antibody mediated glandular dysfunction was supported by the findings of studies in non-obese diabetic (NOD) mice as an animal model of SS. The mice developed histological changes of the salivary glands similar to those in human SS, as well as decreased salivary flow rates.^{27, 28} It was speculated that the glandular dysfunction in NOD mice might have developed before the lymphocytic infiltration of the glands. In Ig μ^{null} NOD mice lacking B lymphocytes, the exocrine glands functioned normally despite T cell infiltration. A decrease in salivary flow, induced by transferring the sera of patients with primary SS or NOD mice with antimuscarinic antibodies to the Ig μ^{null} NOD mice, showed the importance of antibodies in evoking glandular dysfunction.²⁹

The significance of antimuscarinic antibodies in humans was studied by several authors. Bacman *et al* found high levels of antibodies to M3AChR in the sera of patients with primary (60–100%) and secondary (~80%) SS, but not in healthy controls. They also demonstrated the binding of antibodies to the second extracellular loop of protein G coupled M3AChR in the rat lacrimal and salivary glands. In an early step the antibody binding resulted in receptor activation, demonstrated by an increased action of inositol 3-phosphate and diacylglycerol activity through phospholipase C, followed by an increased intracellular Ca²⁺ concentration and activation of protein kinase C. Irreversible binding of the antibody to M3AChR may lead to progressive receptor blockade—probably through desensitisation, receptor internalisation, and intracellular degradation—resulting in down regulation of the exocrine secretion and emergence of clinical symptoms.^{29–33}

Antibodies to M3AChR may also damage the exocrine glands by the activation of inducible NO synthetase and accumulation of NO in the acinar cells,³⁴ although the cytokine induced NO production is more feasible.³⁵ Evidence for an increased NO concentration and confirmation of its salivary origin were reported.³⁴

Waterman *et al* described the functional effects of anti-M3AChR antibodies on the parasympathetic neurotransmission and smooth muscle contraction. The sera from five of the nine patients with primary SS examined inhibited rat bladder strip contractions evoked by carbachol or nerve stimulation. They confirmed the findings of Bacman *et al* by showing that the antibodies acted as a receptor antagonist and demonstrated inhibition of the autonomic functions. In view of the great similarity between the human and rodent muscarinic receptors, they suggested that anti-M3AChR antibodies might have the same effect in humans. Indeed, they found signs of autonomic dysfunction in three of the five patients with primary SS positive for antimuscarinic antibodies.¹⁶

Intracellular signalling

Changes have been described also on the postreceptor level—in the signal transduction. Diminished isoforms of protein kinase C were detected in the acinar cells,³⁶ and the specific water channel protein aquaporin-5 accumulated basally in the cell cytoplasm rather than being transported to the apical membrane.³⁷ A decreased fluid flow was reported in mice as a consequence of defective channel trafficking due to the absence of water channels, which are usually responsible for the rapid water transfer across the lacrimal and salivary gland cell membrane along with the paracellular water movement.³⁸ These findings may represent the primary event in the disease process, or follow the dysfunction of muscarinic receptors, as translocation followed the stimulation of cholinergic receptors.

CONCLUSION

In the past, sicca symptoms in primary SS were attributed solely to glandular destruction by infiltrating lymphocytes, of which T cells predominate. Newer findings point to an important role for cholinergic dysfunction in the disease pathogenesis, most probably mediated mainly by antimuscarinic autoantibodies. Little is known about earlier steps in the production of antimuscarinic antibodies, such as presentation of the putative autoantigen to autoreactive T lymphocytes and subsequent activation of B cells. Further research should provide clues to enable targeting of immune modulation and counterbalancing cholinergic dysfunction as prudent therapeutic approaches in SS.

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