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Characterization of lymphoid infiltrates in chronic obstructive sialadenitis associated with sialolithiasis

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BACKGROUND: Chronic obstructive sialadenitis is characterized by acinar atrophy, lymphocytic infiltrates and progressive fibrosis. The immunological mechanisms involved in the pathogenesis of this disease are, for the most part, unknown. The aim of the present study was to characterize the lymphocytic infiltrates in chronic obstructive sialadenitis associated with sialolithiasis.

METHODS: Paraffin-embedded tissue samples from 23 affected submandibular glands were immunostained for T-cells (CD3, CD4, CD8), cytotoxic T-cells (granzyme B), B-cells (CD20), plasma cells (CD38) and macrophages (Ki-M1P).

RESULTS: CD4-positive subsets were the predominant cells, and they were located mainly periductally. Isolated intraepithelial CD8-positive cytotoxic T-cells associated with ductal epithelial cell destruction were observed in all cases. B lymphocytes were restricted to lymphoid follicles located periductally and around intralobular ducts. In early stages of the disease, a large number of CD38-positive plasma cells were distributed diffusely in the periacinar area. With progression of the disease, conspicuous clusters of plasma cells were located especially between atrophic acini adjacent to fibrotic tissue. An intimate relation between the lymphocytic infiltrates and the ductal epithelium, the target of the inflammatory process, was observed.

CONCLUSION: The composition and distribution of inflammatory cells suggest that intraepithelial infectious agents may be the cause of the inflammatory reaction and the progressive fibrosis in this disease.

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Introduction

Chronic obstructive sialadenitis is the most frequent type of chronic sialadenitis and accounts for about 30% of all cases (1). Intra- and extraductal mechanical factors cause obstruction of the salivary duct system and lead to disturbed salivary secretion. Sialolithiasis is the most common cause of obstructive sialadenitis, and more than 90% of the sialoliths occur in the submandibular gland (2). Chronic obstructive sialadenitis is morphologically characterized by a periductal lymphocytic infiltrate, irregular ectasis of the ducts and acinar atrophy. The persistence and progress of secretory congestion may be associated with accentuated periductal and intralobular fibrosis, destruction of the lobular architecture, parenchymal loss and sclerosis with functional loss of the gland (3).

No special histological classification system for different types of chronic sialadenitis exists. Histologically, they are characterized by acinar atrophy, lymphocytic infiltrates and progressive fibrosis. Clinically relevant factors such as localization in the major or minor salivary glands, aetiological factors (bacterial, viral, radiation-related, immunological factors), the course of the disease (acute, chronic, recurring) and the patient's age and sex determine the classification of chronic sialadenitis (4). As various types of chronic sialadenitis show a similar histomorphological appearance, it may be difficult to distinguish between the different types histologically, particularly in advanced stages with marked fibrosis. Chronic sclerosing sialadenitis of the submandibular gland (Küttner tumour), which is often mistakenly defined as severe chronic sialadenitis of the submandibular gland with tumour-like glandular induration, is a good example of this. Recently, we determined the phenotype of the immunocompetent cells in chronic sclerosing sialadenitis of the submandibular gland. This disease is characterized by an abundance of CD8-positive T cells and cytotoxic destruction of glandular epithelial cells with the features of an autoimmune process (5).

Chronic sclerosing sialadenitis of the submandibular gland is often associated with sialolithiasis (30%). Disturbed salivary secretion and a change of the composition of saliva with the appearance of microliths as a result of acinar

Table 1 Classification of chronic obstructive sialadenitis

Stage 1: slight focal and periductal lymphocyte infiltration, slight periductal fibrosis
Stage 2: moderate diffuse and periductal lymphocyte infiltration, moderate periductal fibrosis and slight intralobular fibrosis, focal acinar atrophy
Stage 3: accentuated diffuse lymphocyte infiltration, periductal and intralobular lymph follicle formation, accentuated periductal and intralobular fibrosis, accentuated acinar atrophy and metaplasia of the duct epithelium
Stage 4: destruction of lobular architecture, marked parenchymal loss and sclerosis

atrophy and destruction of glandular cells and formation of sialoliths seem to be a secondary process in this disease and in other forms of non-obstructive chronic sialadenitis associated with sialolithiasis (6). Secondary formation of sialoliths in chronic sclerosing sialadenitis supports the original opinion of Küttner (7), who described this entity almost one century ago. The existence of sialoliths should not lead to conclusions about the primary obstructive nature of sialadenitis.

The exact aetiopathology and mechanism of atrophy of the glandular cells and lymphocytic infiltration associated with an increase in extracellular matrix in chronic obstructive sialadenitis are unknown. To date, no characterization of the immunological changes in the affected salivary glands has been undertaken.

For a better understanding of the inflammatory process and the fibrosis in chronic obstructive sialadenitis and to enable a detailed differentiation of this disease from other types of chronic sialadenitis, exact knowledge of the composition and distribution of cellular subpopulations is essential. The aim of our study was to determine the phenotype of leucocytes in chronic obstructive sialadenitis associated with sialolithiasis and to study their distribution in the affected submandibular glands.

Materials and methods

Formalin-fixed, paraffin-embedded tissue blocks from submandibular glands of 27 patients were obtained from the files of the Department of Otolaryngology, Head and Neck Surgery of the Philipps University. The submandibular gland of 23 patients (9 females and 14 males, mean age 47 years) had been removed because of chronic sialadenitis associated with sialolithiasis. Symptoms included a history of recurrent painful glandular enlargement, particularly caused by eating. The average duration of symptoms until surgical treatment was 6.8 months, ranging from 1.5 weeks to 3 years. Other types of chronic sialadenitis, such as autoimmune, radiation-related and types associated with generalized inflammatory diseases were excluded. Patients who had salivary gland tumours, leukaemia or malignant lymphomas were also not included in this study. The remaining four examined tissue samples concerned morphologically unaltered tissue samples of the submandibular gland as controls obtained during neck dissection (four males, mean age 60.8 years).

From the tissue samples, 4-μm-thin slices were cut, drawn upon 3-aminopropyltrietoxysilane (APES)-coated slides and dried overnight at 37°C. The following day, the sections were dewaxed in xylene and rehydrated in graded alcohol. The sections were stained with haematoxylin and eosin and Giemsa. The intensity and distribution of lymphocytic and monocytic infiltrates, the occurrence of germinal centres and the degree of fibrosis were reviewed. The extent of these features was graded according to Seifert's staging system (Table 1) (1).

Lymphoid cells and macrophages were identified immunohistochemically by means of the alkaline phosphatase-anti-alkaline phosphatase and the streptavidin–biotin complex methods using monoclonal and polyclonal antibodies. All slides were stained with antibodies against the B-cell antigen CD20 (monoclonal, 1 : 80 dilution, DAKO, Hamburg, Germany) and the plasma cell antigen CD38 (monoclonal, 1 : 50 dilution, DAKO), the T-cell antigens CD3 (polyclonal, 1 : 75 dilution, DAKO), CD4 (monoclonal, 1 : 20 dilution, DAKO), CD8 (monoclonal, 1 : 50 dilution, DAKO) and the macrophage marker Ki-M1P (monoclonal, 1 : 5000 dilution, Department of Haematopathology, University of Schleswig-Holstein, Campus Kiel, Germany) (8). It should be noted that CD38 is also expressed on activated T and B cells. Cytotoxic T cells were detected by means of granzyme B staining (1 : 20 dilution, Hölzel Diagnostika, Cologne, Germany).

To quantify the lymphocytic infiltration, periacinar and periductal cells were stained for CD3, CD4, CD8, CD20 and CD38, counted in consecutive sections and expressed as number per high-power field (0.25 mm^2 with a 10× eyepiece and 40× lens). The incidence of lymphoid follicles displaying germinal centre formation and B-cell nodules were counted in 1 cm^2. B-cell nodules were defined as B-cell focus with at least 50 CD20-positive cells. Negative controls in each stained series included sections with the primary antibody replaced by the buffer. Tonsillar samples were used as positive controls for the immunohistochemical reaction.

Results

The main histomorphological feature was an inflammatory lymphocytic infiltrate of varying intensity (depending on the stage of the disease), which was located especially in the periductal and periacinar area. In 7 cases, submandibular glands removed because of chronic sialadenitis showed stage 2 chronic sialadenitis, 12 cases showed stage 3 and 4 cases stage 4. None of our cases was in stage 1.

Immunohistochemical analysis of the periductal and periacinar lymphocyte subsets revealed that CD3-positive T cells were clearly dominant over CD20-positive B cells. The majority of the lymphocytes were T-helper cells characterized by CD4 expression (Fig. 1). The ratio of CD4- to CD8-positive cells was 1.9 : 1. T cells were also found in intraepithelial sites. Isolated intraepithelial CD8-positive cytotoxic T cells were present in all cases associated with ductal epithelial cell destruction. Granzyme B, an antigen expressed by activated cytotoxic lymphocytes,

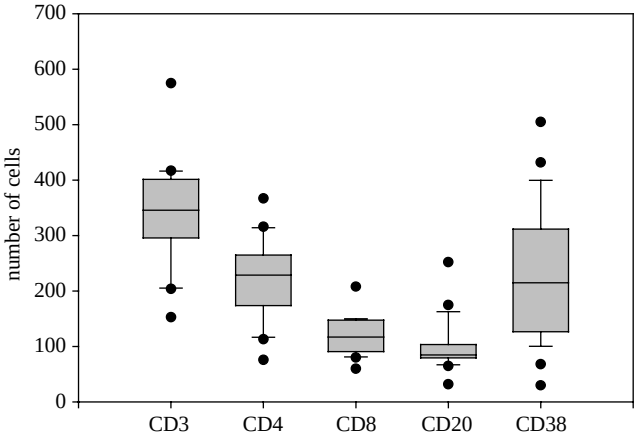

Figure 1 Analysis of the periductal and periacinar lymphocyte subpopulations by quartiles and box plots. Lymphocytes in lymph follicles and B-cell nodules were not considered.

was demonstrated in periductal and periacinar lymphocytes of all cases (Fig. 2a,b). B lymphocytes expressing CD20 were mainly restricted to lymphoid follicles located periductally and around intralobular ducts (Fig. 2c). The great majority of B-cell nodules and lymphoid follicles displaying germinal centre formation occurred in stage 3 of the disease. The ratio of incidence of B-cell nodules in stages 2–4 was 1.8 : 4 : 1. The ratio of incidence of lymphoid follicles displaying germinal centre formation was 1.5 : 6 : 1. Lymphoepithelial invasion by B cells was seen in 5 of 23 cases. In early stages of the disease, CD38-positive plasma cells were distributed diffusely in the periacinar area. With progression of the disease, conspicuous clusters of plasma cells were located especially between atrophic acini adjacent to fibrotic tissue (Fig. 2d). Ki-M1P staining revealed scattered macrophages and monocytic cells distributed over periductal, periacinar and intraepithelial sites. Small foci of epithelioid cells and single Langhans' giant cells were found in small clusters in 12 of 23 cases (Fig. 2e). Only a few isolated CD3-positive periductal lymphocytes were found in four normal samples obtained during neck dissection.

Discussion

Disturbed salivary secretion and a change in the composition of saliva, which is called dyschylia, lead to an increase in salivary viscosity and to a mucous obstruction in the terminal ducts of the salivary gland (9). Dyschylia and an increased formation of microliths in glandular ducts promote the ascent of bacteria and cause focal obstructive atrophy of the glandular parenchyma (10). Ductal secretory congestion is associated with proliferation of the ascending bacteria, which may play the major role in the formation of sialoliths in the presence of microliths (11). Sialoliths lead to further atrophic changes and secretory inactivity of the acini.

While degenerative changes to the acini are the most striking changes in the course of obstructive sialadenitis, there are also obstructive alterations in ductal segments, including ectasis of the ducts and metaplasia and proliferation of ductal cells. Histochemical studies revealed that the lectin-binding capacities increase along the luminar borders of ductal segments and that they display intense keratin immunoreactivity (12). The progression of the obstructive process leads to a marked decrease in carbonic anhydrase, lactoferrin, lysozyme and carcinoembryonic antigen in ductal cells (13, 14). In the course of the disease, ductal cells show changes in the expression of the major histocompatibility complex (MHC) antigens with increased expression of HLA-A–HLA-C. In addition, epithelial HLA-DR expression is significantly enhanced in intercalated ducts, compared to normal controls (15, 16), from which it can be assumed that there is a close relationship between ductal cells and lymphocytes. An exact observation of the morphological changes during the different stages of chronic obstructive sialadenitis revealed an initial increase in lymphocytic infiltration and fibrosis occurring adjacent to ductal cells.

Analysis of the immunological profile of periductal inflammatory infiltrates showed that CD4-positive T cells predominated in all cases examined. There was evidence of T-cell-mediated cytotoxic damage to the ductal cells. B lymphocytes were mainly restricted to lymphoid follicles displaying germinal centre formation located in the proximity of the ductal epithelium. This occurred particularly in stage 3 of the disease. In this stage, conspicuous clusters of plasma cells were also found between atrophic acini. Scattered macrophages and monocytic cells were distributed in periductal, periacinar and intraepithelial sites, where ductal destruction was evident. The periductal origin of the glandular inflammatory reaction and the intimate relation between lymphomonocytic infiltrates and the ducts suggest that the ductal epithelium is the target of the inflammatory process in this disease.

The inflammatory reaction obviously induces fibrotic changes, which encircle the ducts and extend into the interlobular septa. This process is very likely the result of the interaction of various cytokines and growth factors between glandular cells, lymphocytic infiltrates and extracellular matrix. The transforming growth factor beta is one of the most potent local factors for modulating extracellular matrix formation, and it is also a potent immunoregulatory agent enhancing monocyte function and suppressing lymphocyte proliferation and function (17). Analysis of this growth factor in chronic obstructive sialadenitis revealed that this cytokine is markedly overexpressed in the ductal system and that a close correlation exists between its expression and the degree of fibrosis (18).

Lymphoid infiltrates are a common feature in different types of chronic sialadenitis and reactive lesions of the affected salivary glands. They are also common in a variety of neoplastic conditions, including malignant lymphomas and immunoregulatory responses to parenchymal neoplasms (19). Focal lymphocytic infiltration of major and minor salivary glands is frequently seen in healthy volunteers and autopsy cases of individuals without a history of salivary gland dysfunction (20, 21). Histopathological studies of submandibular glands of autopsy cases revealed that the incidence of focal lymphocytic infiltration and also the amount of connective tissue in the submandibular gland increased in relation to the patient's age (22, 23). In a study of submandibular glands of autopsy cases, Kurashima &

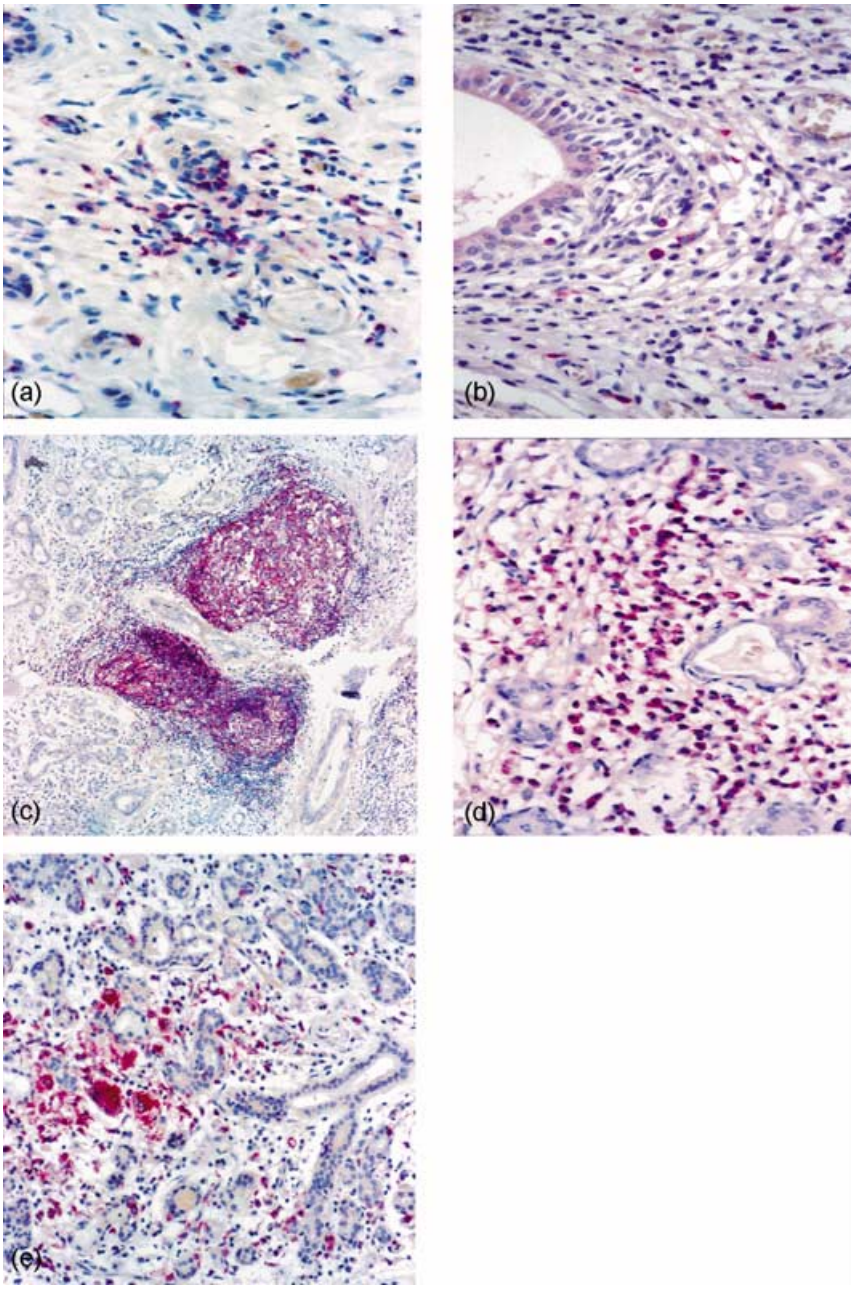

Figure 2 Immunohistochemical analysis of chronic obstructive sialadenitis. (a) periductal and intraepithelial T cells. CD8 staining, 400×, (b) cytoplasmic granzyme B staining of cytotoxic T cells, 400×, (c) germinal centre formation in the proximity of ductal epithelium, CD20 staining 50×, (d) plasma cells between atrophic acini, CD38 staining 400× and (e) macrophages and Langhans' giant cells in the periductal and intraepithelial area, Ki-M1P staining, 100×.

Hirokawa (22) demonstrated a immunohistochemical predominance of T cells, with the majority belonging to the helper T-cell subsets especially in advanced age. The percentage of cytotoxic cells was found to increase in the lesions in the periacinar area. They suggested that focal lymphocytic infiltration in the submandibular glands might be a sign of an immunological disorder based on an autoimmune process associated with the ageing process. In their study, patients with salivary gland tumours, sialolithiasis, autoimmune diseases and malignant lymphomas were excluded. Chronic sclerosing sialadenitis of the submandibular gland (Küttner tumour) shows a different immunological profile of lymphocytic infiltrates compared to the primary chronic obstructive type of sialadenitis. This disease has the features of an autoimmune process with dominance of CD8-positive cytotoxic T cells in close association with ductal epithelium (5).

In summary, the distribution of the T- and B-cell reactions and the immunological changes in different stages of chronic obstructive sialadenitis are not explicable on the basis of primary secretory congestion associated with increased pressure in the ductal system. Experimental

ligation of the ducts of salivary glands revealed that after complete obstruction the expected increasing lymphocytic infiltrates were not seen within the parenchyma (24). These findings suggest that intraepithelial infectious agents are the cause of the inflammatory reaction that leads to progressive fibrosis in chronic obstructive sialadenitis.

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