Histopathological study of the human submandibular gland in graft versus host disease

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Abstract
Major salivary gland dysfunction and severe xerostomia is one of the manifestations of graft versus host disease (GVHD). The histopathological evaluation of the major salivary gland in patients with GVHD has never been reported. The pathological findings of the submandibular glands in a GVHD patient who succumbed to the disease are described. Lymphocytic infiltration, parenchymal destruction, and fibrosis were observed, which may provide the pathophysiological mechanism for the xerostomia and hyposalivation observed in GVHD.

Keywords: graft versus host disease; lymphocyte infiltration; parenchymal destruction; submandibular salivary gland

Graft versus host disease (GVHD) is caused by donor graft T lymphocytes that recognise antigenic disparities between donor and recipient.1,2 Tissue damage associated with GVHD is thought to be caused by T cell mediated cytotoxicity1,2 and it is possible that additional networks of inflammatory cytokines act as mediators.3,4 GVHD is viewed as a three step process: upregulation of HLA and leucocyte adhesion molecules on host target cells, followed by activation of donor immunocompetent T cells by host histocompatibility antigens which then proliferate, and finally these activated T cells secrete cytokines, recruit additional cells, induce the expression of histocompatibility antigens, and focus their attack on recipient targets.5 Xerostomia, hyposalivation, decreased levels of salivary immunoglobulins, and increased incidence of oral infections are integral parts of GVHD.6,7 Salivary gland involvement in GVHD is indicated by increased dryness of the oral cavity, a change in saliva composition, and histopathological changes in the minor labial salivary glands.8–10 These pathological changes resemble the alterations observed in a Sjögren-like syndrome.11,12 In animal models of GVHD, lymphocytic infiltration of the major salivary glands has been demonstrated, mainly around the secretory ducts, where the cells express HLA class I antigens.13,14 To the best of our knowledge, histopathological evaluation of the major salivary gland in human GVHD has never been done.

Using sialometry and salivary gland scintigraphy, we recently assessed the effect of GVHD on the functional status of the major parotid and submandibular salivary glands in patients with GVHD and observed a significant reduction in the major salivary flow rates.15 In this study, we describe the histopathological findings in the submandibular gland in a patient who died from GVHD. The lymphocyte infiltration, parenchymal destruction, and fibrosis observed may explain the hyposalivation, xerostomia, and other oral findings previously reported in GVHD.

Case report
A 39 year old male with Philadelphia positive chronic myeloid leukaemia in the chronic phase was referred for bone marrow transplant. The conditioning protocol included cyclophosphamide 4200 mg × 2 days, total lymph node irradiation 180 cGy × 4 days, and total body irradiation 200 cGy × 6 days. Twenty four hours after conditioning, he was transplanted with 3.4 × 108/kg T cell depleted (CAMPATH-1; rat antihuman CDw2 monoclonal antibody) bone marrow cells from his HLA identical sister. Four weeks after the bone marrow transplant, he developed severe GVHD (grade IV) with watery diarrhoea (> 2 litre/day) and raised serum bilirubin (> 1000 µmol/litre) and urea (> 40 mmol/litre). Concomitantly, he began to complain of severe xerostomia accompanied by difficulties in mastication and swallowing, as well as a taste aberration and a burning sensation in the mouth. Severe intraoral mucositis, erythema, tongue surface depapillation, and lichenoid and lupus-like lesions were observed on physical examination of the oral cavity. Sialometric evaluation (eight weeks after the bone marrow transplant) showed reduced stimulated and unstimulated submandibular/sublingual flow rates (0.05 and 0.01 ml/min, respectively; normal values (mean (SD) are 0.52 (0.06) and 0.07 (0.02) ml/min, respectively).16 He was treated with cyclosporin A (3 mg/kg) and intravenous solnemodrol (2 mg/kg). Three months later, he developed a fever (40°C), tachypnoea, and dyspnoea. Bilateral pneumonia was diagnosed and antibiotic treatment initiated but with no improvement. His condition deteriorated and he died. Necropsy examination showed macroscopically organising diffuse alveolar damage with pleural effusion and tracheal haemorrhage, hepatosplenomegaly, intestinal and interperitoneal haemorrhage, choaemic nephrosis, and left adrenal atrophy.

MICROSCOPY
Microscopic examination showed lymphoplasmacytoid infiltrations of the liver and gastrointestinal tract with total denudation of the intestinal epithelium. There was also profound cholestasis, hepatocyte dropout, sinusoidal...
fibrosis of the liver, and cell necrosis. Inflammatory reaction in the dermal–epidermal junction of the skin was observed.

Histopathological analysis of the submandibular salivary glands revealed lymphocytic infiltrates (mainly of T cell origin, fig 1), both perivascular and dispersed around ductules and between acini, with focal invasion of the ductular epithelium by lymphoid cells. Isolated epithelial cells, lining the affected ductules, showed signs of apoptosis. The infiltrates were accompanied by multiple foci of parenchymal destruction with replacement of the glandular tissue by fibrosis (fig 2).

Discussion

We report for the first time the results of a histopathological examination of the submandibular glands in a patient with GVHD, which revealed scattered lymphocytic infiltration around ductules and between acini and extensive destruction of the normal parenchyma with marked fibrosis. These findings may explain the xerostomia in a GVHD patient who developed other “classical” GVHD-induced oral lesions. Xerostomia, hyposalivation, decreased levels of salivary immunoglobulins, and an increased incidence of oral infections have been reported in GVHD,\(^2\) \(^7\) \(^8\) \(^10\)–\(^12\) \(^17\) and our histological findings are in accordance with previous pathological studies of salivary glands in animal models of GVHD, which showed mononuclear cell infiltration around the secretory ducts where the cells express class I antigens.\(^13\)–\(^16\) They also accord with the findings of lymphocytic infiltration and expression of HLA-DR on the epithelium of minor lip salivary glands in patients with GVHD.\(^4\) \(^18\)

The similarity between the pathological findings in humans and those shown in two rodent model studies\(^15\) –\(^20\) are striking. In those studies, a direct correlation was shown between the severity of the GVHD induced in the animals, the magnitude of the lymphocytic infiltration in the parenchyma of the major salivary glands (accompanied by extensive parenchymal destruction and marked fibrosis), and the level of salivary hypofunction (xerostomia) of these specific glands.

Considering the possible contribution of the preconditioning total body irradiation to the observed xerostomia and lymphocytic infiltration, we feel that the relatively low dose of irradiation received—a total dose of only 7.2 Gy (1.8 Gy \(\times\) 4)—would not have reduced salivary function, even though the major salivary glands were probably within the irradiation field. It is widely accepted that the major salivary glands in humans are not affected significantly by doses lower than 30–40 Gy.\(^21\)–\(^31\) However, when major salivary glands are affected by irradiation, the postirradiation sequelae are usually not characterised by lymphocytic infiltration.\(^12\)–\(^24\) Furthermore, the patient's complaints of dry mouth started concomitantly with the development of GVHD symptoms at four to five weeks postirradiation, while it is well described in published reports that irradiation-induced xerostomia starts by the end of the first week of irradiation and may reach a functional reduction of 50–60% even at that early stage.\(^21\)–\(^29\) \(^31\) In the GVHD animal studies of Nagler et al, in which the animals were exposed to 9 Gy irradiation, a 62.4\% reduction in parotid gland function \((p < 0.01)\) was found in animals which developed acute GVHD, while irradiated control animals, in which GVHD was not induced, did not experience a significant reduction in their parotid flow rate.\(^20\) Additionally, the xerostomia was not the only oral/perioral symptom noted. Lichenoid and lupus-like lesions, as well as mucositis and erythema, were also noted. These could not be attributed to the irradiation effect, nor to oral conditions, as both salivary and oral–mucosal examinations failed to reveal fungal infection.

The pathophysiological mechanism of major salivary gland dysfunction in GVHD may be
caused by HLA upregulation, mononuclear infiltration, and cytokine dysregulation. Recently, various cytokines including interleukin-6 and interleukin-2 have been shown to influence the production of saliva and local IgA immunoglobulins. Therefore, it is highly possible that massive lymphocytic infiltration and aberrant production of cytokines are responsible for the major salivary gland hypofunction in GVHD. Major salivary glands seem to be a target organ for the immunological reaction that takes place in GVHD, leading to their ultimate fibrosis and destruction.

This report is the first to describe lymphocyte infiltration, parenchymal destruction, and fibrosis in human submandibular glands in a patient with GVHD. These pathological findings may underlie the physiological mechanism leading to the hyposalivation and xerostomia observed in GVHD.

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