Expression of Podoplanin in Minor Salivary Glands Increases in Primary Sjögren Syndrome

To the Editor:

Primary Sjögren syndrome (pSS) is a systemic autoimmune disorder of the exocrine glands that results in a reduction of salivary function. Although several hypotheses have been proposed over the years, the etiology of SS is still unknown[1].

Podoplanin is a mucin-type transmembrane protein that is expressed by lymphatic capillary endothelial cells but not by blood vessels. Podoplanin can also be expressed on many different types of tissues including myoepithelial cells (MEC), subsets of lymphocytes, and subsets of dendritic cells. Some studies on mice have indicated that podoplanin expression in MEC is increased in the salivary glands[2,3,4,5]. There has been some disagreement among researchers about the presence of lymphatic vessels in the lobules of major salivary glands[6]. This study was designed to investigate both the lymphatic capillary network and MEC by examining podoplanin expression in the minor salivary glands (MSG) of patients with pSS.

Specimens were obtained through MSG from 8 patients with pSS. The control group consisted of individuals with normal MSG tissues. Informed consent was obtained from all cases. The study complied with the principles of the Declaration of Helsinki and was approved by the local ethics committee.

H&E-stained tissue sections were examined for histopathological changes by an experienced observer. We used the mouse monoclonal IgG1 antibody D2-40 (Biocare) for the detection of the podoplanin. The number of lymphatic capillaries was investigated and the results of the podoplanin expression in the MEC were scored semiquantitatively, with scoring ranging from 0 to +4, and compared to the control group. Based on methods used in previous studies, each of the slides was technically examined under the assumption that the MEC and lymphatics would react with the antipodoplanin. D2-40 immunopositive lymphatic capillaries were counted in each magnification of the field of view under the light micro-

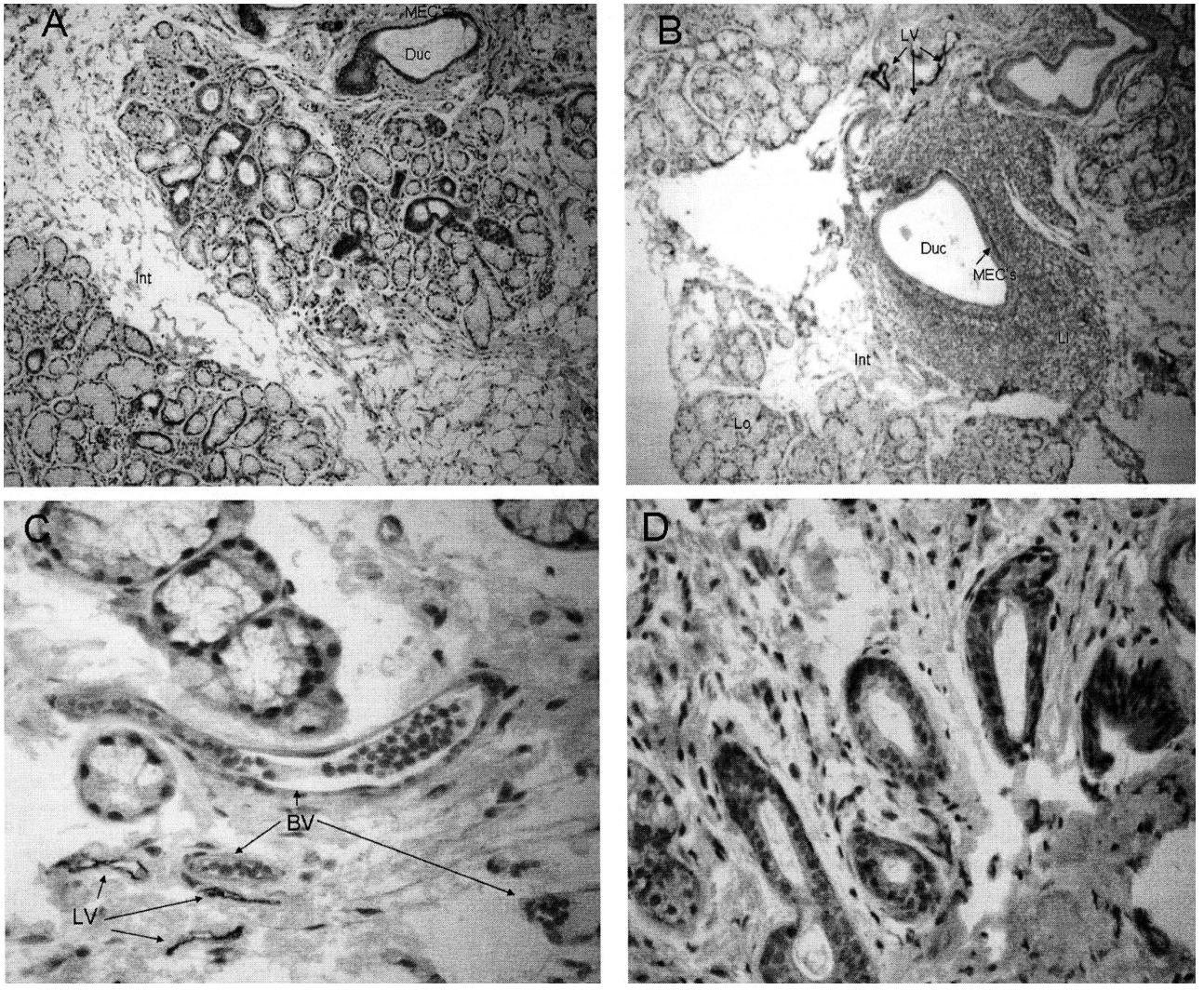

Figure 1. Light micrographs of the minor salivary glands after podoplanin immunostaining (100×). A. Minor salivary gland tissue of sample taken from a subject in the control group; ducts (Duc) and interstitial field (Int) in normal range. There is no lymphatic capillary marked with podoplanin, and there is no involvement in the localization of myoepithelial cells (MEC). B. In a primary Sjögren syndrome (pSS) case, periductal lymphocytic infiltration (LI), lymphatic vessels (LV) in the Int within this lymphocytic aggregation, and intensive linear podoplanin involvement in the MEC localization under the ductal epithelium can be noted. C. In a pSS case (400×), while no staining with podoplanin is seen in the endothelium of the blood vessels (BV) with erythrocytes inside, staining in the LV near this field can be noted. D. Podoplanin involvement can be seen in the MEC in the periductal slide (400×).

Table 1. The clinical and histopathologic characteristics of the study groups.

	Age, yrs	Sex	ANA	SSA (Anti-Ro)	SSB (Anti-La)	Histopathology	Immunohistochemistry of the Minor Salivary Glands		
							Lymphatic Capillaries	Podoplanin Expression in Myoepithelial Cells	Podoplanin Expression in Periductal Lymphocytic Infiltration
pSS									
1	65	F	1/320	+	–	Chisholm III	++	+++	++++
2	32	F	1/1000	+	+	Chisholm III	+++	+++	++++
3	48	F	1/100	+	+	Chisholm III	+++	++	+++
4	42	F	—	+	–	Chisholm III	+++	+++	++++
5	49	F	1/320	+	–	Chisholm III	++	+++	++
6	63	F	1/320	+	+	Chisholm III	+++	++	+++
7	32	F	1/100	+	+	Chisholm III	++	+++	+++
8	38	F	—	+	–	Chisholm III	++	+++	+++
Controls									
1	47	F				Normal	+	+	
2	44	F	—	–		Normal	–	–	
3	34	F	—	–		Normal	+	+	
4	46	F				Normal	–	–	
5	34	F	—	–	–	Normal	–	+	
6	51	F	1/100	–	–	Chisholm I	–	+	
7	70	M	1/100			Chisholm I	+	+	

ANA: antinuclear antibody; pSS: primary Sjögren syndrome.

scope. While no staining was seen in any of the lymphatic vessels in the samples belonging to the control group, staining was seen in > 1 of the lymphatic capillaries samples belonging to the patients with pSS (Figure 1). The intensity of the lymphatic capillaries seen in 1 microscopic field of view has been scored for the cases in groups. Podoplanin expression in the MEC in the MSG of the patients with pSS was found to be significantly higher than the expression in the control group (Table 1). It is also important to note that there was significant anti-IgG collection within the lymphoid aggregates of the periductal area in the samples taken from the patients. This intensity within the lymphoid aggregates has also been scored semiquantitatively, with scoring ranging from 0 to +4 based on the intensity of the podoplanin staining. It was determined that this staining was intensive in the tissues of almost all the patients with pSS (Table 1).

In previous studies, it was established that podoplanin is expressed by the MEC of major salivary glands[3,4,5]. We used human MSG and also investigated the podoplanin expression from MEC. The results show that the podoplanin expression from MEC increases in the MSG of patients with pSS. Podoplanin is also expressed highly in MEC of the breast glands, myofibroblasts of the prostate, fibromyocytes of the testis, and cells of the perineurium. All these cells can use their myofilaments to make contractions. It is thought that podoplanin might play a role in mediating cellular contractile properties and cytoskeletal reorganization. The higher podoplanin expression in the MEC of patients with pSS can be due to either the contractile functions of these cells or to their structural defects.

Our study revealed an increase in the number of lymphatic capillaries in the MSG of patients with pSS. In pSS, an increase in lymphatic capillaries in the salivary glands in the area where the inflammation occurs demonstrates the importance of the lymphatic network in the pathogenesis of the disease. The lymphatic capillaries can play a role through the stimulatory effects of the growth factors and the proinflammatory cytokines that are released in the inflammation. Proliferation of the lymphatic capillaries can be both the cause and the result of chronic inflammation.

We also detected cells expressing podoplanin intensively in the lymphocytic collection fields that were placed in the periductal area. Follicular dendritic cells (FDC) express podoplanin in the tonsils and lymph nodes[7]. Podoplanin is considered an effective marker for FDC[8]. FDC comprises a cell group that is important in the pathogenesis of pSS. It is demonstrated that podoplanin is stably expressed on Th17 cells only, not on other T helper cell subsets *in vitro*. Th17 cells around the ductal epithelial cells might be of critical importance in the initiation of pSS[9,10]. Within this lymphoid aggregate, the cells stained with podoplanin could be the follicular lymphocytic cells and/or Th17 cells that are placed there. Further studies are needed to demonstrate which cell types are responsible for the overall increase in detectable podoplanin.

Although our data are preliminary in nature and based on small case numbers, our findings suggest that the lymphatic system can be important in the immunopathogenesis of pSS. Identification of cells expressing podoplanin within lymphocytic aggregates can help reveal the pathogenesis of pSS.

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