<table>
<thead>
<tr>
<th>Title</th>
<th>Low prevalence of ectopic germinal centre formation in patients with HTLV-I-associated Sjogren’s syndrome.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Nakamura, Hideki; Kawakami, Atsushi; Hayashi, Tomayoshi; Nakamura, Tatsufumi; Iwamoto, Naoki; Yamasaki, Satoshi; Ida, Hiroaki; Eguchi, Katsumi</td>
</tr>
<tr>
<td>Citation</td>
<td>Rheumatology, 48(7), pp.854-855; 2009</td>
</tr>
<tr>
<td>Issue Date</td>
<td>2009-07</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10069/23205">http://hdl.handle.net/10069/23205</a></td>
</tr>
</tbody>
</table>

(c) The Author 2009. Published by Oxford University Press on behalf of the British Society for Rheumatology. All rights reserved.
Letter to the Editor

Low prevalence of ectopic germinal center formation in patients with HTLV-I-associated Sjögren’s syndrome.

Hideki Nakamura¹, Atsushi Kawakami¹, Tomayoshi Hayashi², Tatsufumi Nakamura³, Naoki Iwamoto¹, Satoshi Yamasaki¹, Hiroaki Ida¹, Katsumi Eguchi¹

¹ Department of Immunology and Rheumatology, Unit of Translational Medicine, Nagasaki University Graduate School of Biomedical Sciences, ²Department of Pathology, Nagasaki University Hospital, ³Department of Molecular Microbiology and Immunology, Nagasaki University Graduate School of Biomedical Sciences

Address for reprint requests and correspondence: Hideki Nakamura, MD, PhD Department of Immunology and Rheumatology, Unit of Translational Medicine, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki City, Nagasaki 852-8501, JAPAN Phone: 81-95-819-7260 Fax: 81-958-49-7270 E-mail: nhideki@nagasaki-u.ac.jp

Running title; Ectopic GC in HTLV-I-associated SS

Key words; CXCL13, Ectopic germinal center, Sjögren’s syndrome, HTLV-I, salivary gland biopsy
SIR, We have proposed that HTLV-I infection can be a possible environmental factor for SS based on high prevalence of anti-HTLV-I Ab in pSS in Nagasaki (1-3), and recently confirmed that LSGs of the HTLV-I-seropositive SS patients are less damaged in radiography (4). Amft et al (5) revealed prominent expression of B-cell-attracting chemokine 1 (BCA-1/CXCL13) on endothelial cells and lymphocytic aggregates in the ectopic germinal center (GC) of labial salivary glands (LSGs) in SS, speculating that ectopic GC is associated with the autoantibodies production as well as the salivary destruction (5).

We focused on the presence of ectopic GC formation in situ as well as the expression of CXCL 12/CXCL 13. Sixty-four pSS patients were entered and classification of pSS was determined by the revised criteria proposed by the American-European Consensus group (6). LSGs from a control subject who complained of sicca but did not meet the pSS criteria have been obtained. The presence of anti-HTLV-I Ab was determined by enzyme-linked immunosorbent assay (ELISA; Eitest-ATL kit; Esai, Tokyo, Japan) or particle agglutination assay (Serodia-ATL Kit; Fujirebio, Tokyo, Japan). Informed consent for the usage of samples obtained by the biopsy was obtained from all the participating patients as of the commencement of the study, and the study was conducted with the approval of the human ethical committee of our institution. Immunohistochemistry was performed by the labeled streptavidin-biotin method (Histofine Staining Kit, Nichirei Co., Tokyo, Japan) using mouse anti-CXCL 12 monoclonal Ab or goat anti-CXCL 13 polyclonal Ab (R&D Systems Inc., Minneapolis, MN, USA) (2) with microwave epitope retrieval for the detection of CXCL13. Negative control sections were treated with normal
mouse IgG or normal goat serum. Mann-Whitney’s U test, the Chi-square test or Fisher’s exact probability test were used for statistical analysis. A $P$ value less than 0.05 was statistically significant.

The gender and age were similar in 32 HTLV-I-seronegative (M/F;3/29, age;56.9 ±14.9) and 32 HTLV-I-seropositive (M/F;4/28, age;58.5 ±12.6) pSS patients, as well as in 9 HTLV-I-associated myelopathy (HAM)-pSS patients (M/F;1/8, age;61.6 ±8.9). Sicca symptoms were observed in 83.3%-100% of patients among the groups. Differences in anti-SS-A/SS-B Abs (Mesacup SS-A/Ro test and SS-B/La Test; Medical & Biological Laboratories, Nagoya, Japan) and IgG concentrations at the time of biopsy were not statistically significant irrespective of HTLV-I infection. Strikingly, ectopic GC was low in HTLV-I-seropositive pSS (1/32, 3.1%) as compared with HTLV-I-seronegative pSS (6/32, 18.8%) ($p=0.045$), and 0% in HAM-pSS.

Expression of CXCL13 was observed in 0-10% of MNCs of HTLV-I-seronegative pSS without ectopic GC patients or HTLV-I-seropositive pSS patients (Fig. 1). In HTLV-I-seronegative pSS ($N = 6$) with ectopic GC patients, the expression of CXCL13 was found dominantly in the light zone of ectopic GC. All of the cases showed more than 50% of MNCs in the light zone of ectopic GC expressing CXCL13. One pSS in HTLV-I carrier showed a relatively small size of ectopic GC whose CXCL 13 expression pattern was similar. However, interestingly, the MNCs of HAM-pSS patients demonstrated no expression of CXCL13. In contrast to CXCL 13, CXCL12 was commonly expressed on ductal epithelial cells of all the pSS patients irrespective of anti-HTLV-I Ab. In a normal subject, no expression of CXCL13 was observed with positive expression of CXCL 12 similar with pSS.
The lymphoid aggregates of LSGs are responsible for autoantibodies production that is locally occurred in ectopic GC (5, 7, 8). Radiographic destruction of the ductal structure in HTLV-I-seropositive pSS occurs to a lesser extent than in HTLV-I-seronegative pSS, which is a unique characteristic of the former (4).

The chemokines have been found to regulate ectopic GC formation of SS (5). Xanthou et al (9) also demonstrated significance of lymphoid chemokines for lymphoid structure formation in SS while others have demonstrated an association of CXCL 13 expression and ectopic GC formation in SS (7, 8). Barone et al found a B-cell-dominant expression pattern (8), whereas the selected expression in acinar and ductal epithelial cells was observed by Salomonsson et al. (7), although the exact roles of these results remain unclear.

Our data suggest an important interaction of CXCL 13 and ectopic GC in sialadenitis in SS. The tendency toward low levels of radiographic damage in patients with HAM-pSS suggests that salivary-specific cytotoxicity is modified by HTLV-I infection. Due to even expression of CXCL 12 irrespective of HTLV-I infection, HTLV-I presumably affects the CXCL 13 expression of infected CD4+ T cells. Via inflammatory mediators modulated by HTLV-I Tax protein, dysfunction of MNC-lineage cells due to HTLV-I infection is supposed to play an important role.

**Key message**

Low prevalence of ectopic GC formation is one of the characteristics of HTLV-I-seropositive SS patients associated with CXCL 13 on MNCs.
The authors declare no conflict of interest.

References


Figure legends

Figure 1 Expression of CXCL13 and CXCL12 in HTLV-I-seronegative and HTLV-I-seropositive patients with Sjögren’s syndrome (SS)

Immunohistochemical analysis of CXCL 13 (BCA-1) in minor labial salivary glands (LSGs) was demonstrated (A-D). After pretreatment with microwave epitope retrieval, goat anti-CXCL 13 polyclonal antibody was used to detect the expression of CXCL13. Expression of CXCL12 (SDF-1) was examined using mouse anti-CXCL 12 monoclonal antibody (E-H). A and E. Expression of CXCL 13 and CXCL 12 in minor LSG from an HTLV-I-seronegative SS patient with ectopic germinal center (GC). B and F. Expression of CXCL 13 and CXCL 12 in minor LSG from an HTLV-I-seronegative SS patient without GC. C and G. Expression of CXCL 13 and CXCL 12 in minor LSG from an HTLV-I-seropositive SS patient without GC. These are representative case of 5 patients with HTLV-I-seronegative SS with ectopic GC, HTLV-I-seronegative SS patients without ectopic GC, and HTLV-I-seropositive SS patients without GC, respectively. D and H. Expression of CXCL 13 and CXCL 12 in minor LSG from an HTLV-I-associated myelopathy (HAM)-SS patient. I and J. Expression of CXCL13 and CXCL12 in minor LSG section from a normal subject, respectively. K and L. Expression of CXCL 13 in the light zone and CXCL 12 in mantle zone of human tonsil tissue as a positive control, respectively. Hematoxylin and Methyl green were used for counterstaining of CXCL13 and CXCL12, respectively. (Original magnification x 100)