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Role of the Tonsil in HTLV-I Infection: A Molecular Pathological Analysis

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Human T-cell lymphotropic virus type I (HTLV-I) is etiologically associated with adult T-cell leukemia/lymphoma (ATLL) (1), HTLV-I associated myelopathy (HAM/TSP) (2) and HTLV-I associated uveitis (3). HTLV-I is also suspected to be associated with some types of inflammatory diseases. Little is known, however, about the pathogenic process from the latent infection to the development of these diseases. The lymphoid organ, such as the lymph node and the tonsil might play an important role in developing of the diseases. However, only a few studies have been reported about lymphoid organs in the non-ATLL individuals positive for HTLV-I-Ab (HTLV-I carriers)(4). In HIV-1 infection, the lymphoid organs, such as the lymph node and the tonsil, play an important role during the clinically latent period of infection and the histopathology is studied well. The tissue shows apparent morphologic changes and a large number of infected $CD4^+$ lymphocytes as well as high amount of HIV-1 were present throughout the lymphoid organs (5,6). It is of interest that similar events would be present or not in HTLV-I infection. In this study, we performed a molecular pathological analysis of tonsils of individuals positive or negative for HTLV-I-Ab in order to clarify the role of tonsils as a representative of HTLV-I-infected lymphoid organs.

Samples

One hundred and fourteen tonsils and peripheral blood samples were collected serially from patients who underwent tonsillectomy in a prospective manner in Kagoshima, the southern part of Japan. In addition, three tonsils and peripheral blood samples were collected from individuals who were known to be HTLV-I carriers and underwent tonsillectomy.

Seropositivity and PCR detection of HTLV-I

HTLV-I-Ab in serum was screened by particle agglutination and confirmed by immuno-fluorescence and enzyme-linked immunosorbent assay. The presence of HTLV-I provirus was detected by PCR in extracted DNA from both peripheral blood and tonsils. HTLV-I-Ab was positive in 5 (4.3%) out of 114 serially collected samples. This seropositivity is similar to that of age-matched control, suggesting that HTLV-I infection does not influence the occurrence of chronic tonsillitis.

HTLV-I provirus was detected in both blood and tonsils from all 8 seropositive patients including 3 patients who had been known to be HTLV-I seropositive. All of the DNA samples from the blood and the tonsil of individuals negative for HTLV-I Ab were negative for HTLV-I provirus in PCR. These results indicate that there is HTLV-I infection in the tonsils of HTLV-I carriers, and also suggest that existence of prolonged seronegative virus carrier is unlikely in HTLV-I infection.

Histopathologic characteristics of the tonsils

H&E stained paraffin sections were evaluated histopathologically, and the composition of lymphocyte subsets was evaluated by immunohisto-chemistry using monoclonal antibodies against CD45RO, CD68, CD4, CD8 and CD20 in order to clarify whether there is any difference in histo-pathologic features between the tonsils of HTLV-I infected and non-infected individuals.

All the specimens from 117 patients showed characteristic pathological changes of chronic tonsillitis. In addition, the lymphatic follicle and the marginal zone of tonsil-carrier-samples showed some different characteristics compared with those of tonsil-control-samples. The border between the mantle zone and the marginal zone became unclear, and the area of the mantle zone decreased. To confirm the difference we performed morphometric analyses and clarified following characteristics in the tonsil-carrier-samples; 1) the border between mantle zone and marginal zone became unclear, 2) there was a high number of T-cells in the mantle zone, 3) there was a high number of activated T-cells in the marginal zone, and 4) the mantle zone area decreased in size.

Similar histologic characteristics have been reported in the lymph node of individuals with HTLV-I infection (7,8). These histologic changes may be features of the lymphoid tissue reactive to any systemic or local inflammation in HTLV-I infection because tonsils we examined were taken from the patients with chronic tonsillitis.

Localization of HTLV-I provirus in the tonsil

To estimate the localization of HTLV-I infected cells, fresh frozen sections of tonsil-carrier-samples were divided into two divisions (follicular areas and extrafollicular areas) under the

microscope, and DNA extracted from each area was applied for PCR detection of HTLV-I provirus. In all 8 tonsil-carrier-samples, the extracted DNA from the extrafollicular area was positive for HTLV-I provirus, and the extracted DNA from the follicular area was negative for HTLV-I provirus.

Using fresh frozen sections of five tonsil-carrier-samples and tonsil-control-samples, PCR-ISH was carried out for localization of HTLV-I provirus using the protocol of Matsuoka et al. (9). HTLV-I provirus was detected in some small mononuclear cells of the marginal zone of all five tonsil–carrier-samples. No positive staining was detected in the other areas of tonsil-carrier-samples nor in tonsil-control-samples. Such a deviated localization of HTLV-I may cause histopathologic characteristics in tonsil-carrier-samples when any inflammatory stimulation is added in HTLV-I infected individuals.

Amount of HTLV-I provirus in the tonsil

To investigate whether the tonsil is the reservoir of HTLV-I infection, we performed a quantitative PCR of HTLV-I provirus on extracted DNA from tonsils of HTLV-I carriers by the ABI PRISM 7700 Sequence Detection System (10). The provirus load was compared with that in the peripheral blood and the value of HTLV-I-Ab.

The HTLV-I proviral load in tonsils was lower than that in peripheral blood. However, there was a significant correlation between the HTLV-I proviral load in the tonsil and in the peripheral blood (Pearson correlation coefficient was 0.952, P=0.001). There was also a significant correlation between the HTLV-I proviral load in tonsil and the value of HTLV-I-Ab in peripheral blood (Pearson correlation coefficient was 0.855, P=0.014).

In HIV-1 infection, the number of virus-infected cells is reported to be substantially larger in the lymphoid organ mononuclear cells, such as the lymph node and the tonsil, than in peripheral blood mononuclear cells. We could not deny, however, the possibility of the tonsil as a reservoir of HTLV-I provirus because the virus-replication of HTLV-I must be much slower than that of HIV-1, and the HTLV-I proviral load in the tonsil was significantly correlated with that in the peripheral blood and with the value of HTLV-I-Ab.

Discussion

It has been reported that the tonsil plays an important role in HIV-1 infection (11). There is, however, little research about the tonsil of HTLV-I infected individuals. In this study, we could demonstrate histopathologic characteristics and deviated localization of HTLV-I provirus in the tonsil of HTLV-I infected individuals. Although the histopathological findings seen in HTLV-I infected individuals were somewhat different from the findings of HIV-1 infection, changes of the mantle zone and presence of HTLV-I-infected cells in the marginal zone are similar to HIV-1 infection. Tonsil samples are used for assessment of HIV-1 infection, such as virus RNA titer (12), efficacy of treatment (13), etc. We suppose that analysis of tonsil could be used for the assessment of HTLV-I infection. Further investigation about tonsils of HTLV-I carriers as well as patients with HTLV-I associated diseases may clarify the important role of tonsils in HTLV-I persistent infection and pathogenesis of the related diseases.

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