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Failure in Detection of Epstein-Barr Virus and Cytomegalovirus in Specimen Obtained by Fine Needle Aspiration Biopsy of Thyroid in Patients with Subacute Thyroiditis

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MORI, K., YOSHIDA, K., FUNATO, T., ISHII, T., NOMURA, T., FUKAZAWA, H., SAYAMA, N., HORI, H., ITO, S. and SASAKI, T. *Failure in Detection of Epstein-Barr Virus and Cytomegalovirus in Specimen Obtained by Fine Needle Aspiration Biopsy of Thyroid in Patients with Subacute Thyroiditis.* Tohoku J. Exp. Med., 1998, **186** (1), 13-17 —— To study the possible role of viral infection in the etiology of subacute thyroiditis (SAT), we measured serum virus-specific antibodies to measles, rubella, mumps, type I herpes, chicken pox, human parvovirus B19 and cytomegalovirus (CMV) in 10 patients with SAT during the course of illness. In spite of the presence of IgG to each virus in more than 70% of patients, no significant changes in the IgG titers were observed except those to measles, rubella, chicken pox or CMV in only 10% of patients, respectively. Then we examined the presence of virus DNA in specimens of 9 patients with SAT obtained by fine-needle aspiration biopsy (FNAB) of the thyroid. DNA was amplified to detect that of Epstein-Barr virus and CMV by polymerase chain reaction. However, none of them were detected in all the specimens. Whereas previous studies suggest the involvement of viral infection in the pathogenesis of SAT, we failed to demonstrate significant changes in serum antiviral antibody titers or to detect viral DNA in the specimens obtained by FNAB of the thyroid. Thus further studies are clearly required to establish the definite role of viral infection in the pathogenesis of SAT.
—— subacute thyroiditis; viral infection; serology; polymerase chain reaction © 1998 Tohoku University Medical Press

Subacute thyroiditis (SAT) is characterized by high fever and painful goiter often preceded by a prodrome quite similar to an upper respiratory infection.

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The incidence of SAT usually predominates during the summer months (Saito et al. 1974). Such clinical features imply the possible involvement of viral infection in the etiology of SAT. Volpé et al. (1967) demonstrated significant changes in antiviral antibody titers during the course of SAT, and Eylan et al. (1957) suggested a possible association of mumps infection with SAT. Further, Vejlgaard and Nielsen (1994) reported the presence of antibodies to human parvovirus B19 in a patient with SAT. Thus viral infection has been implicated in the pathogenesis of SAT for a long time.

To confirm the essential role of viral infection in the etiology of SAT, however, the "direct evidence," such as isolation of virus itself and/or viral proteins or nucleic acids, is clearly required, since the proposed hypothesis is mainly supported by "indirect evidence" such as changes in serum antiviral antibody titers. To date, direct detection of viral DNA or RNA in the thyroid has never been reported. Recently polymerase chain reaction (PCR) has been widely used to detect viral DNA or RNA in the human materials obtained from patients with a variety of diseases (Tamm et al. 1993). In the present study, we measured serum virus-specific antibodies to several viruses and further performed PCR to detect viral DNA in specimens of SAT patients obtained by fine needle aspiration biopsy (FNAB) of the thyroid.

Materials and Methods

Ten untreated patients with SAT were included in the present study under the informed consent. Diagnosis of SAT was made by the presence of high fever, painful goiter, accelerated erythrocyte sedimentation rate, thyrotoxicosis and suppressed thyroidal iodide uptake. Sera obtained from SAT patients were analyzed to detect virus-specific IgM and IgG antibodies to measles, rubella, mumps, type I herpes, chicken pox, B19 and cytomegalovirus (CMV) by enzyme-linked immunosorbent assay (ELISA). Changes in serum antiviral antibody titers were monitered during 3 to 6 months after the onset of SAT, which is shown to be enough to detect significant changes in antibody titers in SAT patients (Volpé et al. 1967). DNA was isolated with DNA extraction kit (Wako Pure Chemical, Osaka) from specimens obtained from 9 patients with SAT and 7 with adenomatous goiter as controls by FNAB of the thyroid. The presence of cellular DNA was confirmed by PCR amplification of human glyceraldehyde 3-phosphate dehydrogenase gene. DNA sample (2 μl) was subjected to PCR amplification in the final volume of 25 μl of a solution containing PCR reaction buffer, 200 μM dNTPs, 2 mM $MgCl_2$, 1.25 U Taq DNA polymerase (Toyobo, Tokyo) and 200 nM each primer. For the detection of Epstein-Barr virus (EBV) DNA, primers were designed to amplify 161 base-pair (bp) fragment in the internal repeat 1 of EBV genome (5′-TCCTCGTCCAGCAAGAAGAG-3′ and 5′-CAACTTCAGGCAGCC-TAATCC-3′; Jenson et al. 1987). PCR was performed at 94°C for 2 minutes in 1 cycle followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C and 90

seconds at 72°C. For the detection of CMV DNA, nested PCR was employed. Primers used in the first PCR were designed to amplify 805 bp fragment in the major immediate early region (MIE) of CMV genome (5′-AAGAGAAAGATGGA-CCCTGA-3′ and 5′-ACGACGTTCCTGCAGACTAT-3′; Pritchett 1980). Those used in the second one were designed to amplify 462 bp fragment from the products amplified in the first PCR (5′-AAGGCATTCTGCAAACATCCT-3′ and 5′-TTGCTCACATCATGCAGCTCCT-3′; Pritchett 1980). The first PCR reaction was performed at 94°C for 2 minutes in 1 cycle followed by 30 cycles of 30 seconds at 94°C, 30 seconds at 55°C and 90 seconds at 72°C. The second one was performed at 94°C for 2 minutes in 1 cycle followed by 30 cycles of 30 seconds at 94°C, 15 seconds at 58°C and 90 seconds at 72°C. Each PCR amplification included positive and negative controls. The reaction products were separated by electrophoresis in 2% agarose gel, stained with ethidium bromide, and visualized under a UV lamp. In the preliminary studies, the specificity of the PCR reaction employed in the present study was confirmed, showing no cross-reaction with other viruses, and as little as 1 fg of plasmid DNA containing each viral DNA fragment was amplified and visualized in agarose gel (data not shown).

Results and Discussion

Since Volpé et al. (1967) demonstrated significant changes in the titers of antibodies to viruses such as influenza and mumps in 32 of 71 SAT patients, we measured virus-specific IgM and IgG levels in the sera obtained from 10 patients during the course of SAT, up to 6 months after the onset of the illness. As shown in Table 1, IgM antibodies to measles, rubella, mumps, type I herpes, B19 and CMV were negative in all the patients. Anti-chicken pox IgM was positive (1 : 41) in 2 of 10 patients, however, their titers did not change at all during the course of the illness. The sera of all the patients were positive for IgG to measles, rubella, and chicken pox. Nine patients had anti-CMV IgG. Anti-mumps and anti-type I herpes IgG were positive in 8 patients. Seven patients had anti-B19 IgG. In contrast to the study by Volpé et al. (1967), no significant changes (more than 4-fold dilutions; Volpé et al. 1967) in the titers of IgG to various viruses

Table 1. *Detection of viruses by serology and PCR in patients with SAT*

	Measles	Rubella	Mumps	Herpes I	Chicken pox	B19	CMV	EBV
IgM	0/10	0/10	0/10	0/10	2/10	0/10	0/10	n.d.
IgG	10/10	10/10	8/10	8/10	10/10	7/10	9/10	n.d.
Positive PCR	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0/9	0/9

The cut-off limits for the detection of virus-specific antibodies are as follows (IgM and IgG); Rubella, 1 : 41 and 1 : 204; PB19, 1.0 and 1.0; others, 1 : 41 and 1 : 44. The chicken pox IgM titer was 1 : 41 in both 2 positive cases. DNA isolated from specimens obtained by FNAB of the thyroid was subjected to PCR to detect that of CMV and EBV. n.d., not done.

during the course of SAT were observed except for the followings; anti-measles IgG in 1 patient, anti-rubella IgG in one, anti-chicken pox IgG in one and anti-CMV IgG in one. Thus, in contrast to the previous studies, we failed to demonstrate significant changes in serum antiviral antibody titers in SAT patients. The difference in race and the number of patients examined may contribute to the discrepancy of the results in these studies. However, the previous results should be interpreted carefully because virus-specific IgM was not separated from virus-specific IgG and changes in IgM titers were not evaluated, making it difficult to determine the role of viral infection in the onset of SAT (Volpé et al. 1967). Our data suggest that viral infection did not occur around the onset of SAT. Accordingly, it seems unlikely that viruses examined in the present serological study play a central role in the etiology of SAT. Thus, our results suggest that other viruses or causes may be involved in the pathogenesis of SAT, or the serological screening of virus-specific antibodies may not be suitable enough to determine viruses involved in SAT.

Recently, the PCR technique has been widely used to detect viral DNA in the human materials. For instance, CMV DNA was detected by PCR in synovial fluid of patients with rheumatoid arthritis (Tamm et al. 1993). In the case of thyroid, thyrocytes obtained by FNAB are used for the genetical analysis of various thyroid diseases with PCR (Takano et al. 1997). However, detection of viral DNA in the thyrocytes obtained by FNAB has never been reported. Previous studies imply a possible association of SAT with EBV (Hintze et al. 1964). Accordingly, to determine the presence of DNA of EBV or CMV in the specimens obtained by FNAB of the thyroid, cellular DNA was subjected to PCR amplification. However, PCR failed to detect any viral DNA examined in all the specimens obtained from 9 patients with SAT (Table 1) or 7 with adenomatous goiter (data not shown). Our results seem consistent with a previous report showing that EBV genomes were detected by PCR in the formalin-fixed, paraffin-embedded thyroid tissues of only 2 of 30 patients with thyroid lymphoma or 1 of 28 with chronic thyroiditis (Tomita et al. 1995). To draw a conclusion concerning an association of SAT with EBV and CMV, however, existence of viral DNA in the thyroid clearly needs to be examined in larger numbers of SAT patients.

In conclusion, we cannot find evidence supporting the hypothesis of the viral contribution to the etiology of SAT. In spite of our negative results, however, involvement of viruses or environmental factors in the pathogenesis of SAT cannot be ruled out. Further studies are clearly required to establish the definite role of viral infection in the etiology of SAT.

References

1) Eylan, E., Zmucky, R. & Sheba, C. (1957) Mumps virus and subacute thyroiditis. *Lancet*, **1**, 1062-1063.
2) Hintze, G., Fortelius, P. & Railo, J. (1964) Epidemic thyroiditis. *Acta Endocrinol.*,

45, 381–401.

3) Jenson, H.B., Farrell, P.J. & Miller, G. (1987) Sequences of the Epstein-Barr virus (EBV) large internal repeat from the center of a 16-kilobase-pair palindrome of EBV (P3HR-1) heterogeneous DNA. *J. Virol.*, **61**, 1495–1506.
4) Pritchett, R.F. (1980) DNA nucleotide sequence heterogeneity between the Towne and AD169 strains of cytomegalovirus. *J. Virol.*, **36**, 152–161.
5) Saito, S., Sakurada, T., Yamamoto, M., Yamaguchi, T., Yoshida, K. & Yoshinaga, K. (1974) Subacute thyroiditis: Observations on 98 cases for the last 14 years. *Tohoku J. Exp. Med.*, **113**, 141–147.
6) Takano, T., Sumizaki, H. & Amino, N. (1997) Detection of CD44 variants in fine needle aspiration biopsies of thyroid tumor by RT-PCR. *J. Exp. Clin. Cancer Res.*, **16**, 267–271.
7) Tamm, A., Ziegler, T., Lautenschlager, I., Nikkari, S., Moettoenen, T., Luukkainen, R., Skurnik, M. & Toivanen, T. (1993) Detection of cytomegalovirus DNA in cells from synovial fluid and periphral blood of patients with early rheumatoid arthritis. *J. Rheumatol.*, **20**, 1489–1493.
8) Tomita, Y., Ohsawa, M., Kanno, H., Matsuzuka, F., Kuma, K. & Aozasa, K. (1995) Sporadic activation of Epstein-Barr virus in thyroid lymphoma. *Leuk. Lymphoma*, **19**, 129–134.
9) Vejlgaard, T.B. & Nielsen, O.B.F. (1994) Subacute thyroiditis and parvovirus B19 infection. *Ugeskr. Laeger*, **156**, 6039–6040.
10) Volpé, R., Row, V.V. & Ezrin, C. (1967) Circulating viral and thyroid antibodies in subacute thyroiditis. *J. Clin. Endocrinol. Metab.*, **27**, 1275–1284.