Incidences of Antibodies to *Yersinia enterocolitica* : High Incidence of Serotype O5 in Autoimmune Thyroid Diseases in Japan

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Abstract

Antibodies to *Yersinia enterocolitica* serotype O3, O5, O6 and O9 were measured by the micro-agglutination method in 445 healthy subjects and patients with Grave's disease (n=70), Hashimoto's disease (n=45) and thyroid tumor (n=29). In contrast to previous reports, the incidence of antibodies to serotype O3 in each group of patients with thyroid diseases was not significantly different from that in healthy subjects. However, the incidence of antibodies to serotype O5 was significantly higher in patients with Graves' disease (81.4%, $P<0.001$) and Hashimoto's disease (91.1%, $P<0.001$) than in healthy subjects (58.9%). Significantly increased incidence of antibodies to serotypes O6 and O9 was observed only in patients with Hashimoto's disease (40.0% and 51.1% vs healthy subjects 24.7% and 29.9%, respectively). Patients with thyroid tumor showed no increase in any serotype of *Yersinia enterocolitica*. No correlations was found between the titers of anti-*Yersinia* antibodies and anti-thyroglobulin or anti-microsomal antibodies. These data indicate an association between thyroid autoimmunity and antibodies to *Yersinia enterocolitica*. These results are different from those in reports from other countries, suggesting that serotype specificity may be influenced by racial or genetic factors.

Graves' disease and Hashimoto's disease are considered to be caused by genetic autoimmune aberration which may be influenced by other factors. Delivery is known to be a precipitating factor (Amino and

Miyai, 1983) and postpartum onset of Graves' disease (Amino *et al.*, 1987) and Hashimoto's disease (Amino *et al.*, 1982) is frequently observed clinically. Some environmental factors, such as bacteria and virus, are also involved in triggering autoimmune diseases (Glynn, 1976), but the exact relation between infection with micro-organism and thyroid autoimmunity is still unknown.

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Infection with the gram-negative bacterium *Yersinia enterocolitica* induces gastrointestinal symptoms and is sometimes complicated by extra-intestinal symptoms, such as arthritis, erythema nodosum, carditis and iritis (Arvaston *et al.*, 1971). After infection, development of autoantibodies to nuclear antigen, smooth muscle, parietal cells and renal tubular epithelial cells is frequently observed and thus *Yersinia* infection has been thought to induce general humoral immune stimulation (Gripenberg *et al.*, 1978). In this connection, Bech *et al.* (1974) first reported a high incidence of antibodies to *Yersinia enterocolitica*, especially serotype O3, in patients with Graves' disease, Plummer's disease and non-toxic goiter in Denmark. Since then, a similar association between *Yersinia* antibodies and thyroid disease has been reported from Sweden (Lidman *et al.*, 1974, Lidman *et al.*, 1976), Finland (Leino *et al.*, 1988) and West Germany (Wenzel *et al.*, 1988). A cross-reaction between antigens of *Yersinia enterocolitica* and thyroid epithelial cells has been suggested (Lidman *et al.*, 1976), and an interesting finding in that *Yersinia enterocolitica* has thyrotropin (TSH) binding sites (Weiss *et al.*, 1983, Heyma *et al.*, 1986).

In contrast, no significant association between antibodies to *Yersinia* and thyroid diseases was observed in the United Kingdom (Keddie *et al.*, 1977), Australia (Reynolds *et al.*, 1978) or Spain (Saéz *et al.*, 1979). Results on the relation of thyroid diseases with antibodies to serotype O9 are also conflicting (Lidman *et al.*, 1976, Shenkman and Bottone 1976, Bech *et al.*, 1977, Leino *et al.*, 1988).

In Japan there have been no reports, to our knowledge, on *Yersinia* antibodies in patients with thyroid diseases. Therefore, we measured antibodies to *Yersinia enterocolitica*, including the new serotype O5, in patients with thyroid diseases in Osaka.

Materials and Methods

Subjects

We measured antibodies to *Yersinia enterocolitica* in 445 normal subjects, 70 patients with Graves' disease, 45 with Hashimoto's disease and 29 with thyroid tumors (22 with cancer and 7 with adenoma). The antithyroid microsomal antibodies of normal subjects were measured to exclude cases of subclinical autoimmune thyroiditis (Amino *et al.*, 1986).

Anti-*Yersinia* antibodies

Yersinia enterocolitica, serotypes, O3, O5, O6 and O9, were cultured on heart-infusion agar plate for 48 hours at 25°C. Then they were harvested and suspended in distilled water. After incubation for 2 hours at 37°C, the heavy suspension was heated as a flowing steam for 30 minutes at 100°C. The *Yersinia* obtained were washed 3 times with physiological saline and resuspended in distilled water. Finally, the *Yersinia* suspension was mixed with an equal volume of ethyl alcohol and this antigen solution was stored at 4°C. For antibody assay, the antigen solution was centrifuged at 1,500×g for 10 minutes and the organisms were resuspended in phosphate buffered saline (PBS: pH 7.4) containing bovine serum albumin (10 mg/ml). The *Yersinia* antigen solution was adjusted to an optical density of 0.35 at 550 nm. The antibody titer was measured by the micro-Widal agglutination method. Fifty μ l of serum sample was diluted serially 2-fold in micro-plates and 50 μ l of antigen solution was added to each well. The plates were first incubated at 37°C for 1 hour and then overnight at room temperature. The titer of antibodies was recorded as the end-point of agglutination. A titer of 8 or more was considered as positive.

Anti-thyroid antibodies

Anti-thyroglobulin antibody (TGHA) and anti-thyroid microsomal antibody (MCHA) were assayed by the hemagglutination method described previously (Amino *et al.*, 1976).

Results

The incidences of antibodies to *Yersinia enterocolitica*, serotype O3, O5, O6 and O9 in

Table 1. Incidence of antibodies to *Yersinia enterocolitica* in healthy subjects and patients with thyroid diseases

	No. examined	% Incidence of antibodies			
		Serotype			
		O3	O5	O6	O9
Healthy subjects	445	29.4	58.9	24.7	29.9
Graves' disease	70	27.1	81.4***	28.6	37.1
Hashimoto's disease	45	40.0	91.1***	40.0*	51.1**
Thyroid tumor	29	41.4	72.4	34.5	34.5

Difference from healthy subjects: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

healthy subjects, and patients with thyroid diseases are shown in Table 1. Unexpectedly, high incidences of antibodies, especially to serotype O5, were observed even in healthy subjects. In contrast to reports from Scandinavia and the United States, the incidence of antibodies to serotype O3 in patients with thyroid diseases was not different from that in healthy subjects. However, the incidence of anti-serotype O5 antibodies was significantly increased in patients with Graves' disease and Hashimoto's disease, but not in patients with thyroid

tumor. An increased incidence of antibodies to serotypes O6 and O9 was observed only in patients with Hashimoto's disease (Table 1). Figure 1 shows the distribution of titers of anti-O5 antibodies in healthy subjects and patients with Graves' disease and Hashimoto's disease.

There was no relation between the titers of anti-*Yersinia* antibodies and MCHA or TGHA. Figure 2 shows the relation of the titers of antibodies to serotype O5 and MCHA in patients with Graves' disease.

Fig. 1. Incidence of antibodies to *Yersinia enterocolitica* serotype O5 in healthy subjects (open columns) and patients with Graves' disease (shaded columns) and Hashimoto's disease (closed columns).

Fig. 2. Relation of antibodies to *Yersinia enterocolitica* serotype O5 and antithyroid microsomal antibodies (MCHA) in patients with Graves' disease.

Discussion

There are several different O antigens of *Yersinia enterocolitica*, and the antibodies to O3 in thyroid diseases have been studied extensively. The incidence of anti-O3 antibodies in thyroid disease has been reported to be high in Denmark (Bech *et al.*, 1974, Bech *et al.*, 1977), the United States (Shenkman and Bottone, 1976) and Finland

(Leino *et al.*, 1988). However, this has not been found to be so in the United Kingdom (Keddie *et al.*, 1977), Australia (Reynolds *et al.*, 1978) and Spain (Saez *et al.*, 1979). Patients with subclinical autoimmune thyroiditis (Yoshida *et al.*, 1978) were not excluded from healthy subjects in any of these studies. In this study we measured antithyroid microsomal antibodies (MCHA) in all subjects and excluded MCHA-positive subjects from the group of healthy controls. However, the incidence of anti-O3 antibody in healthy subjects was found to be 29.4% in Japan, which is higher than that in Europe or the United States, and we found no difference between its incidences in healthy subjects and the groups with thyroid diseases. This finding supports the results from the United Kingdom, Australia and Spain. Results on the incidence of anti-O9 antibody in thyroid diseases are also conflicting: the incidence is reported to be high in the United States (Shenkman and Bottone, 1976), but normal in Denmark (Bech *et al.*, 1977)

Spain (Saéz *et al.*, 1979) and Finland (Leino *et al.*, 1988). We found that the incidence was high only in Hashimoto's disease in Japan. The antibody to serotype O5 has not been examined before. We found that its incidence was high in Graves' disease and Hashimoto's disease.

The relation of anti-*Yersinia* antibodies to the nature of the thyroid disease is also important. In Denmark (Bech *et al.*, 1977) and the United States (Shenkman and Bottone, 1976), a significant association

was found between these antibodies and not only autoimmune thyroid disease but also non-autoimmune related diseases, such as toxic and nontoxic nodular goiter. A recent report from Finland (Leino *et al.*, 1988) indicated that IgA class antibodies were present mainly in cases of autoimmune thyroid disease, not in those of other thyroid diseases (toxic nodular goiter, nontoxic nodular goiter, thyroid adenoma and thyroid carcinoma). In this study we found an association only with autoimmune thyroid disease, suggesting that anti-*Yersinia* antibodies are related to thyroid autoimmunity but not to thyroid neoplasms.

There are several possible explanations for the association of anti-*Yersinia* antibodies with thyroid diseases. The first is the cross reaction of an antigen(s) of *Yersinia enterocolitica* with thyroid cells, as suggested by Lidman *et al.* (1974, 1976). However, the high incidence of anti-*Yersinia* antibodies in non-autoimmune thyroid diseases, the absence of a relationship of these antibodies to anti-thyroid antibodies and the serotype differences in different countries do not indicate this. An interesting recent finding was the demonstration of TSH binding sites on *Yersinia enterocolitica* (Weiss *et al.*, 1983, Heyma *et al.*, 1986). The high incidence of anti-*Yersinia* antibodies in thyroid diseases other than Graves' disease is also not compatible with this explanation, although the influence of *Yersinia* infection on the initiation of Graves' disease cannot be completely excluded.

A second possibility is a common genetic susceptibility to both *Yersinia* infection and thyroid autoimmunity. The different findings on antibodies to serotype O3 in various countries may be explained by differences in race.

A third possibility is general immune stimulation by *Yersinia* infection (Gripenberg *et al.*, 1978). This infection may break through the homeostatic immune surveil-

lance system and induce thyroid autoimmunity. There is yet little evidence supporting this and further studies are needed to elucidate the pathological relation between *Yersinia* infection and thyroid autoimmunity.

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