

Cell-Mediated Immunity to *Yersinia enterocolitica* Serotype 3 in Patients with Thyroid Diseases

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The cellular immunity to *Yersinia enterocolitica* serotype 3 and crude human thyroid extract in 64 patients with thyroid diseases and 25 controls was studied by the leucocyte migration test. In the patient group as a whole and in patients with Graves' disease and nontoxic diffuse goitre a significantly reduced leucocyte migration towards *Yersinia* was found when compared with the controls. In controls the migration index was not related to the presence or titre of circulating yersinia antibodies, whereas the migration index of patients with yersinia antibodies was lower than the migration index of patients without yersinia antibodies as well as that of the controls. The leucocyte migration inhibition in two patients with recent yersiniosis was normal during the recovery phase.

In the presence of thyroid extract leucocyte migration inhibition differed only significantly in Graves' disease. However, a significantly positive correlation between inhibition of migration by thyroid extract and by *Yersinia* was found, while no correlation could be demonstrated in the controls.

The cell-mediated immunity towards *Yersinia* in thyroid diseases thus demonstrated adds further evidence to the association between *Yersinia* and thyroid disease.

Key words: cell migration inhibition; immunity, cellular; thyroid disease; *Yersinia* infections.

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In thyroid diseases, particularly Graves' disease and Hashimoto's thyroiditis, a variety of thyroid autoantibodies together with evidence of delayed type hypersensitivity to thyroid antigens indicates an altered immune response in these diseases (37). Recently, a high frequency of agglutinating antibodies to the O-antigen of *Yersinia enterocolitica* serotype 3 (*Y. ent.*) has been found in sera from patients with various thyroid diseases (7, 8, 29). In an attempt to discover the nature of the relation between *Y. ent.* and thyroid disease we found it of interest to study the cell-mediated immunity of thyroid patients to *Y. ent.* A preliminary report has been published (9).

MATERIAL AND METHODS

Patients. The material comprised 64 patients, 56 females and 8 males, and their age varied from 17 to 73 years (mean 47.6 years). The patients were classified in subgroups as listed in Table 1. The thyroid function was evaluated by means of serum thyroxine, serum triiodothyronine, serum T_3 -resin test, serum thyrotropin, 131-iodine uptake by the thyroid gland and a thyrotropine-releasing hormone test. Thyroid microsomal antibodies were detected by immunofluorescence technique (36) and a titre >4 was regarded as positive. The specificity of the

Table 1

The frequencies of thyroid antibodies and agglutinating antibodies to *Yersinia enterocolitica* serotype 3 in patients with thyroid diseases

Patients	No.	Thyroid antibodies	Antibodies to yersinia titres	
			≥10	≥80
Nontoxic diffuse goitre	20	1	14	2
Nontoxic nodular goitre	17	7	10	2
Toxic adenoma	8	0	5	3
Graves' disease	19	13	13	6
Controls	25	1	12	4

reaction was controlled by parallel examinations for antibodies against human gastric mucosa and rat kidney. Anti-thyroglobulin antibodies were detected by passive haemagglutination reaction (15). As anti-thyroglobulin antibodies were found only in three cases, thyroid antibodies in the following refer to anti-microsomal thyroid antibodies. Antibodies to *Y. ent.* were determined by an agglutination technique (19). The thyroid gland was examined by palpation and Tc⁹⁹ scintigraphy to classify the goitre as either diffuse, nodular or a solitary adenoma. Graves' disease was defined as diffuse goitre and hyperthyroidism often associated with exophthalmos and/or pretibial myxoedema. All the patients were investigated at time of diagnosis before treatment, and none had symptoms compatible with yersiniosis.

Controls. The control group consisted of 25 persons without clinical and laboratory evidence of thyroid disease and without a family history of thyroid disorders. Thirteen were females and 12 males, aged 17 to 39 years (mean 29.5 years). Furthermore, two patients with symptoms of recent yersiniosis were studied and tested several times during the recovery phase.

Antigens. Thyroid tissue was obtained and pooled from five patients operated for diffuse nontoxic goitre. The tissue specimens were immediately placed in liquid nitrogen and

stored at -80°C. Under sterile conditions the specimens were dissected, minced and homogenized in Hanks's balanced salt solution in a Virtis homogenizer at 0°C. The homogenate was left overnight at 4°C under constant stirring and then filtered through cheese cloth. The protein content was estimated by the method of Lowry et al. (23), adjusted to 2 mg per ml and stored at -80°C.

The renal tissue was obtained from the tumour-opposite poles of four kidneys with small localized hypernephroma. The specimens were macro- and microscopically normal. Homogenization was performed as described above. The protein concentration was adjusted to 1 mg per ml before storage.

The yersinia antigen consisted of heat-killed, whole *Y. ent.* suspended in isotonic saline and kept at a stock solution of 10¹⁵ bacteria per ml at 4°C.

Leucocyte migration test. The leucocyte migration test was performed by the capillary technique developed by Søborg & Bendixen (30) and slightly modified as recently described (10). Each antigen concentration was tested in quadruplicate. The leucocyte migration area was measured and the migration index (MI) calculated from the average migration area with antigen ($\bar{M}_x$) and without antigen (M_0):

$$MI = \frac{\bar{M}_x}{M_0}$$

The antigen concentration, which clearly and consistently discriminates between an immunologically specific inhibition and a "toxic" inhibition of the migration, was determined for each antigen by titration. The thyroid antigen was tested at concentrations of 0.1 to 0.4 mg per ml, the renal antigen at 0.2 to 0.8 mg per ml and yersinia antigen at 10^6 to 10^9 bacteria per ml. The patients and controls were tested with each antigen in at least two concentrations.

Statistical evaluation. To analyse the distribution of migration indices of controls and patients a statistical evaluation for difference in position as well as dispersion was used. That is, the Fisher exact probability test modified after the Westenberg interquartile range test (14) and Wilcoxon-Mann-Whitney test.

RESULTS

Table 1 shows the frequency of thyroid antibodies and yersinia antibodies in patients and controls. In all patients the MI at antigen levels of 10^6 , 10^7 and 10^8 bacteria per ml were reduced when compared with controls ($P=0.03$, 0.01 and 0.02 respectively, Fig. 1).

Patients with Graves' disease had lower MI than controls at antigen levels of 10^6 and 10^7 bacteria per ml ($P=0.03$ and 0.03) and patients with diffuse nontoxic goitre showed reduced MI at an antigen level of 10^7 bacteria per ml ($P=0.02$). In contrast, no significant differences between MI of controls and patients with nontoxic nodular goitre or toxic adenoma were found (Table 2). No correlation between MI and the presence of anti-microsomal and/or anti-thyroglobulin antibodies could be demonstrated, and no relation to the thyroid status or duration of disease before treatment was noted. In controls the MI to *Y. ent.* was not related to the presence or titre of yersinia antibodies, whereas the MI of patients with detectable yersinia antibodies was significantly lower than the MI of controls and of patients without yersinia antibodies (Fig. 2). In the two persons with recent yersiniosis and antibody titres ranging from 360 to >1200 the leucocyte migration inhibition was normal during recovery (1.01 at 10^6 and 0.88 at 10^7 bact./ml).

The leucocyte migration inhibition in the presence of thyroid antigen is shown in Fig. 3. A statistically significant difference from

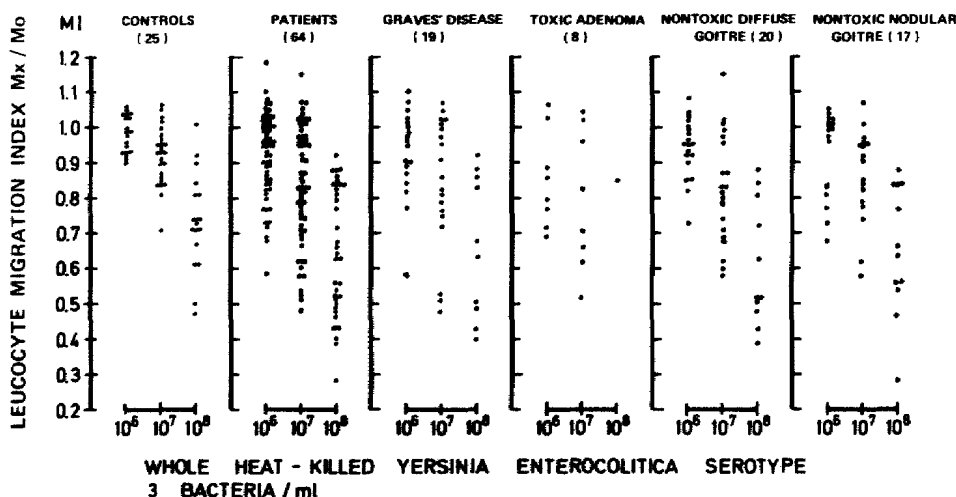


Fig. 1. Dose response of leucocyte migration inhibition with whole heat-killed *Yersinia enterocolitica* serotype 3. Statistical test used was Fisher's exact test.

Table 2

Leucocyte migration indices in presence of yersinia antigen at 10^7 cells/ml

Group	No.	MI (median and range)	P*
Controls	25	0.93 (1.06-0.71)	—
Graves' disease	19	0.86 (1.07-0.48)	0.03
Nontoxic diffuse goitre	20	0.83 (1.15-0.58)	0.02
Nontoxic nodular goitre	17	0.91 (1.07-0.58)	0.20
Toxic adenoma	8	0.77 (1.04-0.52)	0.05
Total patients	64	0.84 (1.15-0.48)	0.01

* Statistical evaluation by Fisher's exact probability test modified after the Westenberg interquartile range test.

controls was found only in Graves' disease ($P=0.009$) without relation to exophthalmos (three patients) or the presence of anti-thyroglobulin and/or anti-microsomal antibodies. However, both in the patient

group as a whole and in the subgroups, a significantly positive correlation between migration inhibition of thyroid and yersinia antigens (10^6 and 10^7 cells/ml) was found, while no correlation could be demonstrated in controls ($r=0.46 \pm 0.12$ and $r=0.04 \pm 0.07$ respectively, $P<0.001$ at following concentrations: yersinia 10^7 cells/ml and thyroid extract 0.2 mg/ml).

No significant difference between the MI of renal antigen in patients and controls was found.

DISCUSSION

Antigen-induced inhibition of human leucocyte migration has been used as an *in vitro* test of delayed hypersensitivity (13, 30). The inhibition is due to a release from sensitized T lymphocytes of leucocyte migration inhibition factor (LIF) acting on the motility of leucocytes (11, 12, 28). There is evidence of *in vitro* delayed type hypersensitivity in Graves' disease and Hashimoto's thyroiditis in response to thyroid antigens but not in nontoxic goitre, although this disease has not been studied extensively (4, 18, 24, 25, 26, 31, 33). The present study confirmed these findings.

Yersinia causes one of the commonest enteric infections in Scandinavia but is not a resident in the normal intestinal flora (20).

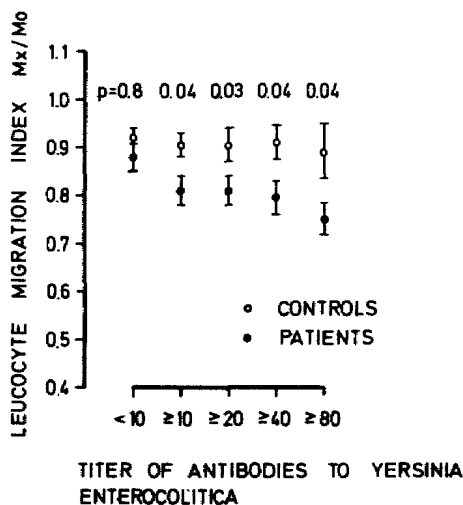


Fig. 2. Correlation between the leucocyte migration inhibition with yersinia at concentration of 10^7 cells/ml and the titre of circulating antibodies to yersinia in the sera of patients and controls. Each point represents the mean $\pm$ s.e.m., and the statistical test used was the Wilcoxon-Mann-Whitney test. Thirteen controls were at titre <10 , 12 at titre ≥ 10 , nine at titre ≥ 20 , seven at titre ≥ 40 and four at titre ≥ 80 . The number of patients at same titres were 23, 41, 30, 21 and 13 respectively.

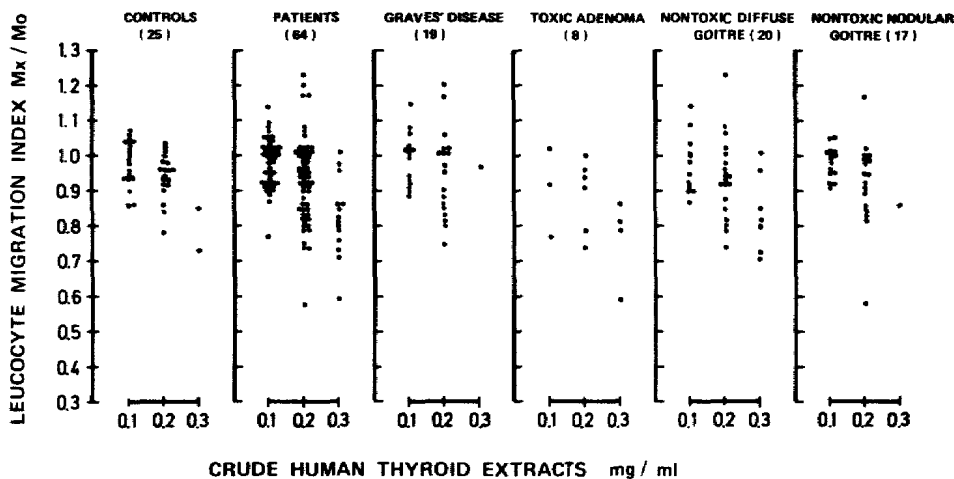


Fig. 3. Dose response of leucocyte migration inhibition with crude human thyroid extract. The statistical test used was Fisher's exact test.

During infection with *Y. ent.* an endotoxin of lipopolysaccharide (LPS) is released. LPS is known as a B-cell mitogen and able to substitute for T cells in the induction of a primary immune response (5). Recently, LPS has been shown to interfere with the T-cell function (16) and enhance the T-cell dependent delayed type hypersensitivity when given after antigenic stimulation (17), as well as having a direct adjuvant effect on the function of the T cells (27). Further, it has been shown that the immunological reaction to LPS is genetically determined both in mice and man (1, 15, 34, 35) and a genetically determined increased susceptibility to infections with *Y. ent.* in man is quite possible.

Recently, an association between agglutinating antibodies to *Y. ent.* and thyroid disease was demonstrated (7, 8, 29). In the present study we add further evidence of this association by the finding of a delayed type hypersensitivity towards *Y. ent.* in patients with thyroid disease in contrast to controls or patients with yersiniosis, although the patients and controls were not entirely comparable in age and sex. One explanation of these findings is a cross-reaction between antigenic determinants of *Y. ent.* and the

thyroid cell. This is supported by immunofluorescence studies (21, 22) which show a high frequency of specific thyroid cell-membrane directed antibodies in sera with agglutinating yersinia antibodies.

Another theory implicates *Y. ent.* in the pathogenesis of thyroid disease. Many antigens circulate in low concentrations in otherwise healthy persons. In such circumstances only T lymphocytes may be tolerant, while B lymphocytes are able to respond to autoantigens suitably presented to them with the help of T lymphocytes (2). However, it is assumed that autoimmunity is prevented by certain T-lymphocyte populations (T suppressor lymphocytes), which control and suppress the immune responses (3). It has been demonstrated (6) that the fraction of circulating B lymphocytes with membrane-attached thyroglobulin antibody is larger in Graves' disease than in healthy persons, and furthermore, a depressed level of total and "activated" T lymphocytes in patients with Graves' disease has recently been shown (32). Therefore, the potent immune stimulation of LPS by infection with *Y. ent.* might initiate an autoimmune reaction towards thyroid tissue

and yersinia in genetically predisposed persons, while the suppressor T lymphocytes in most people are sufficient to prevent this reaction.

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