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Abstract

Function of pituitary-thyroid axis was studied in rats with experimentally induced thyroiditisWistar strain female rats were immunized with homologous thyroid extract in Freund's complete adjuvant and with simultaneous intradermal injection of pertussis vaccine concentrate. They received booster shots at the first and third week after the initial immunization. Serum thyroid hormones and TSH were measured just before, and at weekly intervals after, the initial immunization. Histological examination of the thyroid gland at the second week after immunization showed slight infiltration of macrophages in the thyroid follicles. From the third to the fourth week, massive lymphoid cell infiltration and destruction of follicular architecture developed in all immunized rats. Serum R3 levels slightly decreased during the second week, increased transiently during the third week, then decreased again thereafter. Serum T4 levels decreased slightly durinf the fourth week. Serum TSH levels were not elevated significantly during the third week, but the response to TRH was significantly increased at this time. Basal TSH levels were increased during the fourth week. The TRH test was a sensitive method capable of detecting minimal failure of thyroid function undetected by other routine measuremens of thyroid hormones and TSH.

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STUDIES ON THYROID FUNCTION IN RATS WITH EXPERIMENTALLY INDUCED THYROIDITIS

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Abstract. Function of pituitary-thyroid axis was studied in rats with experimentally induced thyroiditis. Wistar strain female rats were immunized with homologous thyroid extract in Freund's complete adjuvant and with simultaneous intradermal injection of pertussis vaccine concentrate. They received booster shots at the first and third week after the initial immunization. Serum thyroid hormones and TSH were measured just before, and at weekly intervals after, the initial immunization. Histological examination of the thyroid gland at the second week after immunization showed slight infiltration of macrophages in the thyroid follicles. From the third to the fourth week, massive lymphoid cell infiltration and destruction of follicular architecture developed in all immunized rats. Serum T3 levels slightly decreased during the second week, increased transiently during the third week, then decreased again thereafter. Serum T4 levels decreased slightly during the fourth week. Serum TSH levels were not elevated significantly during the third week, but the response to TRH was significantly increased at this time. Basal TSH levels were increased during the fourth week. The TRH test was a sensitive method capable of detecting minimal failure of thyroid function undetected by other routine measurements of thyroid hormones and TSH.

Key words: thyroid function, thyrotropin (TSH), experimental thyroiditis, thyroid hormones.

Patients with chronic thyroiditis show wide variation in their thyroid function, ranging from hypothyroidism to hyperthyroidism. We have reported that the TRH test was useful in detecting minimal failure in thyroid function of patients with euthyroid chronic thyroiditis and with asymptomatic autoimmune thyroiditis (1, 2). In patients with asymptomatic thyroiditis, thyrotrophs were increased in number and size, showed dilated Golgi area and large nuclei, and the morphological evidence of thyrotropic activity correlated well with the high serum TSH concentration (3). However the relationship between the degree of thyroid lesion, circulating thyroid hormone levels and pituitary responsiveness to TRH is unclear during the development of experimental thyroiditis.

In this paper, we assess the function of pituitary-thyroid axis of rats with thyroiditis induced experimentally by immunization with homologous rat thyroid extract.

MATERIALS AND METHODS

Wistar strain female rats, weighing about 200 g, were used. The thyroid homogenate was prepared from the thyroid glands of normal rats by homogenization with a glass homogenizer and filtered through a double layer of gauze. The homogenate was centrifuged at 20,000 g for 30 min and the resulting supernatant was used for immunization after sonication. The protein concentration of the thyroid extract was adjusted to about 10 mg/ml with saline. A 0.1 ml aliquot was emulsified in Freund's complete adjuvant and injected intradermally into the back of each rat. One hundred microliters of pertussis vaccine (Takeda Pharmaceut. Co. Ltd., Osaka), containing 200 billion killed organisms/ml, was injected intradermally at the same time to enhance development of thyroiditis (4, 5). Booster shots were given at the first and third weeks after the initial immunization. Control rats were injected only with Freund's complete adjuvant and pertussis vaccine in the same schedule. The immunized rats and control rats were divided into five groups and sacrificed at the 2nd, 3rd, 4th or 5th week after the initial immunization. Synthetic TRH (200 ng/rat) was injected into a jugular vein and 2 ml of blood was collected from the jugular vein at 0, 10, 20 and 45 min after TRH injection under urethane anesthesia. The TRH injection and the first blood sampling were begun at 2:00 p.m.. Thyroid tissues were removed and fixed in Bouin's solution. Histological sections were prepared and stained with hematoxylin and eosin. Serum TSH was measured by the double antibody radioimmunoassay kit supplied by the National Institute of Arthritis, Metabolism and Digestive Disease (NIAMDD). Serum thyroid hormones (T3 and T4) were measured by radioimmunoassay. Student's t test was used for statistical evaluation of the data.

RESULTS

Pathological findings. All immunized rats developed a thyroid lesion at the 2nd week after the initial immunization. The lesion was characterized by slight acinar disruption with desquamation of follicular epithelial cells and invasion of macrophages into colloid follicles (Fig. 1). At the 3rd and 4th weeks after immunization, extensive mononuclear cell infiltration and destruction of the normal architecture of the thyroid gland, similar to human autoimmune thyroiditis, were present (Fig. 2). At the 5th week, the mononuclear cell infiltration was less and reorganization of acinar architecture was apparent. In the control group, the thyroid glands were histologically normal.

Thyroid function. Serum thyroid hormone levels of all immunized rats remained within the normal range during immunization and any changes were statistically insignificant. Serum T3 levels of immunized rats decreased slightly during the 2nd week and increased transiently during the 3rd week after the

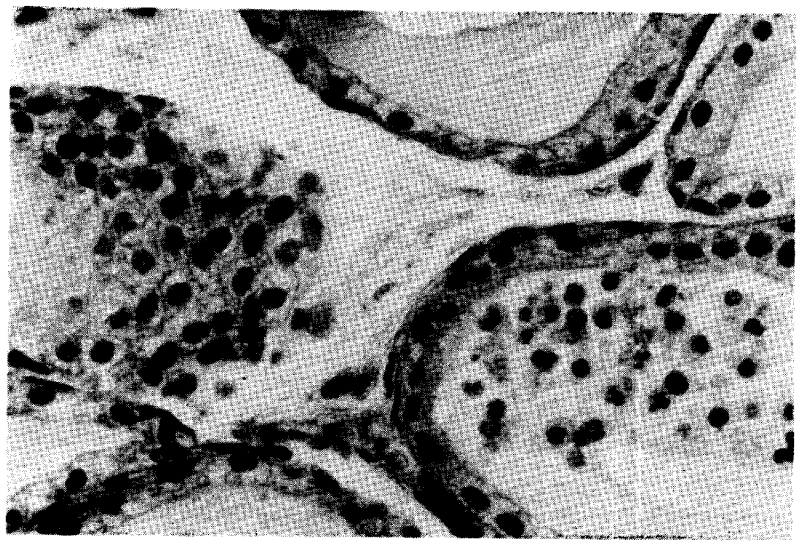

Fig. 1. Histology of rat thyroid gland at the second week after the initial immunization, showing slight acinar disruption with desquamation of follicular epithelial cells and invasion of macrophages into the colloid follicles. ×400.

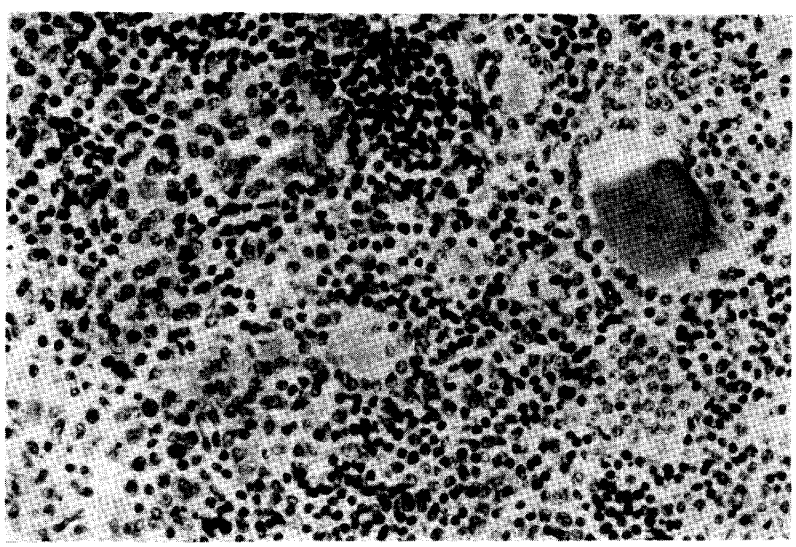

Fig. 2. Histology of rat thyroid gland at the 4th week after immunization, showing extensive infiltration of mononuclear cells and destruction of the normal architecture, resembling human autoimmune thyroiditis. ×200.

initial immunization (Fig. 3). Serum T4 levels tended to decrease gradually from the 2nd week in immunized rats, but the change was not statistically significant (Fig. 4).

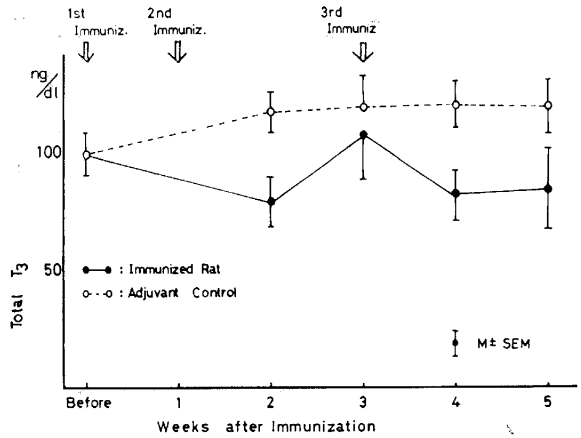

Fig. 3. Serum T3 levels before and after immunization, showing the slight decrease at the 2nd week and transient increase at the 3rd week, the changes are statistically insignificant. The solid line indicates the immunized group and the dotted line indicates the control group. Each point represents mean±SEM of five rats.

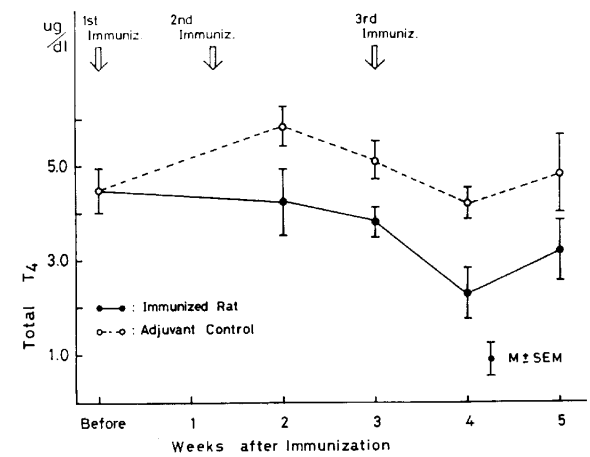

Fig. 4. Serum T4 levels in the immunized rats decrease gradually from the first week and reach the lowest level at the fourth week, but the changes are statistically insignificant.

Serum TSH levels and response to TRH. Serum TSH levels of the immunized group began to increase from the 2nd week after the initial immunization and were significantly increased at the 4th week (670 ± 182 ng/ml, $p<0.05$). At the 5th week, serum TSH levels were lower than at the 4th week, but the change was not statistically significant (Fig. 5). Gradual increase of basal TSH levels in the control group was due to aging of the rats. The maximum response of serum TSH to TRH was significantly higher in the immunized group at the 3rd week

(2206±681 ng/ml, $p<0.05$) than in the control group (1175±385 ng/ml) (Fig. 6). Maximum TSH responses to TRH in the immunized rats at the 2nd and 4th weeks were also increased but the changes were not statistically significant.

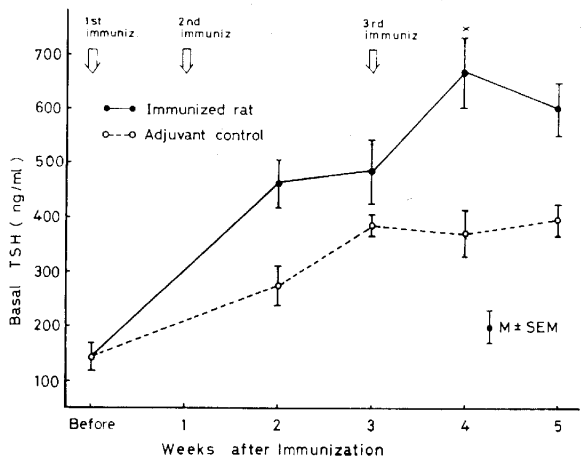

Fig. 5. Basal TSH levels before and after immunization. Serum TSH levels of the immunized rats begin to increase from the second week and increase significantly at the fourth week ($p<0.05$). Gradual increase in serum TSH in control group is due to aging of rats. The solid line indicates the immunized group and the dotted line indicates the control group. Each point represents mean±SEM of five rats.

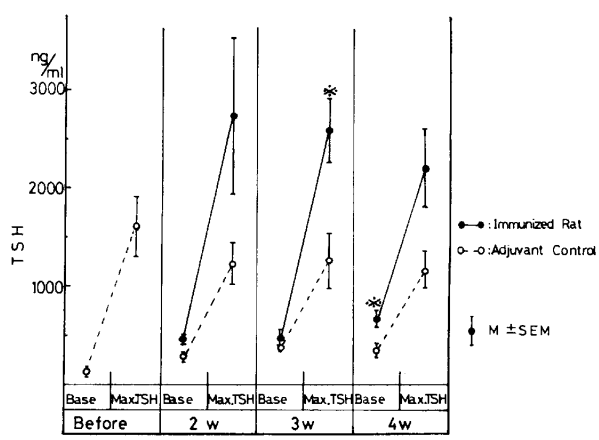

Fig. 6. Maximum TSH response to TRH injection before and after immunization. Maximum response at the third week is significantly higher than that in the control group ($p<0.05$). The solid line indicates the immunized group and the dotted line indicates the control group. Each point represents mean±SEM of five rats.

DISCUSSION

Immunological aspects of experimentally induced thyroiditis in animals have been studied extensively by many investigators (4–7). However, there have been few reports on function of pituitary-thyroid axis in animals with experimentally induced thyroiditis. In the present study, we immunized rats with crude homologous thyroid extract and examined function of pituitary-thyroid axis and histological changes in the thyroid gland. Massive lymphoid cell infiltration and destruction of follicular architecture were observed at the 3rd and 4th weeks after the initial immunization. Basal TSH levels increased in the immunized rats in response to destructive changes of the thyroid glands. Significantly increased TSH response to TRH administration was observed in these rats at the 3rd week, although basal TSH levels were within the normal range. These findings suggested that pituitary TSH responsiveness to TRH was increased at this stage in response to a minimal decrease in thyroid hormones because of thyroid damage induced by immunization. These observations in the immunized rats were similar to the abnormality of function of pituitary-thyroid axis in patients with euthyroid chronic thyroiditis or asymptomatic autoimmune thyroiditis (1, 2). Bonnyns *et al.* (3) reported that pituitary thyrotrophs were increased in number and size, and showed dilated Golgi area and large nuclei; moreover that such morphological evidence of thyrotropic activity correlated well with elevated serum TSH concentrations in patients with asymptomatic autoimmune thyroiditis. Torizuka *et al.* (8) reported similar findings in guinea-pigs 3 to 4 weeks after single immunization with homologous thyroid extract. They suggested that transient hypofunction of the thyroid gland at this early stage after immunization stimulated pituitary TSH secretion and that elevated TSH stimulated the thyroid gland to become hyperplastic.

Beall *et al.* (9) reported that thyroid stimulating activity was found in the serum of a baboon immunized with the microsomal fraction of human thyroid extract. There was no change in serum free T4 concentration and the thyroid stimulating activity was neutralized by anti-human TSH serum. In our study, serum TSH levels tended to decrease during the 5th week after the immunization. This may have been due to improvement in the thyroid lesions after cessation of immunization. In fact, histological examination of the thyroid glands at the 5th week showed decreased lymphoid cell infiltration and regeneration of thyroid follicles. Although massive lymphoid cell infiltration, resembling human autoimmune thyroiditis, was observed during the 3rd to 4th week after the initial immunization, the experimentally induced lesion was self limiting and did not progress after cessation of immunization.

In conclusion, an enhanced putuitary response to TRH occurred earlier than the increase in basal TSH levels which developed in response to minimal

thyroid damage induced by immunization. Although the change in circulating thyroid hormone levels after immunization were within normal range, hyperresponsiveness to TRH was probably due to hypertrophy of pituitary thyrotrophs induced by a minimal decrease in serum thyroid hormone levels. The changes in function of pituitary-thyroid axis induced in the short course of the development of experimental thyroiditis showed a close resemblance to the findings in human euthyroid chronic thyroiditis.

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