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Iodine-Induced Autoimmune Thyroiditis in NOD-H-2^{h4} Mice

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Excess iodine ingestion has been implicated in induction and exacerbation of autoimmune thyroiditis in human populations and animal models. We studied the time course and sex-related differences in iodine-induced autoimmune thyroiditis in NOD-H-2^{h4} mice. This strain, derived from a cross of NOD with B10.A(4R), spontaneously develops autoimmune thyroiditis but not diabetes. NOD-H-2^{h4} mice were given either plain water or water with 0.05% iodine for 8 weeks. Approximately 54% of female and 70% of male iodine-treated mice developed thyroid lesions, whereas only 1 of 20 control animals had thyroiditis at this time. Levels of serum thyroxin (T4) were similar in the treatment and control groups. Thyroglobulin-specific antibodies were present in the iodine-treated group after 8 weeks of treatment but antibodies to thyroid peroxidase were not apparent in the serum of any of the animals. Levels of thyroglobulin antibodies increased throughout the 8-week iodine ingestion period; however, no correlation was seen between the levels of total thyroglobulin antibodies and the degree of thyroid infiltration at the time of autopsy. The thyroglobulin antibodies consisted primarily of IgG2a, IgG2b, and IgM antibodies with no detectable IgA, IgG1, or IgG3 thyroglobulin-specific antibodies. The presence of IgG2b thyroglobulin-specific antibodies correlated well with the presence of thyroid lesions.

INTRODUCTION

In many countries the levels of iodine ingested in the food are far beyond the recommended level. While the RDA (recommended daily allowance) for a person per day is 150 μg, in countries where the salt is iodinated the actual consumption may be as much as fourfold higher (1). In humans, epidemiological evidence suggests an increased incidence of thyroiditis associated with iodine supplementation (2–4).

A connection between higher levels of iodine ingestion and autoimmune thyroiditis has been demonstrated in several animal models. Excess dietary iodine accelerated development of thyroid-associated lymphoid tissue in BB rats (5) and increased the incidence of spontaneous thyroiditis in BB rats, chickens, and hamsters (6–8).

To explore the mechanism by which ingestion of iodine contributes to the development of autoimmune thyroiditis, we examined the effects of iodine supplementation in the drinking water of NOD-H-2^{h4} mice. In this new mouse model the animals spontaneously develop thyroiditis with a low incidence but supplementation of iodine in the drinking water was said to increase the percentage of positive mice (Linda Wicker, personal communication). The purpose of the present study was to establish the autoimmune characteristics in this animal model. Specifically we intended to investigate the presence and chronology of the thyroid-specific antibody response, the effect of gender on thyroiditis, and the isotype involved in thyroglobulin response.

MATERIALS AND METHODS

Animals

NOD-H-2^{h4} mice derived from a cross of NOD with B10.A(4R) spontaneously develop autoimmune thyroiditis but not diabetes. The original breeding stock was a gift of Dr. Linda Wicker (Merck) and bred in our animal facility under specific pathogen free conditions.

Iodine Treatment

Six- to 8-week-old NOD-H-2^{h4} mice were given either plain water or water with 0.05% iodine for 8 weeks.

Tissue and Serum Collection

Mice were bled at biweekly intervals. Blood was incubated at room temperature for 2 hr and serum was collected after centrifugation. Serum was stored at −80°C until use. At the end of the experiment mice were sacrificed by CO_2 inhalation and thyroids were collected, fixed in buffered formalin for 2 days, and submitted for histological staining.

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TABLE 1

Effect of Iodine Ingestion on the Development of Thyroiditis in NOD-H-2^{h4} Mice[a]

			Grade of lesion				
Treatment	Sex	*n*	0	1	2	3	4
0.05% NaI	F	15	7 (46%)	2 (13%)	2 (13%)	2 (13%)	2 (13%)
0.05% NaI	M	10	3 (30%)	2 (20)%	0	3 (30%)	2 (20%)
None	F	10	10 (100%)	0	0	0	0
None	M	10	9 (90%)	0	0	0	1 (10%)

[a] Mice were given either iodine-containing water or plain water for 8 weeks. Upon sacrifice thyroids were removed, sectioned, and stained with an H&E stain. Lesions were graded on a scale of 0–4 based on the percentage of thyroid infiltrated. Results are the number (and %) of mice that had lesions in each group.

Histology

Thyroids were sectioned and stained with hematoxylin and eosin (H & E) in the Comparative Medicine facility of the Johns Hopkins School of Medicine.

RIA for Serum Thyroxin (T4) Levels

Thyroxin (T4) levels in mouse serum were detected by using the Clinical Assays GammaCoat M total T4 RIA kit (INCSTAR, Stillwater, MN).

Thyroid Peroxidase (TPO) Fluorescent Staining

Sections of normal mouse thyroids were blocked by a 30-min incubation at room temperature with 1% bovine serum albumin (BSA) (Sigma) in PBS (BSA-PBS) followed by a 1-hr incubation at room temperature with mouse serum diluted 1:100 in 1% BSA-PBS. Binding of mouse serum to the cytoplasm of the thyrocyte epithelial cells was detected with goat anti-mouse fluorescinated antibody.

Thyroglobulin-Specific ELISA

Mouse thyroglobulin was purified as described earlier (9). Ninety-six-well Immunolon II plates (Costar) were coated with thyroglobulin in coating buffer (pH 9.6) at a concentration of 10 μg/ml overnight at 4°C. Wells were blocked by a 30-min incubation at room temperature with 1% BSA-PBS. Plates were then washed 3 times in PBS-Tween 20 (0.05%) and incubated at 4°C overnight with mouse serum diluted 1:100 in 1% BSA-PBS. Plates were again washed 6 times with PBS-Tween 20 and incubated for 1 hr at room temperature with alkaline phosphatase-conjugated goat anti-mouse isotype-specific antibodies (Cappel, Durham, NC) diluted 1:1000 in 1% BSA-PBS. Plates were washed 10 times with PBS-Tween and color was detected with *p*-nitrophenyl phosphate substrate (Sigma) in a MRX plate reader (Dynatech Laboratories). Optical density (OD) was read at 405 nm.

Western Immunoblot for Thyroglobulin Binding

Mouse thyroglobulin was loaded at 40 μg/well into a 7.5% gel and run at 100 V until the dye front ran off the gel. The protein was then transferred onto a nitrocellulose membrane at 100 V for 1 hr. The membrane was blocked by overnight incubation with 1% BSA-PBS at 4°C. The membrane was incubated with mouse serum diluted 1:100 in 1% BSA-PBS for 1 hr at room temperature, washed five times with PBS-Tween 20 (0.05%), and incubated with goat anti-mouse alkaline phosphatase diluted 1:1000 in 1% BSA-PBS for 1 hr at room temperature. The membrane was washed eight times with PBS-Tween and color was developed by a short incubation with Sigma FAST BCIP/NBT substrate (Sigma).

RESULTS

Iodine Effect Following 8 Weeks of Ingestion

The mouse groups that received iodine in the drinking water had an increased incidence of thyroiditis with extensive thyroid lesions compared to the control groups (Table 1). While 10% of control males had thyroiditis at the time of autopsy, the incidence of thyroid lesions increased to 54% in the treated females and 70% in males (Table 1). Although the males had a slightly higher pathology index of thyroid lesions due to iodine in the drinking water (Table 2), the difference between the treatment males and females was not statistically significant. Despite the presence of thyroid lesions that were quite extensive in some of the treatment mice (Fig. 1), the levels of serum thyroxin (T4) were not significantly different in the treatment female and male groups compared to the control female and male groups (Table 2). Ingestion of iodine also significantly increased the levels of thyroglobulin-specific serum antibody levels in treated mice compared to the control groups (Table 2). No antibody to thyroglobulin was observed in the control female group but a low level of antibody was observed in several ani-

TABLE 2

Levels of Thyroxine (T4), Pathology Index, and Levels of Thyroglobulin-Specific Serum Antibodies in Control and Iodine-Treated Mice[a]

Group	Treatment	*n*	Pathology Index[b]	Mean serum T4 (mg/dl)	Mean thyroglobulin-specific antibody (OD)
1 Females	0.05% NaI	15	1.33 ± 0.39	5.59 ± 0.27	0.32 ± 0.08
2 Males	0.05% NaI	12	1.90 ± 0.53	6.23 ± 0.21	0.33 ± 0.09
3 Females	None	10	0	7.65 ± 0.38	0.02 ± 0.01
4 Males	None	10	0.50 ± 0.50	6.30 ± 0.36	0.09 ± 0.04

[a] Data are average ± SD.
[b] Pathology index is the average grade of lesion.

mals in the control male group. Again, no difference was observed between the males and the females in the treated group.

Since the levels of T4 in the serum had quite a large degree of variation, it was important to see if lower levels of T4 correlated with a higher grade of thyroid lesion. However, no correlation between the grade of lesion and serum T4 was apparent ($r = 0.297$).

Thyroid Peroxidase Antibodies

Indirect immunofluorescence assays of normal mouse thyroid sections with serum from control and iodine-treated mice did not show cytoplasmic staining characteristic of TPO antibodies in either group (data not shown).

Time Course of Serum Thyroglobulin-Specific Antibodies during 8 Weeks of Iodine Ingestion

The mice were bled on a biweekly basis and serum was measured for total anti-thyroglobulin antibodies. The levels of thyroglobulin-specific antibodies started increasing after 2 weeks of iodine ingestion in the treated females and after 4 weeks in the treated males (Fig. 2). The levels increased steadily throughout the

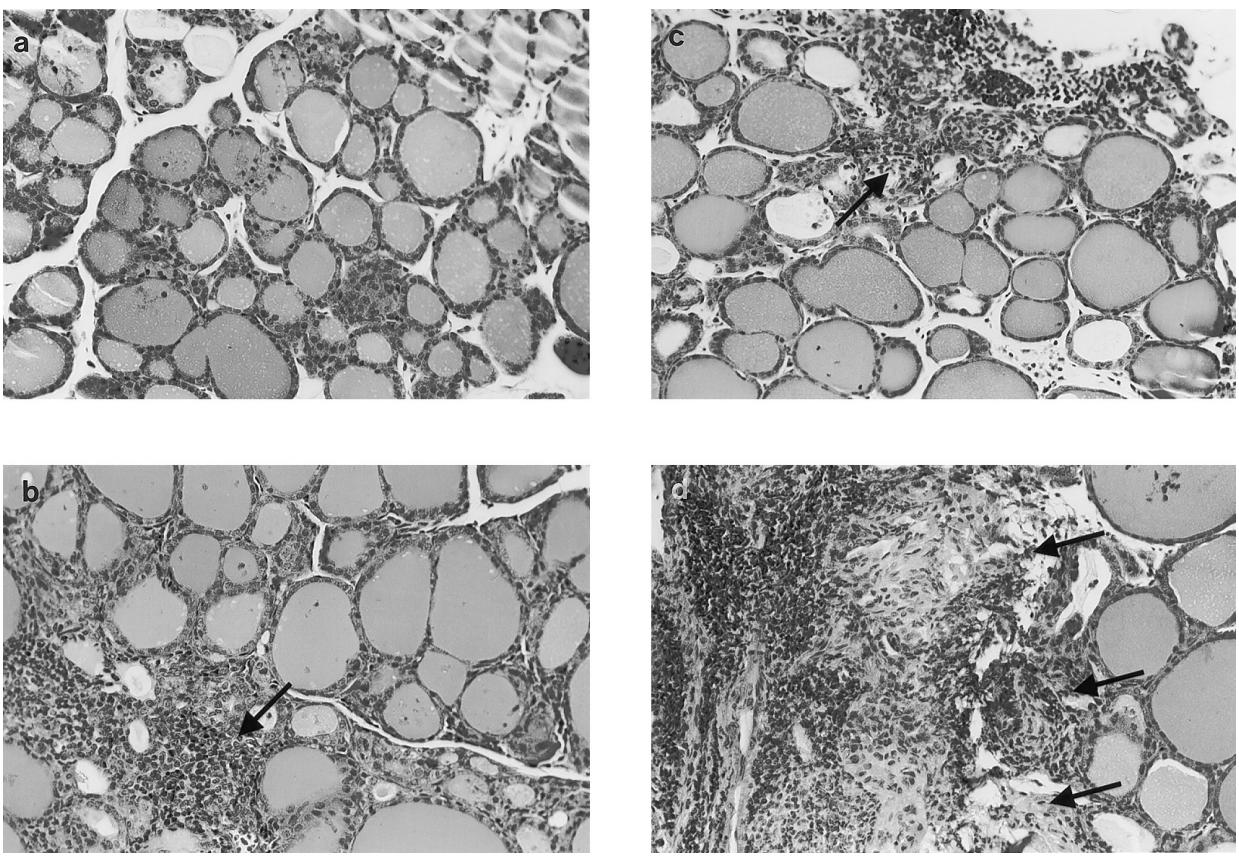

FIG. 1. Iodine-induced lesions in NOD-H-2^{h4} mice. Histological staining of thyroids from (a) control mouse, (b, c) iodine-treated mice with lesion grade 2, and (d) iodine-treated mouse with lesion grade 4. Arrows are pointing to the area of infiltration and thyroid follicle destruction.

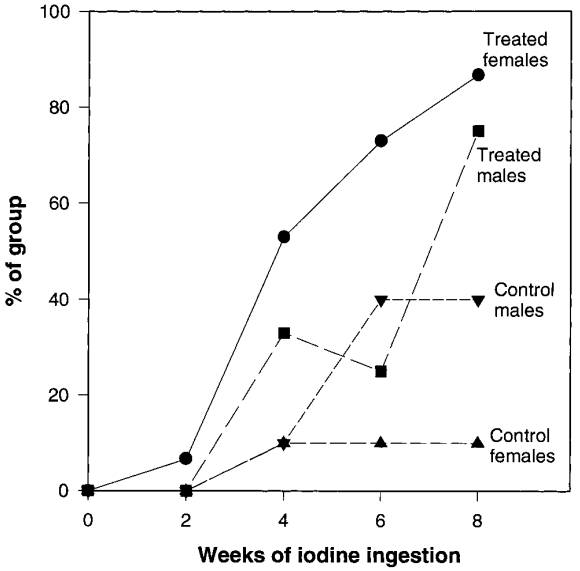

FIG. 2. Time course of thyroglobulin antibody appearance in iodine-ingesting and control NOD-H-2^{h4} mice. Mice were bled biweekly and serum was tested by ELISA to thyroglobulin as described under Materials and Methods.

8 weeks of the experiment in both the male and the female treatment groups. In contrast, very few animals in the control female group had thyroglobulin-specific antibodies. In the control male group, a somewhat higher percentage of mice had thyroglobulin-specific antibodies (Fig. 2).

Following 8 weeks of iodine treatment all mice had high levels of thyroglobulin-specific serum antibodies. These antibodies bound the major thyroglobulin band of approximately 330 kDa on a Western immunoblot but also detected smaller breakdown thyroglobulin fragments (Fig. 3c). Serum from control mice did not contain sufficient antibody to bind any of the thyroglobulin bands (Fig. 3d).

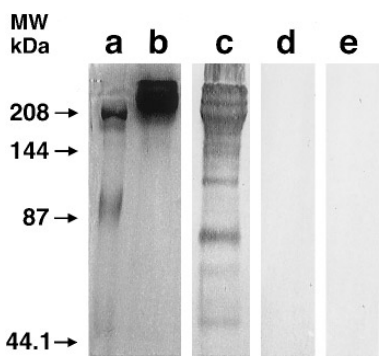

FIG. 3. (a) Molecular weight marker and (b) mouse thyroglobulin run on a PAGE gel and stained with Coomassie total protein stain as described under Materials and Methods. Mouse thyroglobulin was transferred onto nitrocellulose filter (as described under Materials and Methods) and reacted with (c) iodine-treated mouse serum diluted 1:100, (d) control mouse serum diluted 1:100, or (e) no serum.

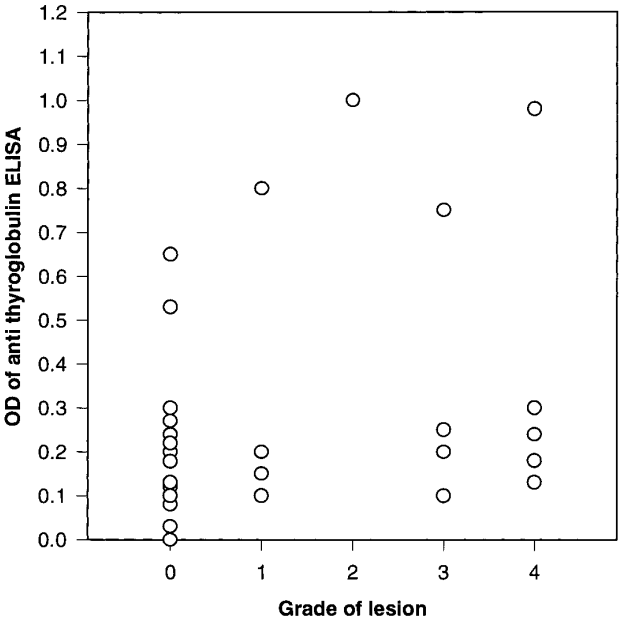

FIG. 4. Correlation between grade of lesion and level of total serum thyroglobulin-specific antibodies in the iodine-ingesting NOD-H-2^{h4} mouse group.

Serum Thyroglobulin-Specific Antibody Levels and Correlation with Grade of Lesion

Because of the variability in thyroglobulin-specific antibody in both treatment groups (see Table 2), we tested the possibility that high amounts of thyroglobulin-specific antibodies correlate with a higher grade lesion. However, there was no correlation ($r = 0.382$) between the thyroglobulin-specific antibody and the grade of lesion (Fig. 4).

Isotype Profile of Thyroglobulin-Specific Antibody Levels Following 8 Weeks of Iodine Ingestion

In measuring isotypes of the serum antibodies that are specific to thyroglobulin, it became apparent that the antibody in the treatment group is primarily in the IgG2a and IgG2b isotypes while control mice had mostly IgM antibodies (Fig. 5). The presence of thyroglobulin-specific IgG2a and IgG2b in the control group stems from the presence of one control male mouse that had grade 4 lesions and a high titer of thyroglobulin-specific antibodies (Fig. 5, Table 2). No IgA or IgG3 was detected and very low amounts of IgG1 were present. The presence of IgG2a antibodies did not correlate with the appearance of thyroid lesions (data not shown) but the presence of IgG2b antibodies was directly correlated with the occurrence of lesions (Table 3).

DISCUSSION

The NOD-H-2^{h4} mouse was obtained by repeated backcrossing of the NOD strain, which spontaneously

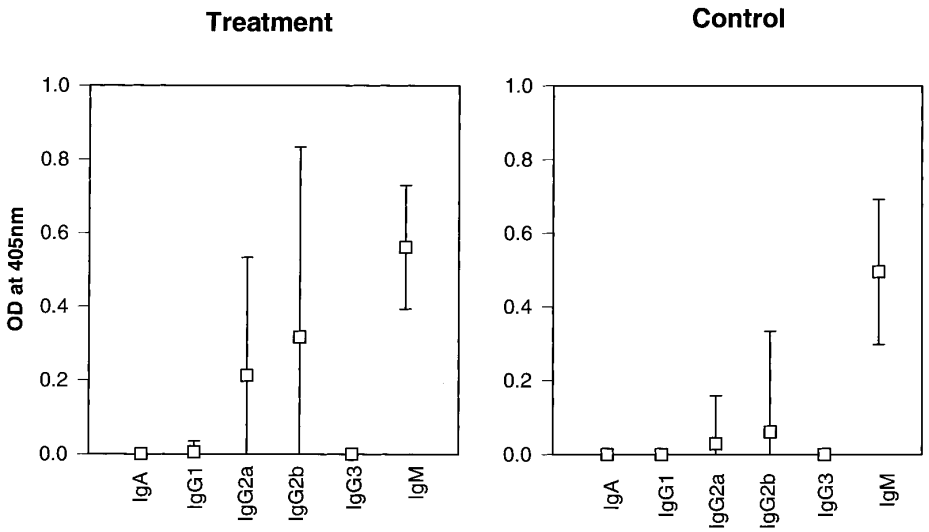

FIG. 5. Isotypes of thyroglobulin-specific serum immunoglobulin levels in 8-week treatment and control NOD-H-2^{h4} mice.

develops diabetes but has a very low incidence of thyroiditis, with B10.A(4R) first-generation (F1) mice crossed to the NOD strain with selection for $K^{d/k}$ heterozygotes at each generation (Linda Wicker, Merck, personal communication). The purpose of this backcrossing procedure was to hybridize a mouse strain that has the tendency to develop a low incidence of thyroid autoimmune disease (the NOD parent) with a mouse strain (the B10.A(4R)) that has the H-2 genetic background (I-Ak) favoring development of experimental thyroiditis (10). The resultant mouse strain, NOD-H-2^{h4}, does not develop diabetes but demonstrates thyroiditis. When supplemented with 0.05% of iodine in the drinking water this mouse strain has an increased incidence of thyroiditis (Linda Wicker, personal communication).

We now report that ingestion of 0.05% iodine in the drinking water for 8 weeks increased the percentage of thyroiditis in the mouse group from 10 to 70%. TPO antibodies were not apparent in either the control or the iodine-treated group. Also the levels of thyroxin (T4) were not affected by iodine ingestion. Although the total level of thyroglobulin-specific antibodies was increased throughout the iodine supplementation, specific correlation with the grade of thyroid lesion was seen with IgG2b thyroglobulin-specific antibodies. The effect of an environmental stimulus, such as excess iodine ingestion in this case, on development of thyroiditis demonstrates the need for both a suitable genetic background and the environmental stimulus in the development of an autoimmune disease.

TABLE 3

Thyroglobulin-Specific Serum IgG2b and Grade of Lesions in 8-Week Iodine-Treated and Control Mice

	Presence of thyroglobulin-specific IgG2b	
	+	–
Lesions grade 2 or higher		
+	11	1
–	0	25

The finding that female mice do not have a higher incidence of thyroiditis points to a difference between the human disease and the animal model since in the human disease the incidence in females is significantly higher than in males (11). Another difference between the human disease and the animal model is the lack of TPO binding in the mouse model. In general, the presence of TPO antibodies has not been reported in the induced or spontaneous animal models of thyroiditis (except monkeys), and the ability to develop TPO antibodies might be uniquely present in primates (12). The results suggested that TPO antibodies are not involved in the pathogenesis of thyroiditis in this model. Despite these two differences, the NOD-H2^{h4} mouse model has an important similarity to the human disease, namely, spontaneous development of thyroiditis when susceptible genetic background is present in combination with an environmental stimulus (such as dietary iodine).

The levels of thyroxin (T4) were not decreased in animals that had a high degree of thyroid infiltration (Table 2). This finding is not surprising because the thyroid can store enough thyroglobulin to last for 2 months or more (13). The results suggest, moreover, that thyroxin-binding antibodies are not present (14).

IgM thyroglobulin-specific antibodies were found in both the treatment and the control animals. The presence of these natural IgM autoantibodies is the reason for the lack of correlation between levels of total thyroglobulin-specific antibodies and grade of lesion. However, the level of IgG2b thyroglobulin-specific antibodies was shown to have value in predicting the presence of thyroiditis (Table 3).

Preferential production of IgG2a and IgG2b isotypes that bind thyroglobulin, together with a lack of IgA or IgG1 response, suggests that the response is a T helper 1 type response. T helper 1 (Th1) cells help B cells to produce IgG2a while T helper 2 (Th2) cells help B cells to produce IgG1 and IgA (15, 16). Also, since TGF-β is the cytokine that generates IgG2b antibodies (17), the presence of which is correlated with lesions, it is very likely to be directly involved in the generation of thyroid lesions. Our future studies will attempt to further support the hypothesis that iodine-induced thyroiditis in the NOD-H-2^{h4} mouse involves a Th1 type response and to elucidate the role of TGF-β in thyroid damage.

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