Iodine Deficiency Induces Thyroid Autoimmune Reactivity in Wistar Rats*


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ABSTRACT

The last 2 decades it has become clear that iodine deficiency has a modulating effect on the thyroid autoimmune response in humans. Also, in animals that spontaneously develop autoimmune thyroid disease, evidence is accumulating that a low iodine intake can modulate thyroid autoimmune reactivity. However, it is still not clear what the effect of a low iodine intake on thyroid autoimmune reactivity is in normal nonautoimmune animals. To study the relationship of a dietary low iodine intake on the thyroid autoimmune reactivity in nonautoimmune animals, normal Wistar rats (female) were kept on an enriched iodine diet (daily iodine intake of 100 μg iodine), a "for our area normal" (conventional) diet (COD; daily iodine intake of 17 μg iodine), a low iodine diet (LID; 2 days of 1% KClO3, followed by iodine-deficient drinking water/pellets), or an extremely low iodine diet (LID+; 1% KClO3 continuously in the drinking water and iodine-deficient pellets). The enriched iodine diet rats were euthyroid (T3, ~8 nM/liter; T4, ~50 nM/liter; TSH, ~2 ng/ml), had a normal thyroid weight (~12.5 mg), and showed only minimal signs of local thyroid immune reactivity: low numbers of intrathyroidal dendritic cells (DC; ~35 DC/mm²), CD4+ cells (~2 cells/mm²), and CD8+ cells (~2.5 cells/mm²) were found in combination with low anticolloid antibody production (incidence of positive animals, 12.5%).

The COD resulted in a normal thyroid function. The rats were euthyroid (range of T3, 1.6-1.2 nM/liter; T4, ~50 nM/liter; TSH, ~2 ng/ml) and had a normal thyroid weight (~12.5 mg). However, some signs of thyroid autoimmune reactivity were found (number of intrathyroidal DC, ~40/mm²; ~3 CD4-positive (CD+) cells/mm²; ~2 CD8+ cells/mm²; together with a 30% incidence of anticolloid antibodies).

The LID and LID+ not only induced goiter formation (thyroid weight, 27.3 ± 4.2 mg (mean ± SD) after 12 weeks of LID and 38.4 ± 5.3 mg after 4 weeks of LID+ and low production of T3 by the thyroid [28 ± 3 nM/liter (mean ± SD) after 12 weeks of LID and 14 ± 5 nM/liter after 2 weeks of LID+], but also induced various signs of thyroid autoimmune reactivity. These consisted of: 1) influxes of DC into the thyroid [63.4 ± 3.8 DC/mm² (mean ± SD) after 12 weeks of LID and 64.4 ± 9.0 DC/mm² after 3 weeks of LID+]; 2) the formation of homotypic clusters of these DC; 3) influxes of intrathyroidal CD8+ and CD4+ T-cells [10.3 ± 3.5 CD4+ cells/mm² (mean ± SD) after 3 weeks of LID, 18.0 ± 4.7 CD4+ cells/mm² after 4 weeks of LID+, 7.6 ± 4.1 CD8+ cells/mm² after 6 weeks of LID+]; and 4) increases in the production of anticolloid antibodies (incidence of 60% positive animals after 18 weeks of LID and 73% after 10 weeks of LID+).

These data, collected from normal nonautoimmune female Wistar rats, show that, as in humans, iodine deficiency precipitates thyroid autoimmune reactivity. (Endocrinology 133: 1197-1204, 1993)

During the last 2 decades, it has become clear that a high dietary iodine intake may aggravate thyroid autoimmune reactivity in both humans as well as experimental animals suffering from thyroid (autoimmune) disease. In experimental animals (1-4) and humans (5-8) with preexisting thyroid abnormality, such as an iodine-deficient goiter, a single administration of a high dose of iodine resulted in an attack of thyroiditis positive for antithyroglobulin (anti-Tg) and antimicrosomal antibodies. In the Cornell C strain of chicken (9) and the BB rat (10), it was shown that a high dietary intake of iodine led to an increase in the incidence and severity of the thyroiditis of these animals.

Evidence is also accumulating that iodine deficiency is able to precipitate thyroid autoimmune reactivity in humans. A higher incidence of anti-Tg antibodies has been described in areas of iodine deficiency (11). The majority of patients suffering from diseases linked to iodine deficiency, such as endemic goiter (12, 13) and endemic cretinism (14-16) show immunoglobulins G in their serum that affect the growth of thyrocytes in vitro (the so-called thyroid growth-stimulating immunoglobulins (TGIs)). It must be noted, however, that the character and meaning of these TGIs are still under debate (12, 17); some investigators have found these TGIs in the absence of TSH receptor antibodies (18), whereas others consider the TGI effect to be a classical TSH receptor stimulator effect of thyroid-stimulating immunoglobulins (19).

Thyroids of iodine-deficient goiter patients contain aggregates of epitheloid cells and dendritic cells (DC) (20). DC are the antigen-presenting cells par excellence (21) and play a major role in the initiation of immune responses. They have been described as the first cells arriving in thyroids before thyroid autoimmune reactivity develops (22, 23). Additionally, DC are capable of transferring autoimmune thyroiditis in an experimental animal model (24) and are, thus, considered to be responsible for the initiation of the thyroid autoimmune response. Others consider an abberant major histocompatibility complex (MHC) class II expression on thyrocytes as the primary event in thyroid autoimmune disease (25).

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In animals, the effect of a low dietary iodine intake on thyroid autoimmune reactivity has only been studied in the existing models of autoimmune thyroid disease. To our knowledge, it has not been determined whether iodine deficiency precipitates thyroid autoimmune reactivity in non-autoimmune normal euthyroid animals. Such a study might be a better reflection of the effect of dietary iodine intake on thyroid autoimmune reactivity in average human populations. Therefore, we developed an animal model in which normal female Wistar rats were kept on four iodine regimens, namely an iodine-enriched diet (EID), a *for our area normal* (conventional) diet (COD), a low iodine diet (LID), and an extremely low iodine diet (LID*), for periods of up to 18 weeks, starting from the third week of life. The extent of iodine deficiency/sufficiency was determined by urinary iodine excretion. The severity of hypothyroidism was measured by serum TSH and thyroid hormone levels. Goiter formation was determined by measuring thyroid weight. Thyroid autoimmunereactivity was measured by counting the number of intrathyroidal infiltrated leucocytes (MHC class II-positive DC, T-cells, and B-cells) and determining the incidence of anticolloid and anticytoplasmic antibodies. The effects of the iodine diet on the serum levels of immunoglobulins G that stimulate the growth of thyrocytes in vitro (the so-called TGI) will be discussed in another report.

**Materials and Methods**

**Animals and diets**

Female Wistar rats were purchased from INO (Kuiswijk, The Netherlands) and kept at the central experimental animal housing facilities of Erasmus University under standard conditions. Directly after weaning (at the age of 3 weeks), five consecutive cohorts of rats (n = 30–60) were each kept on four dietary iodine regimens: 1) On the EID, rats received conventional pellets (Am-II Hope Farms bv, Woerden, The Netherlands) *ad libitum* as well as an extra iodine supplementation of 6.5 mg KI/liter added to the drinking water; consequently, iodine intake was about 100 μg iodine/day. On the COD, the rats received conventional pellets (Am-II Hope Farms bv, Woerden, The Netherlands) *ad libitum* as well as 15 μg iodine/kg body weight/day, and on the LID, rats received 1% KClO₃ in the drinking water for a period of 2 days and thereafter distilled water and iodine-deficient pellets (Modified Remmington diet, Hope Farms) *ad libitum*. On the LID*, rats continuously received 1% KClO₃ in their distilled drinking water and iodine-deficient pellets (Modified Remmington diet, Hope Farms) *ad libitum*.

Wistar rats of the 5 cohorts were killed by aortic exsanguination under ether anesthesia after 3, 6, 9, 12, or 18 weeks of the diet, in such a way that 5–18 animals/time group could be used for evaluation. Serum was prepared for T₃, T₄, and TSH evaluation and anticolloid antibody determination. T₃, T₄, and TSH were measured by conventional RIAs (Dr. W. M. Wiersinga and Mrs. M. Broenink, Academic Medical Center, Amsterdam, The Netherlands). For the rat TSH RIA, the rat TSH reference preparation (NIDDK rTSH RP-2) was used (176 times more potent than NIDDK rTSH RP-1 kindly provided by Dr. Parlow from the University of California-Los Angeles Medical Center, Torrance, CA). Thyroid glands were removed, weighed, and stored at −80 C until immunohistochemical examination.

**Iodine measurement in rat urine**

Rat urine was collected for 24 h by keeping the rats in metabolic cages. Iodine excreted in the urine was measured using a modified method described by Benotti et al. (26). In brief, the urine samples were destructed by adding an equal volume of 30% chloric acid and incubating the samples at 200 C for several hours. The iodine reacted as a catalyzing agent in the redox reaction between cerium ammonium sulfate and arsenic acid. During the reaction, the yellow Ce₄⁺ was converted into the colorless Ce³⁺, which was measured using an enzyme-linked immunosorbent assay reader at 405 nm.

**Immunohistological examination of thyroids**

Immunohistological examination was performed according to the method of Green et al. (27) with minor modifications. Of each frozen thyroid, one lobe was semiserially cut into 6-μm thin sections. The sections were air dried overnight, fixed in acetone at room temperature for 10 min, incubated with either a monoclonal antibody specific for MHC class II molecules, CD4-positive (CD4⁺) cells, CD8⁺ cells, or B-cells (see Table 1) for 60 min, washed with PBS containing 0.2% BSA (Sigma Chemical Co., St. Louis, MO) for 10 min, further incubated with a rabbit antimonouse immunoglobulin-horseradish peroxidase-labeled conjugate (Dakopatts, Denmark) for 30 min, and diluted 300 times in PBS containing 1% normal rat serum and 0.2% BSA. After washing the slides in PBS containing 0.2% BSA for 10 min and rinsing them with 0.1 M sodium acetate buffer, pH 6.0, the sections were incubated with a metal-enhanced 3,3'-diaminobenzidine (DAB) solution containing 0.01% DAB (Sigma), 0.15% H₂O₂ (Sigma), 1% sodium azide (Sigma), and 0.006% imidazole (Sigma), and 0.8% sodium chloride in 0.1 M acetate buffer, pH 6.0, for 3–5 min. Hydrogen peroxide was added to a final concentration of 0.01%. After DAB development, the sections were washed briefly in 0.1 M Tris-HCl buffer, pH 7.6, and immersed in a 0.5% solution of cobalt chloride in 0.1 M Tris-HCl buffer, pH 7.2, at room temperature for 4 min. The slides were either counterstained with 0.1% nuclear fast red or 5% aluminium sulfate for 2 min or incubated with acid phosphatase at 37 C for 30 min and subsequently counterstained with hematotoxylin. The slides were dehydrated and embedded in DePex mounting medium (Gurr, BDH Ltd., Poole, England).

For the quantification of intrathyroidal infiltrated cells, four sections of each thyroid, at intervals of at least 100 μm, were reacted with the appropriate marker monoclonal antibody. Positive infiltrated cells were counted using light microscopy at a magnification of ×400. The surface area of the thyroid sections in which the infiltrated cells had been counted was measured using a camera attached to a Leitz Diaplant light microscope (Rockleigh, NJ) and a Videoplan image processing system (Kontron, Bild Analyse GmbH, Germany). The numbers of infiltrated DC (identified as strong MHC class II-positive cells with cytoplasmic processes and weak or absent acid phosphatase activity), CD4⁺ T-cells, and CD8⁺ T-cells were expressed per mm² surface area of a thyroid section. It is of importance to note that similar results were obtained when the numbers of DC and T-cells were not expressed per mm² surface area of the section, but were expressed per observed thyroid follicle in the plane of section or per surface area of thyrocyte parenchyma.

**Antithyroid antibody determination**

Six-micron thin frozen porcine thyroid sections were cut, air dried overnight, and fixed in cold acetone (−20 C) for 10 min. Porcine thyroid tissue was used, because it gave optimal results compared to rat or human tissue. The sections were precoated with normal rabbit serum (DAKO Immunoglobulins, Denmark), diluted 50 times in PBS with 1% BSA, for 10 min. Subsequently, the rat sera were applied (in duplicate, diluted 10 times in 0.9% NaCl), and the slides were incubated at room temperature for 1 h. The sections were then sequentially incubated with monoclonal antibodies and peroxidase-labeled anti-rat immunoglobulin G (DAKO Immunoglobulins, Denmark), diluted 50 times in PBS with 1% BSA, and peroxidase-labeled anti-rat immunoglobulin M (DAKO Immunoglobulins, Denmark). The plates were developed with the colorimetric method of Green et al. (27) with minor modifications. Of each frozen thyroid sections were air dried overnight, fixed in acetone at room temperature, reacted with the monoclonal antibodies (Table 1), and incubated with peroxidase-labeled anti-rat immunoglobulin G or M. The slides were washed with PBS containing 0.2% BSA, incubated with 0.05% DAB (Sigma), 1% nickel sulfate (Merck, Rahway, NJ), 0.068% imidazole (Sigma), and 0.8% sodium chloride in 0.1 M acetate buffer, pH 6.0, for 3–5 min. Hydrogen peroxide was added to a final concentration of 0.01%. After DAB development, the sections were washed briefly in 0.1 M Tris-HCl buffer, pH 7.6, and immersed in a 0.5% solution of cobalt chloride in 0.1 M Tris-HCl buffer, pH 7.2, at room temperature for 4 min. The slides were either counterstained with 0.1% nuclear fast red or 5% aluminium sulfate for 2 min or incubated with acid phosphatase at 37 C for 30 min and subsequently counterstained with hematotoxylin. The slides were dehydrated and embedded in DePex mounting medium (Gurr, BDH Ltd., Poole, England).

**TABLE 1. Monoclonal antibodies used for the detection of leukocytes**

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>Specificity</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OX6 (Seralab, UK)</td>
<td>MHC class-II</td>
<td>28</td>
</tr>
<tr>
<td>W3/25 (Seralab, UK)</td>
<td>CD4 antigen</td>
<td>29</td>
</tr>
<tr>
<td>B115-4 (Holland Biotechnology, Netherlands)</td>
<td>CD4 antigen</td>
<td>30</td>
</tr>
<tr>
<td>OX8 (Seralab, UK)</td>
<td>CD8 antigen</td>
<td>31</td>
</tr>
<tr>
<td>HIS 14 (F.G.M. Kroese)</td>
<td>B cells</td>
<td>32, 33</td>
</tr>
</tbody>
</table>
temperature for 60 min. After washing in PBS, the sections were incubated with rabbit antirat immunoglobulins, which were labeled with fluorescein isothiocyanate (DAKO Immunoglobulins), for 30 min (diluted 25 times in PBS with 1% BSA). After this second step and rewashing, the slides were embedded in aquamount (Gurr) and examined using a fluorescence microscope. Three control slides were included: one without incubation of rat serum, another incubated with a rat serum previously scored as negative, and a third incubated with a rat serum previously scored as positive. The staining intensity of each serum was arbitrarily and blindly scored (under code, by two independent investigators) as negative, positive, or strongly positive.

Statistical analysis

Differences in thyroid weight; serum $1_{3}$, $1_{4}$, and $1_{5}$; and number of intrathyroidal MHC class II-positive DC, in clusters of these cells and T-cells were compared by Wilcoxon's rank sum test. The incidence of anticolloid antibody production was tested by $\chi^2$ analysis.

Results

Thyroid weight

The thyroid weight of animals kept on EID and COD increased slightly from 6.7 ± 1.1 to 13.3 ± 2.9 mg (mean ± SD) after 6 weeks of diet, as could be aspected from the normal growth development, and the thyroid/body weight index remained constant at 0.07. The absolute thyroid weight after 6 weeks of diet remained 13-14 mg for the rest of the experimental period (Fig. 1A).

In animals kept on LID, however, a greater increase in thyroid weight was found, which was statistically significantly different from the thyroid weights of animals kept on EID or COD ($P < 0.05$, by Wilcoxon's rank sum test) After 6 weeks of diet, thyroid weight was 20.1 ± 2.8 mg (mean ± sd), and the goiter reached a maximal value after 18 weeks of diet (26.3 ± 7.3 mg (mean ± SD; Fig. 1A).

It is noteworthy that the increase in thyroid weight was neither in time nor extent related to increases in serum TSH levels in the LID group (Fig. 1A vs. B; see also below).

Rats receiving a LID$^*$ showed more pronounced goiter development. As early as after 2 weeks of the diet, statistically significantly higher thyroid weights were found compared to those in rats kept on EID, COD, or LID ($P < 0.05$, by Wilcoxon's rank sum test). After 4 weeks of diet, the thyroid reached a maximum weight of 26.4 ± 5.3 mg (mean ± sd), which remained constant thereafter (Fig. 1A).

Thyroid endocrine status and urinary iodine excretion

In animals kept on EID, the $1_{4}$ output from the thyroid was normal (Fig. 1D). Serum $1_{3}$ levels, however, were low (Fig. 1C). Similar low serum $1_{3}$ levels have been described by other investigators in rats treated with a high iodine dose (300–500 μg iodine/day) (34, 35). Serum TSH levels in the EID rats were in the normal range [between 2.1 ± 0.4 (mean ± sd) and 2.5 ± 0.3] during the entire experimental period (Fig. 1B). The urinary iodine excretion of the Wistar rats receiving the EID was high; excretion of 50 μg I/day was measured after 1 week of diet, and excretion reached a plateau after 3 weeks (6 weeks of life) of treatment with 100 μg I/day. These latter values reflected the estimated daily intake.

In animals receiving a COD, serum $1_{4}$ levels started to increase during the first 3 weeks of diet, reaching a constant level of 50 nm/liter for the remaining experimental period (Fig. 1D). Serum $1_{3}$ levels were high [1.6 ± 0.2 nm/liter (mean ± sd)] at the start of the experiment. They gradually decreased after 3 weeks of diet from 1.6 ± 0.1 to 1.2 ± 0.1 nm/liter after 12 weeks of diet and stayed at that level thereafter (Fig. 1C). Such relatively high serum $1_{4}$ levels in prepubertal rats compared to adult rats have been described by others previously (35), and these researchers regarded the high thyroid hormone levels as necessary for optimal brain development at the prepubertal age. The need for high serum thyroid hormone levels in the young rats was reflected by their urinary iodine excretion. The rats on a COD showed a relatively long period of low urinary iodine excretion: an output of 1.6 ± 0.3 μg I/day (mean ± sd) at the age of 3 weeks until the age of 6 weeks, when urinary iodine excretion had reached 7.6 ± 1.5 μg I/day, the latter value reflecting the daily iodine intake. This excretion level was maintained during the remaining experimental period. Serum TSH levels in the COD animals were in the normal range during the entire period of observation (Fig. 1B).

In animals kept on a LID, the $1_{4}$ output of the thyroid gland gradually failed, and the animals became subclinically hypothyroid after 12 weeks of diet. Serum $1_{4}$ levels were as
low as 28.0 ± 2.5 nm/liter (mean ± sd) and remained lower than the levels in EID and COD animals (P < 0.05; see Fig. 1D). A very slight, but statistically significant, rise in serum TSH levels could be observed after 6 weeks of a LID compared to the TSH levels found in EID and COD rats, but a more clear rise in TSH levels was not found until 18 weeks of LID (6.4 ± 1.8 vs. 2.0 ± 0.4 and 2.3 ± 0.3 ng/ml compared to EID and COD animals, respectively; Fig. 1B). Serum T4 levels remained in the normal range (Fig. 1C). The low iodine intake was well reflected by the urinary iodine excretion. After 1 week of diet, the excreted urinary iodine was under the lower limit of detection of our assay.

The animals kept on LID+ became severely hypothyroid. After 2 weeks of a LID+, a decline in T4 levels from 37.4 ± 7.5 to 13.3 ± 2.2 nm/liter (mean ± sd) was seen, and levels remained low during the remaining experimental period (Fig. 1D). Also, very low serum T4 levels were found that were below the detection limit of the assay (≤0.03 nm/liter) after 2 weeks of diet (Fig. 1C). The serum TSH levels in animals on a LID+ were already high after 2 weeks of diet (24.3 ± 2.1 ng/ml) and reached maximal values after 8 weeks of diet (88 ± 9.9 ng/ml) before declining slightly to 60.4 ± 9.2 ng/ml after 10 weeks (Fig. 1B). The urinary iodine excretion was extremely low; as early as after 1 week of the diet, iodine could not be detected in the excreted urine. Observations were stopped after 10 weeks of the LID+ diet, because by then the animals had become severely ill due to their hypothyroidism.

Signs of immune reactivity in the thyroid

At the start of the experiment, only a few DC were found in the thyroids of the animals. The DC were scattered throughout the thyroid and were localized in the interstitial connective tissue, but never between the thyrocytes. The DC were not only found as single cells, but they also made contact with other DC and with mast cells present in the interstitial tissue to form small cellular clusters (homotypic clusters; Fig. 2A). A few CD4+ and CD8+ T-cells were also present and were, like the DC, found scattered throughout the thyroid in the interstitium. Sometimes, however, the T-cells could be observed between the thyrocytes as part of the thyroid follicle lining. The T-cells were never grouped together or found clustered to other cells, but were present as single cells (Fig. 2B). B-Cells could not be detected in any of the young rat thyroids examined.

In animals receiving an EID, the intrathyroidal DC remained at a low constant level during the entire experimental period (range, 30–40 DC/mm²; Fig. 3A). Also, the number of intrathyroidal CD4+ and CD8+ T-cells remained low (range, 1.4 ± 0.9 to 2.0 ± 0.5 [mean ± sd] for CD4+ cells and 1.7 ± 1.6 to 2.8 ± 1.0 for CD8+ cells; Fig. 3, C and D). It must be stressed that similar results were obtained when the numbers of DC and T-cells were not expressed per mm² surface area of the section, but per observed thyroid follicle in the plane of section or per surface area of thyrocyte parenchyma.

In the thyroids of rats kept on a COD, the number of DC increased to about 45 DC/mm² in the first weeks. This increase occurred at the same time as the increase in the serum T4 level. By 9 weeks and thereafter, the number of intrathyroidal DC dropped to a lower level of 35 DC/mm² and remained at this level (Fig. 3A). The number of intrathyroidal CD4+ and CD8+ T-cells remained low during the entire experimental period in rats receiving a COD (range, 0.6 ± 0.5 to 4.4 ± 2.9 CD4+ cells/mm² and 1.2 ± 0.8 to 5.2 ± 1.8 CD8+ cells/mm²; Fig. 3, C and D).

The LID caused a slightly stronger accumulation of DC and T-cells in the thyroid compared to values in the EID and COD rats. There was a clear increase in DC numbers after 12 weeks of diet, and values of 65 DC/mm² were found. This increase occurred at the same time as goiter development in these animals. After 18 weeks, the number of DC dropped to 45 DC/mm² (Fig. 3A). Not only could an increase in DC numbers be found during LID, but the cells also became more active to form homotypic clusters. During the first weeks of the LID, the number of intrathyroidal small cellular
iodine deficiency and thyroid autoimmune reactivity

clusters of DC had increased from 0.6 ± 0.8 clusters/mm² (mean ± sp) at the start of the experiment to 3.9 ± 1.4 clusters/mm² after 3 weeks of diet. Thereafter, a gradual decrease was seen (Fig. 3B). The numbers of CD4+ and CD8+ T-cells increased to 10.3 ± 3.5 CD4+ cells/mm² (mean ± sp) and 7.6 ± 4.1 CD8+ cells/mm² after 3 and 6 weeks of LID, respectively. After a drop in numbers, relatively high values were again reached at 18 weeks of the diet (6.6 ± 2.5 CD4+ cells/mm² and 10.2 ± 4.2 CD8+ cells/mm²; Fig. 3, C and D).

The LID+ caused a pronounced increase in cell infiltration of DC, CD4+, and CD8+ T-lymphocytes and homotypic clustering of DC. Values of about 65 DC/mm² were already seen after 3 weeks of the LID+. After 6 weeks, a decline was observed, and a value of 40 DC/mm² was reached after 12 weeks of diet (Fig. 3A). A pronounced peak of clusters of DC occurred in the same period, reaching a maximal value of 8.1 ± 2.5 clusters/mm² (mean ± sp) after 4 weeks of the LID+ (Fig. 3B). Not only was an accumulation of DC induced in the thyroids of LID+ animals, but there was a rapid and clear increase in the number of intrathyroidal CD4+ and CD8+ T-cells (reaching maximal values of 18.7 ± 6.0 CD4+ cells after 8 weeks of diet and 25.7 ± 6.3 CD8+ cells after 6 weeks of diet; Fig. 3, C and D). These T-cells were, as in the animals kept on the other diets, single cells present in the interstitium and in between the thyrocytes.

antithyroid antibody production

At the start of the experiments, the incidence of anticolloid antibodies in the Wistar rat colony was 4% (n = 24). Anticytoplasmic antibodies were not detected. In rats receiving the EID, the incidence of anticolloid antibodies remained low and did not exceed an incidence of 12.5% from 6 weeks of the diet onward (n = 8–16; Fig. 4). Anticytoplasmic antibodies were not detected during the entire observation period in rats receiving an EID.

Keeping the rats on a COD resulted in an incidence of anticolloid antibodies of 30% (n = 25) after 5 weeks and onward (Fig. 4). This incidence, however, was not statistically significantly different from the incidence of anticolloid antibodies in EID rats (P > 0.05, by χ² analysis). Anticytoplasmic antibodies were sporadically found: in 1 of 17 rats after 3 weeks of diet and in 1 of 9 rats after 6 weeks of diet. The other rats were negative.

The LID resulted in a similar increase in anticolloid antibody production than the COD, and an incidence of 33% (n = 18) was found after 5 weeks of the diet. After 18 weeks, however, the incidence was statistically significantly different from that in the EID rats and had reached 60% (n = 10; P < 0.05 compared to EID rats, by χ² analysis; Fig. 4). At that time, the rats had become subclinically hypothyroid and developed a goiter (see above).

In rats on the LID, anticytoplasmic antibodies were not detected.

The LID+ stimulated the production of anticolloid antibodies in our Wistar colony to an even greater extent than the LID. After 10 weeks of the diet, all of the rats (n = 7) showed anticolloid antibodies in their serum (Fig. 4). At that time, the rats were severely hypothyroid, showing clearly enlarged thyroids.

Only a few rats showed anticytoplasmic antibodies in their serum: after 2 weeks of a LID+, 1 of 18, and after 6 weeks of diet, 1 of 7. The other rats were negative.

Discussion

This study in normal Wistar rats shows that the dietary iodine intake influences not only thyroid weight and thyroid...
In experimental animals (36); however, autoreactive T-cells be noted that immunization with Tg can induce thyroiditis pathogenic effects of anti-Tg antibodies are not clear. It must bodies. The higher incidence of anticolloid antibodies in the and T-cells) and a very low production of anticolloid anti-low local thyroid immune response (few intrathyroidal DC and the clustering of the thyroid infiltrated DC, and 3) increased anticol- genic. This might have triggered the DC to accumulate and start a thyroid autoimmune response. It has indeed been suggested that a high thyrocyte metabolism might be involved in initiating a thyroid autoimmune response in the OS chicken. An intrinsically high thyroid metabolism has been described in the OS chicken as a primary genetic abnormality of the target organ that forms one of the basic conditions for susceptibility to thyroid autoimmune disease (39). In our experiments, the stimulation of iodine-deficient Wistar thyrocytes by the relatively high levels of serum TSH might also have played a role. Indeed, the serum TSH levels of the LID* rats correlated well with the influxes of DC, the clustering of these cells, and the incidence of anticolloid antibodies. Chiavoto et al. (40) observed that TSH enhances the expression of the antigen TPO (41) on the cell surface of the rat thyroid cell line FRTL-5, whereas TSH also induces a higher production of the autoantigen Tg from thyrocytes. In addition, when serum TSH levels were suppressed in BB/ Wor rats by T4 treatment, lower levels of Tg antibodies and a decreased incidence of thyroiditis were found (42).

The reported data here also indicate that there is a marked contrast between Wistar rats, on the one hand, and BB rats and OS chickens, on the other hand, with regard to their response to deficient or sufficient iodine diets. In the OS chicken and the BB/W rat, both well established models of spontaneous autoimmune thyroid disease, a low iodine intake ameliorated the thyroid autoimmune reaction, whereas an increase in the incidence of thyroiditis was found during a high iodine intake (43, 44).

Iodine intakes in the studies on the BB rat and OS chicken (5,000–10,000 µg I/day) are considerably higher than the iodine supplementation used in our Wistar study (100 µg I/day), which could explain the observed discrepancy. However, recent experiments performed by us (to be published) show that a relatively low excess of iodine (100 µg I/day) is sufficient to induce a marked and enhanced leukocytic infiltration in the thyroids of BB rats. This means that the discrepancy between Wistar rats and BB rats/OS chickens in their responses to high and low iodine diets must be due to specific differences between the animals, which also relates to their different susceptibilities for spontaneous thyroid autoimmunity. It must be noted, however, that although the Wistar rat is not as severely genetically predisposed to thyroid autoimmune disease as the BB rat, the animal does show some susceptibility to thyroid autoimmune reactivity, such as the presence of thyroid-specific T-cells (45).

In several studies, the thyroid autoimmune-promoting effects of the high iodine diets in BB rats and OS chickens are generally attributed to the direct effects of iodine on the thyroid autoimmune response itself. Iodine increases the antigenicity of Tg (46, 47) [although this is also disputed (48, 49)] or directly stimulates the function of macrophages (50), T-cells (51), and B-cells (52). To explain our results (the virtual absence of thyroid autoimmune reactivity in Wistar rats on a high iodine diet), one has to assume that either iodine has no such autoimmune-stimulating effects in the Wistar rat or that the autoimmune-promoting effects of iodine are overruled by autoimmune suppressor mechanisms specific for the Wistar rat (tolerance induction). Since our hormone profile, but also thyroid autoimmune reactivity. An iodine-insufficient diet in our experimental animals resulted in 1) intrathyroidal accumulation of DC and T-cells, 2) clustering of the thyroid infiltrated DC, and 3) increased anticolloid antibody production.

The Wistar rats supplemented with iodine showed a very low local thyroid immune response (few intrathyroidal DC and T-cells) and a very low production of anticolloid antibodies. The higher incidence of anticolloid antibodies in the iodine-deficient goitrous Wistar rats is in accordance with the higher prevalences of anti-Tg antibodies reported in endemic goiter patients with iodine deficiency (11). The direct pathogenic effects of anti-Tg antibodies are not clear. It must be noted that immunization with Tg can induce thyroiditis in experimental animals (36); however, autoreactive T-cells are considered more important in this phenomenon than thyroid autoantibodies. In a recent report, thyroid peroxidase (TPO), not Tg, is thought to be of greater importance as an autoantigen in autoimmune thyroid disease (37). In the thyroid autoimmune-prone BB rat, anticytoplasmic antibodies (anti-TPO antibodies) are indeed more frequent (10%) than in Wistar rat strains (3%) (our unpublished data). The experiments reported here also show that this low frequency of anticytoplasmic (anti-TPO) antibodies did not increase due to iodine deficiency or iodine excess, the anticytoplasmic antibodies are, thus, considered of minor importance in inducing thyroid autoimmune reactivity.

The observed increases in the number of intrathyroidal DC and homotypic clusters of these cells in the rat goiters find their human parallel in the reported increases in DC infiltration and clustering of these cells in thyroids of iodine-deficient endemic goiter patients (20).

The toxic effects of KClO4 on thyrocytes, inducing thyroid antigen release and explaining the thyroid autoimmune effects of the iodine-deficient diets in our rats, can practically be excluded, firstly because KClO4 was only given for the first 2 days of the diet in our LID group, whereas the increases in leucocyte infiltration and anticolloid antibody production in this animal group were found only after 3–4 weeks and 12–18 weeks of LID, respectively, and secondly because thyroid cell death could not be observed in our histological specimen of LID and LID+ thyroids (on the contrary, an active high columnar epithelium was evident).

The mechanisms of the influxes of DC and lymphocytes into the Wistar thyroids during iodine deficiency are speculative. DC are the antigen-presenting cells par excellence (38) and are the most potent inducers of experimental autoimmune thyroiditis after transfer (24). They are the first cells to appear in the thyroid during the development of thyroiditis in the BB rat (23). It is likely that the macrophage-like DC accumulating early in the BB rat thyroid pick up thyroid antigens and travel with these via the lymph to the draining lymphnode to initiate the autoimmune response (38).

We hypothesize that the high thyroid metabolic activity and the enhanced proliferation rate of the thyrocytes induced by the iodine deficiency played a role in the attraction of DC by an enhanced expression and release of thyroid autoantigens. This might have triggered the DC to accumulate and
histology showed that the thyrocytes of Wistar rats kept on an EID had a very low level of metabolic activity [the cells showed a flattened appearance, with prominent colloid spaces (to be published)], it could also be envisaged that the iodine intake of 100 μg/day during the iodine supplementation is the most optimal intake for the Wistar rat and that this intake allows the Wistar thyrocytes to function at a low metabolic level.

If the metabolic activity of thyrocytes is indeed coupled to their autoantigenicity (see discussion above), one could imagine that a dietary intake of 100 μg iodine/day in Wistar rats is capable of keeping thyroid autoimmune reactivity at a very low level, despite the direct stimulating effects of iodine on immune cells. Future studies need to unravel these possible mechanisms.

In conclusion, the data reported here show that an insufficient dietary iodine intake is capable of precipitating a thyroid autoimmune response in normal nonautoimmune Wistar rats, as in normal human populations.

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