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## Induction of Autoimmune Thyroiditis in Chickens by Dietary Iodine

**Abstract.** *Clinical studies have suggested that excess dietary iodine promotes autoimmune thyroiditis; however, the lack of a suitable animal model has hampered investigation of the phenomenon. In this study, different amounts of potassium iodide were added to the diets of chicken strains known to be genetically susceptible to autoimmune thyroiditis. Administration of iodine during the first 10 weeks of life increased the incidence of the disease, as determined by histology and the measurement of autoantibodies to triiodothyronine, thyroxine, and thyroglobulin. Further support for the relation between iodine and autoimmune thyroiditis was provided by an experiment in which iodine-deficient regimens decreased the incidence of thyroid autoantibodies in a highly susceptible strain. These results suggest that excessive consumption of iodine in the United States may be responsible for the increased incidence of autoimmune thyroiditis.*

N. BAGCHI\*

T. R. BROWN

E. URDANIVIA

Department of Medicine, Division of Endocrinology, Wayne State University, Detroit, Michigan 48201

R. S. SUNDICK

Department of Immunology and Microbiology, Wayne State University

\*To whom correspondence should be addressed at Hutzel Hospital, 4707 St. Antoine, Detroit 48201

Clinical studies have suggested an association between increased consumption of iodine and a sharp rise in the incidence of autoimmune thyroiditis in recent years. For example, the prevalence of histologic thyroiditis in thyroidectomy specimens was found to be low in iodine-deficient areas in the United States (1) and to increase after the introduction of iodine prophylaxis (2). Other clinical studies revealed that treatment with iodine or iodine-containing agents increased the incidence of thyroid autoantibodies (3, 4). Conversely, treatment

of animals with iodine alone did not cause autoimmune thyroiditis (5). Interestingly, large parenteral doses of iodine accompanied by chronic stimulation with thyrotropin caused inflammatory changes in the thyroid gland of hamsters (6). The apparent contradiction between the clinical and animal data prompted the present study. In addition, it seemed important to develop an appropriate animal model for studying the relation between iodine and autoimmune thyroiditis. We report that dietary iodine induced autoimmune thyroiditis in genetically susceptible chickens and that iodine depletion had an ameliorative effect.

We chose two genetically susceptible chickens as our experimental models in view of the well-known involvement of genetic factors in autoimmune thyroid disease. The Cornell C strain (CS) has a low incidence of mild autoimmune thyroiditis through the first several months of life, but by 1 year 25 percent of females have autoantibodies to triiodo-

Table 1. Effect of dietary iodine on autoantibody responses in CS chickens. Newly hatched CS chicks were maintained on a normal diet and water with or without added KI. Thyroglobulin autoantibody titers were determined by passive hemagglutination (20). Antibodies to T<sub>3</sub> and T<sub>4</sub> were assayed by an electrophoretic-autoradiographic technique (7). Chicken serum (50  $\mu$ l) was incubated with 200 nCi (1  $\mu$ l) of [<sup>125</sup>I]T<sub>3</sub> or [<sup>125</sup>I]T<sub>4</sub> (specific activity, 750 to 1250  $\mu$ Ci/ $\mu$ g; New England Nuclear) at 37°C for 30 minutes. Electrophoresis was performed on 125 by 245 mm polyester film (Gel Bond, FMC Corp.) using 1 percent agarose (High M<sub>r</sub>, Bio Rad) in 0.15M tris-HCl (pH 8.0) at 10°C and 15 V/cm for 2 hours. The slabs were dried under a stream of warm air and overlaid on Kodak XAR-5 film for 16 hours. The autoradiographs were aligned with the dried gels and the distribution of labeled hormone was determined by cutting out and counting the radioactivity in the appropriate regions. Reproducibility was  $\leq$ 4 percent. Mean values for antibody-positive birds at 10 weeks of age were 18.9 percent T<sub>3</sub> antibody and 26 percent T<sub>4</sub> antibody. The data were subjected to chi-square analysis with Bonferroni correction (21); chicks treated with either dose of iodide did not differ from each other, but the treated groups together had significantly higher titers of antibodies to Tg, T<sub>3</sub>, and T<sub>4</sub> than the untreated group commencing at 6 weeks of age ( $P < 0.05$ ). Values are numbers of chicks.

Treatment	Incidence of Tg antibody*				Incidence of T <sub>3</sub> and T <sub>4</sub> antibodies at 10 weeks†
	4 weeks	6 weeks	8 weeks	10 weeks	
None	0 of 42	1 of 42	0 of 42	0 of 42	2 of 42
Iodine (2 mg/dl)	0 of 44	7 of 44	6 of 44	4 of 44	12 of 44
Iodine (20 mg/dl)	1 of 44	12 of 44	6 of 44	8 of 44	12 of 44

\*Log<sub>2</sub> titer,  $>3$  by passive hemagglutination.

†At least 0.5 percent of the total radiolabeled T<sub>3</sub> or T<sub>4</sub> bound to  $\gamma$ -globulin.

Table 2. Effect of dietary iodine on lymphocytic infiltration in thyroids of CS chickens. Newly hatched CS chicks, maintained on a normal diet and water with or without added KI ( $n = 42$  to 44), were killed at 10 weeks. At necropsy the thyroid glands were fixed, embedded, stained with hematoxylin and eosin, and sectioned. The sections were coded, placed on a projecting microscope fitted with a  $\times 10$  objective, and projected onto graph paper mounted at a distance of 61 cm. The relative amount of infiltration was determined by calculating the area (square centimeters) of the projected image that was infiltrated by mononuclear cells. The percentage of infiltration was determined by dividing the area of infiltrated thyroid by the total area of the cross section and multiplying the result by 100. Each group differed significantly from the other two groups ( $P < 0.003$ , Student's  $t$  test with Bonferroni correction).

Treatment	Number of sections examined	Infiltration	
		Relative amount (mean $\pm$ standard error)	Percentage
None	202	3.06 $\pm$ 0.28	1.28
Iodine (2 mg/dl)	303	6.53 $\pm$ 0.39	2.72
Iodine (20 mg/dl)	417	17.53 $\pm$ 2.87	7.30

thyronine ( $T_3$ ), thyroxine ( $T_4$ ), and thyroglobulin (Tg) (7).  $T_3$  and  $T_4$  are the active thyroid hormones and are highly iodinated haptenic groups on the Tg molecule (7, 8). The related Obese strain (OS) develops severe disease characterized by complete destruction of the thyroid gland 4 weeks after hatching (9). Its disease is characterized by mononuclear cell infiltration of the thyroid gland and autoantibodies against  $T_4$  and Tg (10). Both avian strains exhibit the unusual characteristic of thyroid function being partially independent of thyroid-stimulating hormone (11).

The CS chickens were separated into three groups at hatching, fed a regular diet, and given drinking water containing 0, 2, or 20 mg of potassium iodide per deciliter. Blood was withdrawn periodically, and the animals were killed at 10 weeks so that their thyroid glands could be analyzed for lymphocytic infiltration.

Tg,  $T_3$ , and  $T_4$  antibodies increased in both groups that received iodine (Table 1), and the degree of lymphocytic infiltration of the thyroid glands increased in a dose-dependent manner (Table 2). Iodine did not cause hypothyroidism at the doses used, as assessed by serum  $T_3$  and  $T_4$  radioimmunoassays at 4 weeks. To determine whether the increase in thyroid autoantibodies was due to a generalized enhancement of immune responses by iodine (12), we injected each bird with sheep red blood cells (SRBC's) and measured the titer of immunoglobulin G (IgG) to the cells. There were no statistically significant differences in antibody responses among the various groups (Table 3).

We then tested the hypothesis that reduction in the thyroidal uptake of iodine prevents or reduces thyroid autoantibody responses and thyroiditis in the highly susceptible OS chickens. Since

low-iodine diets are not palatable to chickens, we provided a regular diet with  $KClO_4$  added to the drinking water to prevent the transport of iodine to the thyroid gland. This regimen, which was begun at hatching, significantly reduced Tg antibody at 3 weeks of age (Table 4). At 5 weeks a combination of  $KClO_4$  and exogenous  $T_4$  was needed to suppress synthesis of antibodies to Tg (Table 4). Sex had an effect in only one treatment group: seven male chicks treated with  $T_4$  and  $KClO_4$  had a titer of  $0.8 \pm 0.4$  (mean  $\pm$  standard error), while the ten female chicks so treated had a titer of  $3.4 \pm 1.3$ . Antibodies to  $T_3$  and  $T_4$  were suppressed significantly by treatment with  $T_4$  alone or a combination of  $T_4$  and  $KClO_4$ . Since  $T_4$  administration leads to suppression of thyrotropin secretion, this combined regimen would be expected to be most effective in reducing thyroidal uptake of iodine. Examination of the thyroid at 3 and 7 weeks of age showed severe infiltrative changes in all groups. The lack of a pronounced effect of iodine depletion on infiltration may have been due to the in ovo transfer of iodine from the hens, which were maintained on a diet supplemented with Protamone, a highly iodinated casein. This diet increases the iodine content of the feed from 0.5 to 7 ppm (13) and the iodine content of the egg 4.5 times (14). Therefore, depletion of thyroidal iodine by  $KClO_4$  would be a slow process in the newly hatched birds because of the unusually high initial levels of iodine and the increased capacity of OS chicks to accumulate iodine in their thyroid glands (13, 14).

Table 3. Effect of dietary iodine on the IgG response to SRBC's. Newly hatched CS chicks were maintained on a normal diet and water with or without added KI. They were injected with  $2 \times 10^9$  SRBC's at 8 weeks of age and with  $10^9$  SRBC's at 9 weeks. At 10 weeks they were bled. Serum was tested for IgG antibody by an indirect Coomb's hemagglutination assay (22) in which twofold dilutions of serum were treated for 1 hour with 0.2M 2-mercaptoethanol to inactivate IgM antibodies, incubated with 1 percent SRBC's, and incubated with rabbit antibody to chicken IgG. Analysis of variance did not show any significant differences among groups. Each titer is expressed as the mean  $\pm$  standard error of  $\log_2$ 's.

Treatment	$n$	Sex	IgG anti-body titer
None	19	M	6.9 $\pm$ 0.2
	23	F	7.0 $\pm$ 0.2
Iodine (2 mg/dl)	28	M	6.7 $\pm$ 0.2
	15	F	7.0 $\pm$ 0.2
Iodine (20 mg/dl)	19	M	6.9 $\pm$ 0.2
	25	F	6.8 $\pm$ 0.2

Table 4. Effect of low iodine regimens on titers of antibodies to Tg,  $T_3$ , and  $T_4$  in OS chickens. When chicks were 3 and 5 weeks of age blood was withdrawn and examined for antibodies to Tg (33 to 36 chicks per group at 3 weeks and 17 to 18 chicks per group at 5 weeks). The data were analyzed by one-way analysis of variance and a modified least significant difference test (23). At 3 weeks all three treatment protocols resulted in significantly reduced titers of antibody to Tg ( $P < 0.05$ ). At 5 weeks only the chickens treated with  $T_4$  plus  $KClO_4$  exhibited reduced titers ( $P < 0.05$ ). Antibodies to  $T_3$  and  $T_4$  were assayed by an electrophoretic-autoradiographic technique (see legend to Table 1). Serum from birds fed  $T_4$  were stripped of  $T_3$  and  $T_4$  by ion-exchange chromatography (7) to eliminate the possibility of false negatives. Results for  $T_3$  and  $T_4$  antibodies were considered positive when at least 5 percent of the total radiolabeled  $T_3$  or  $T_4$  bound to antibody. Since these data were not normally distributed, they were analyzed by a Kruskal-Wallis one-way analysis of variance and then by a Mann-Whitney  $U$ -Wilcoxon rank-sum  $W$  test. Groups treated with  $T_4$  and  $T_4$  plus  $KClO_4$  expressed less  $T_3$  and  $T_4$  antibodies than the control group or the  $KClO_4$ -treated group ( $P < 0.02$ ). Each titer is expressed as the mean  $\pm$  standard error of  $\log_2$ 's.

Treatment	Tg antibody titer		Incidence of antibody at 6 weeks	
	3 weeks	5 weeks	$T_3$	$T_4$
Normal diet and water	4.7 $\pm$ 0.4	6.8 $\pm$ 0.5	7 of 18	9 of 18
$T_4$ (50 $\mu$ g per 100 g of feed)	1.5 $\pm$ 0.3	4.7 $\pm$ 0.8	1 of 16	4 of 16
$KClO_4$ (0.1 percent in water)	2.8 $\pm$ 0.4	5.3 $\pm$ 0.5	7 of 17	8 of 17
$T_4$ + $KClO_4$	1.1 $\pm$ 0.3	1.9 $\pm$ 0.7	1 of 18	3 of 18

The results demonstrate the critical role of iodine in promoting thyroiditis in genetically susceptible chickens. Other studies have shown that iodination of Tg leads to an increased T<sub>3</sub> and T<sub>4</sub> content (15), an increased sedimentation coefficient (16), and an altered configuration (17) of the molecule. Such changes may be accompanied by increased immunogenicity of the molecule. This is an attractive hypothesis because treatment with iodine increases antibodies not only to Tg but also to T<sub>3</sub> and T<sub>4</sub>. Alternatively, iodine may act by altering antigen presentation by the thyroid epithelial cells (18), modifying the function of thyroid-targeted immune cells or by other mechanisms. The thyroid abnormality of young CS chicks (11) may predispose them to iodine-induced thyroiditis.

These results are of relevance to the study of human autoimmune thyroiditis and suggest that elevated levels of dietary iodine may exacerbate or induce the disease in genetically susceptible individuals, such as females in families with a high incidence of thyroid disorders. In this context, it is important to note that the daily intake of iodine in the United States is two to five times the recommended allowance (19).

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## Evidence That the v-sis Gene Product Transforms by Interaction with the Receptor for Platelet-Derived Growth Factor

**Abstract.** A scheme for partial purification of biologically active v-sis-coded protein from cells transformed with simian sarcoma virus (SSV) has made possible a functional comparison of the transforming protein with platelet-derived growth factor (PDGF). The SSV-transforming gene product is capable of specifically binding PDGF receptors, stimulating tyrosine phosphorylation of PDGF receptors, and inducing DNA synthesis in quiescent fibroblasts. Each of these activities was specifically inhibited by antibodies to different regions of the v-sis gene product. Moreover, viral infection of a variety of cell types revealed a strict correlation between those cells possessing PDGF receptors and those susceptible to transformation by SSV. These findings provide evidence that SSV-transforming activity is mediated by the interaction of a virus-coded mitogen with PDGF receptors.

FERNANDO LEAL

Laboratory of Cellular and Molecular Biology,  
National Cancer Institute,  
Bethesda, Maryland 20205

LEWIS T. WILLIAMS

Howard Hughes Medical Institute,  
University of California,  
San Francisco 94143

KEITH C. ROBBINS

STUART A. AARONSON  
Laboratory of Cellular and Molecular Biology,  
National Cancer Institute

The discovery that the onc gene (v-sis) of simian sarcoma virus (SSV) encodes a protein closely related in its predicted amino acid sequence to a major component of human platelet-derived growth factor (PDGF) provided the first evidence that onc gene products were involved in growth factor-mediated proliferative pathways (1). Subsequent studies have shown that the product of the v-sis gene is a PDGF-2-like precursor poly-

peptide that assumes a homodimer configuration soon after its synthesis (2, 3). In contrast, human PDGF preparations contain two distinct but related polypeptide chains, one of which is encoded by the human sis proto-oncogene (4). Mitogenically active PDGF also exhibits a dimer structure, but whether it is composed of homo- or heterodimers of the two polypeptide chains is not known. Thus, while there are strong structural similarities between the processed sis product and PDGF, the functional relation of the SSV-transforming gene product to the growth factor is not yet established.

In the present study, we used SSV-transformed cells as the source of v-sis-coded protein and devised a means of functionally characterizing cell-associated molecules that can be unequivocally identified as products of the v-sis gene. We also sought to determine whether the transforming properties of the v-sis gene product are directly exerted through interaction with the cellular PDGF recep-

Fig. 1. Mitogenic activity of products of the v-sis gene. Crude membranes were prepared from hypotonic lysates of HF/SSV cells that were untreated (A) or exposed to 10<sup>-6</sup>M monensin for 16 hours (B). Membranes were boiled, clarified, and tested for mitogenic activity after treatment with the IgG fraction of preimmune serum (○) or with anti-serum to sis-N (□, ■) or to sis-C (△, ▲) bound to protein A-Sepharose beads (17). In some cases (■, ▲), antisera were incubated before immunoprecipitation with the homologous peptide after binding to protein A-Sepharose beads. [<sup>3</sup>H]Thymidine incorporation was determined by scintillation counting.

