Hypothyroidism in transgenic mice expressing IFN-γ in the thyroid

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IFN-γ has been implicated with contradictory results in the pathogenetic process of autoimmune (Hashimoto's) thyroiditis, the most common cause of hypothyroidism in adults. To test whether the local production of IFN-γ can lead to thyroid dysfunction, we have generated transgenic mice that express constitutively IFN-γ in the thyroid follicular cells. This expression resulted in severe hypothyroidism, with growth retardation and disruption of the thyroid architecture. The hypothyroidism derived from a profound inhibition of the expression of the sodium iodide symporter gene. Taken together, these results indicate a direct role of IFN-γ in the thyroid dysfunction that occurs in autoimmune thyroiditis.

Autoimmune (Hashimoto's) thyroiditis, the third most prevalent autoimmune disease in the United States (1), is the most frequent cause of hypothyroidism in adults (2). Both environmental factors (such as iodine) and multiple genes scattered throughout the genome (especially MHC genes) contribute to susceptibility to autoimmune thyroiditis. Much of the pathogenesis of the hypothyroid state, however, remains unknown (2). IFN-γ has been implicated in the pathogenetic process with contradictory results. Some studies have indicated a role for IFN-γ in promoting thyroiditis. For example, $CD4^+$ T cell clones isolated from intrathyroidal lymphocytic infiltrates of patients with autoimmune thyroid diseases produced high levels of IFN-γ (3, 4). In immunohistochemical studies, IFN-γ-producing lymphocytes were described adjacent to thyrocytes (5); systemic injection of IFN-γ induced thyroiditis in susceptible mice (6), whereas the injection of mAb to IFN-γ retarded the development of disease (7). Other studies, in contrast, indicated that IFN-γ is not required for the experimental induction of autoimmune thyroiditis, because thyroiditis can occur in mice with disrupted IFN-γ (8) or INF-γ receptor (9) genes.

To study more directly the role of IFN-γ in the pathogenesis of experimental thyroiditis and to test whether the local IFN-γ production can lead to hypothyroidism, we produced transgenic mice that specifically express IFN-γ in the thyroid by placing it under control of the thyroglobulin promoter.

Materials and Methods

Construction of *Thyr*-IFN-γ Transgenic Mice. The *thyr*-IFN-γ transgene was made by joining the rat thyroglobulin promoter, the murine IFN-γ complementary DNA, and part of the human growth hormone gene. The rat thyroglobulin promoter was first amplified by PCR to include nucleotides -763 to $+5$ from the transcription start site, using recognition primers that included sites for *Eco*RI–*Hin*dIII at the 5′ end and for *Eco*RI at the 3′ end. The 3.6-kb transgene was excised by *Hin*dIII digestion, purified by electroelution, and resuspended at 10 ng/μl in 10 mM Tris/0.1 mM EDTA, pH 7.4. The transgene was injected into fertilized eggs from (CBA $\times$ C57BL/6)F_1 females and maintained by mating hemizygous founder animals to wild-type C57BL/6 mice. All transgenic and control mice used in the experiments were between 3 and 10 months of age.

Analysis of *Thyr*-IFN-γ Transgene Expression. To assess the thyroid-specific expression of IFN-γ, total RNA was extracted from thyroids, liver, lymph nodes, salivary glands, spleen, heart, and testicles by using the RNAzol B kit (Tel-Test, Friendswood, TX) and reverse-transcribed by using the SuperScript system (Life Technologies, Gaithersburg, MD). PCR amplification then was performed with MIF01 (5′-cAcggcAcAgTcATTgAAAg-3′) and MIF02 (5′-ccTTgcTgTTgcTgAAgAAg-3′) primers, which interact with the middle of exon 1 and exon 3 of the murine IFN-γ gene, respectively, and produce an amplicon of 258 bp. If genomic DNA is contaminating the RNA sample, an amplicon of 1,531 bp also is produced. PCR products were fractionated by electrophoresis through a 1.5% agarose gel, transferred to Zeta-Probe GT membrane (Bio-Rad), hybridized to ^{32}P-labeled murine IFN-γ cDNA probe, and detected by using a Storm PhosphorImager (Molecular Dynamics). IFN-γ expression by thyroid cells also was analyzed by immunohistochemistry by using a rabbit anti-mouse IFN-γ (PBL, West Caldwell, NJ).

Thyroid Pathology, Serum Thyroxine, Serum Thyrotropin (Thyroid Stimulating Hormone, TSH), and Radioactive Iodine Uptake. Thyroids were fixed overnight in 10% neutral buffered formalin and embedded in paraffin by following standard procedures. Four-micron sections then were placed on Colormark slides (Erie Scientific, Portsmouth, NH), deparaffinized, rehydrated, and stained with hematoxylin/eosin. Thyroid lesions were graded based on the extent of thyroid architecture disruption: 0, normal thyroid; +1, involvement of less than 25% of the gland; +2, between 25% and 50%; +3, between 50% and 75%; and +4, complete architectural disruption. The mononuclear cell infiltrate was characterized by immunohistochemistry by using rat anti-mouse CD3, CD4, CD8, and B220 primary antibodies (Sigma). Serum total thyroxine was determined by a commercially available competitive RIA kit (Incstar, Stillwater, MN). Serum TSH was performed by a commercial laboratory (Analytics, Gaithersburg, MD). Radioactive iodine uptake was measured by injecting under anesthesia 10 μCi of carrier-free ^{125}I contained in 100 μl of sterile saline into the tail vein of each mouse. Four hours after injection, mice were sacrificed, and the thyroid lobes were excised, weighed, and counted for 1 min in a gamma counter. For subtraction of background radioactivity, a small piece of quadriceps muscle from each mouse was excised, weighed, and counted as above.

Abbreviations: TSH, thyroid stimulating hormone; CIITA, class II transactivator; TG, thyroglobulin; TPO, thyroperoxidase; TSH-R, TSH receptor; NIS, sodium iodide symporter.

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Effect of Thyroidal IFN-γ on MHC Class I, Class II, and Class II Transactivator (CIITA) Gene Expression. To assess whether IFN-γ, when produced ectopically by the thyroid cell, was capable of inducing classical IFN-γ-responsive genes in thyroid cells, total RNA was extracted from mouse thyroids as described above, separated on a 1% agarose gel containing 2 M formaldehyde, and transferred to Zeta-Probe GT membrane. The membrane was hybridized sequentially with probes for MHC class I, MHC class II, and CIITA genes, described previously (10, 11). Levels of mRNA were quantitated with a Storm PhosphorImager (Molecular Dynamics) and normalized to murine β-actin mRNA obtained from the same filter to correct for amount of loading. MHC class II expression also was analyzed by immunohistochemistry by using a monoclonal mouse anti-mouse I-A^b (PharMingen).

Effect of Thyroidal IFN-γ on the Expression of Thyroglobulin (TG), Thyroperoxidase (TPO), TSH Receptor (TSH-R), and Sodium Iodide Symporter (NIS). To study the effect of thyroidal IFN-γ on the expression of thyroid-specific/restricted genes, total RNA was extracted from mouse thyroids and analyzed by Northern blot, as above. The probes for TG, TPO, TSH-R, and NIS are described (11). Data were normalized to values for murine glyceraldehyde-3-phosphate dehydrogenase mRNA obtained from the same filter. NIS expression also was assessed at the protein level by immunoblotting, using the procedure and the high-affinity antibody directed against NIS C terminus described previously (12). Data were normalized to values for murine actin.

Other Mouse Strains and the FRTL-5 Cell Line. Line A transgenics were crossed to RAG-2 knockout mice (Taconic Farms) to test the role of mature T and B lymphocytes in the production of the thyroid phenotype. F_1 mice, all hemizygous for the RAG-2 deletion, were screened by Southern blot with the IFN-γ cDNA probe as described to identify the *thyr*-IFN-γ transgenics. Transgene-positive mice were intracrossed, and the F_2 progeny were screened again by Southern blot to identify the *thyr*-IFN-γ transgenics and by PCR to identify the RAG-2 hemizygous and knockout mice, as described (13). Line A *thyr*-IFN-γ transgenic mice also were crossed to IFN-γ receptor knockout mice (The Jackson Laboratory) to test the requirement of the presence of the IFN-γ plasma membrane receptor. In addition to the usual Southern blot screening to identify the *thyr*-IFN-γ transgenics, F_2 mice were screened by PCR to identify the IFN-γ receptor hemizygous and knockout mice, as described (14). Rat thyroid FRTL-5 cells (Interthyr Research Foundation, Baltimore; ATCC CRL8305) were grown in Coon's modified F-12 medium containing 5% heat-treated, mycoplasma-free calf serum (Life Technologies) and 1 mM nonessential amino acids (Life Technologies) supplemented with a mixture of six hormones, including bovine TSH (1×10^{-10} M), insulin (10 μg/ml), cortisol (0.4 ng/ml), transferrin (5 μg/ml), glycyl-L-histidyl-L-lysine acetate (10 ng/ml), and somatostatin (10 ng/ml). FRTL-5 cells were a fresh subclone (F1) with the properties described (11); they were diploid and between their fifth and 20th passages. Rat IFN-γ (Life Technologies) was added to the culture medium at final concentrations of 1, 10, 100, and 1,000 units/ml. The culture medium containing rat IFN-γ was changed every 2 days.

Results

Construction of *Thyr*-IFN-γ Transgenic Mice and Transgene Expression. The murine IFN-γ was expressed under control of the rat thyroglobulin promoter (Fig. 1*A*), which supports transcription specifically in thyroid follicular cells (15, 16). Three founder animals, designated A, B, and C and bearing 22, 18, and 15 copies of the transgene, respectively, were maintained by mating to

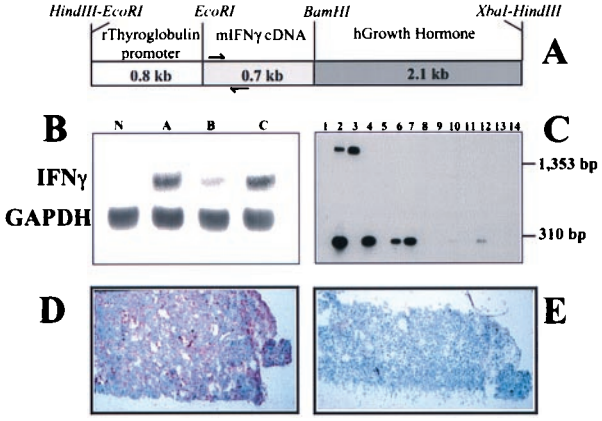

Fig. 1. Thyroid-specific expression of IFN-γ in transgenic mice. (*A*) Organization of *thyr*-IFN-γ transgene. The thyroglobulin promoter, IFN-γ cDNA, and growth hormone splice donor and acceptor sequences are indicated by rectangles. The primers used for reverse transcriptase–PCR are indicated by arrows. (*B*) Thyroidal expression of IFN-γ in normal C57BL/6 mice (N) and in the three transgenic lines (A, B, and C), as assessed by Northern blot analysis. (*C*) Expression of IFN-γ in thyroids and other tissues: water control (lane 1); genomic DNA from line A transgenic (lane 2); genomic DNA from normal C57BL/6 (lane 3); thyroid RNA from line A transgenic with (lane 4) and without (lane 5) reverse transcriptase; thyroid RNA from line B transgenic (lane 6), line C transgenic (lane 7), and normal C57BL/6 (lane 8); and RNA from liver (lane 9), lymph nodes (lane 10), salivary glands (lane 11), spleen (lane 12), brain (lane 13), and testicles (lane 14). Sizes of DNA standards, in base pairs, are indicated on the right. (*D*) Immunohistochemical analysis of the expression of IFN-γ by thyroid cells. (*E*) Control slide without addition of the primary antibody.

C57BL/6 mice. *Thyr*-IFN-γ transgenic mice exhibited significant growth retardation (20% smaller than control littermates) and reduced fertility (litters of 2–3, instead of the typical 6–7). The thyroidal expression of IFN-γ was significantly higher in line A and C transgenics than in line B transgenics (Fig. 1*B*) ($P < 0.05$ by two-way ANOVA). Ectopic IFN-γ production by thyrocytes was confirmed at the protein level by immunohistochemical study (Fig. 1 *D* and *E*). IFN-γ was expressed specifically in the thyroid of transgenic mice and not in other tissues (Fig. 1*C*), with the exception of spleen and, at very low levels, lymph nodes.

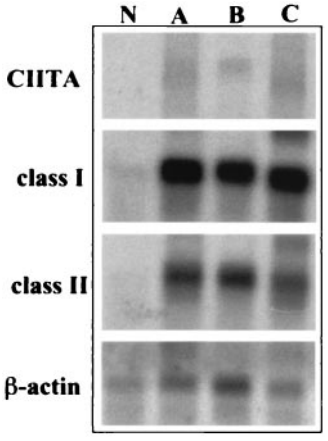

Fig. 2. Ability of transgenic IFN-γ to induce in the thyroid cells the expression of MHC class I, MHC class II, and CIITA genes. Northern blot analyses were performed by using 20 μg of total RNA extracted from thyroids of normal C57BL6 mice (N) and the three transgenic lines (A, B, and C). Blots were sequentially hybridized with probes for MHC class I, MHC class II, CIITA, and β-actin. The ratio of binding of each probe to β-actin was calculated to correct for the amount of loading.

Table 1. Score (mean ± SD) of thyroid lesions

	Number of mice	Mean ± SD
C57BL/6 controls	9	0
Line A transgenics	37	3.32 ± 0.7
Line B transgenics	11	2.36 ± 1.1
Line C transgenics	24	2.91 ± 0.8
Line A/RAG-$2^{+/-}$	4	3.25 ± 0.5
Line A/RAG-$2^{-/-}$	5	3.40 ± 0.5
Line A/IFN-γ $receptor^{+/-}$	5	3.00 ± 0.7
Line A/IFN-γ $receptor^{-/-}$	5	0

Aberrant Thyroidal Expression of MHC Class I, Class II, and CIITA Genes. Transgenic expression of INF-γ by the thyrocytes was capable of activating in the thyrocytes these classical IFN-γ-responsive genes. In fact, it induced the expression of the CIITA gene, increased the expression of the MHC class I gene, and caused aberrant expression of the MHC class II gene in each of the three transgenic lines (Fig. 2). Immunohistochemistry revealed that thyrocytes were the cells expressing MHC class II (data not shown).

Disruption of Thyroid Architecture and Hypothyroidism. *Thyr*-IFN-γ transgenic mice showed extensive disruption of the thyroid architecture (Table 1), with loss of the typical follicular structure (Fig. 3*A*). The remaining thyroid follicles were small and hypercellular, resembling the morphologic pattern observed during embryonic life (Fig. 3*B*). Thyroid cells showed abundant eosinophilic cytoplasm and were proven to be epithelial in origin because they stained for cytokeratin (data not shown). No cytological or histological sign of malignancy was ever observed. A limited mononuclear cell infiltration was present, either scattered in the interstitium between thyroid follicles or organized in small foci (Fig. 3*C*). These mononuclear cells were mainly $CD4^+$ T cells. $CD8^+$ T cells were rare, and $B220^+$ cells were absent (data not shown). Fig. 3*D* shows a normal thyroid for comparison. *Thyr*-IFN-γ transgenic mice developed primary hypothyroidism as evidenced by reduced serum thyroxine, reduced thyroidal uptake of radioactive iodine, and increased serum TSH (Table 2). The severity of hypothyroidism correlated with the amount of IFN-γ expressed in the thyroid gland, which was more severe in line A and line C transgenic mice and not present in line B transgenics.

Hypothyroidism Derives from a Selective Suppression of NIS Gene Expression. The transgenic expression of IFN-γ in the thyroid gland did not significantly affect the expression of the TG gene. The TSH-R and TPO genes were slightly increased, with no difference, however, among the three lines (Fig. 4). The most striking effect, instead, was on the expression of NIS. The NIS signal was weak and required long exposure times, indicating that NIS transcription was decreased in line A and C transgenics. In contrast, it was slightly increased above control in line B mice. The modulation of NIS expression by IFN-γ was confirmed at the protein level, using a high-affinity NIS antibody (Fig. 5). The immunoblot showed again the dramatic suppression of NIS expression in line A and C transgenics.

The Effect of IFN-γ on NIS Expression Is Dose- and Time-Dependent. To confirm *in vitro* the effect of IFN-γ on NIS expression observed *in vivo*, we treated rat thyroid FRTL-5 cells with various doses of IFN-γ for 2 days (the time most commonly used in tissue culture experiments) (17) and also for 6 days to mimic a more chronic stimulation similar to the *in vivo* scenario. In the 2-day treatment, IFN-γ decreased NIS expression at the highest concentration (1,000 units/ml), whereas it had no effect at lower concentrations (100 units/ml or less) (Fig. 6), findings consistent with a recent report (18). In the 6-day treatment, IFN-γ again decreased NIS expression at 1,000 units/ml but, interestingly, it induced the opposite effect at lower doses, where NIS expression was increased (Fig. 6). The molecular mechanisms underlying this dose- and time-dependent effect of IFN-γ on NIS expression are under investigation, but it is intriguing to note that this *in vitro* effect correlates with the *in vivo* observation. Line B transgenic mice that express the lowest thyroidal levels of IFN-γ show NIS levels slightly increased with respect to control littermates. In contrast, in line A and C transgenic mice NIS expression is greatly reduced.

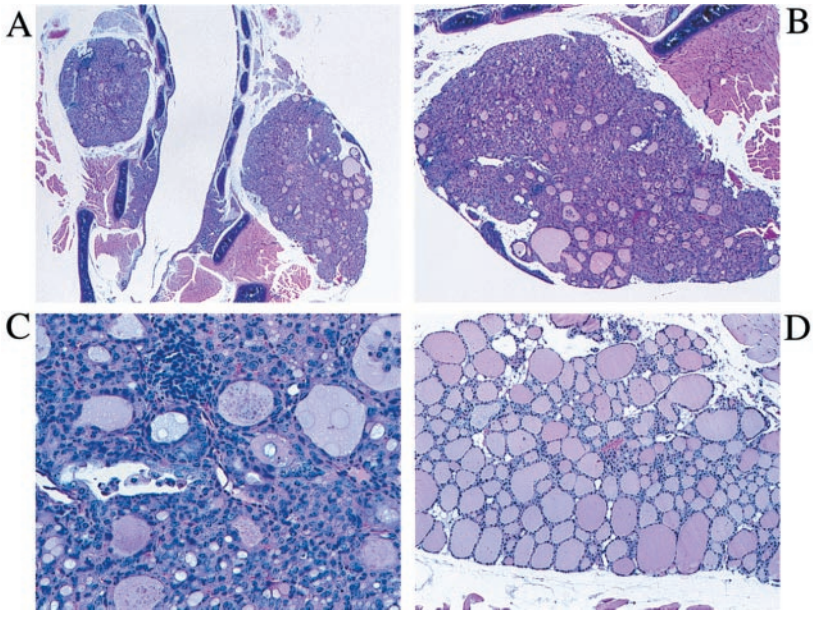

Fig. 3. Histological analysis of the thyroid glands in *thyr*-IFN-γ transgenic and control mice. Hematoxylin/eosin staining of thyroid glands from line A transgenic mice at ×10 magnification (*A*), ×20 magnification (*B*), and ×40 magnification (*C*). A normal thyroid from C57BL6 mouse is shown for comparison (*D*).

Table 2. Serum total T4 (μg/dl), serum TSH (μg/ml), and radioactive iodine uptake (RAIU, in cpm/mg thyroid) in *thyr*-IFN-γ transgenic and control mice

	Line A		Line B		Line C	
	Control	Transgenic	Control	Transgenic	Control	Transgenic
Serum total T4						
Mean ± SD	5.58 ± 1.50**	2.78 ± 1.36	5.34 ± 0.93	5.14 ± 1.71	5.83 ± 1.21**	2.39 ± 1.25
No. of mice	50	53	37	38	27	35
Serum TSH						
Mean ± SD	0.162 ± 0.06§§	9.09 ± 3.12	0.15 ± 0.05	0.13 ± 0.07	0.14 ± 0.04§§	15.28 ± 5.83
No. of mice	5	6	5	5	5	5
RAIU						
Mean ± SD	1,757,340 ± 244,358*	318,257 ± 188,052	1,537,821 ± 234,679	1,724,317 ± 203,120		
No. of mice	4	10	4	9	Not done	Not done

**, $P < 0.0001$, when comparing by unpaired *t* test in each line the means of total T4 in transgenics and controls. §, $P < 0.01$ and §§, $P < 0.001$, when comparing in each line the THS and RAIU means of transgenics and controls by Mann–Whitney test.

The Thyroid Phenotype Is Not Caused by Infiltrating Lymphocytes and Requires the IFN-γ Receptor. To test whether the observed thyroid phenotype is a direct effect of IFN-γ on thyroid cells rather than the consequence of lymphocytic infiltration of the thyroid gland, we crossed the *thyr*-IFN-γ transgenic mice with RAG-2 knockout mice, which lack functional T and B lymphocytes. The F_2 intracross provided four *thyr*-IFN-γ transgenic/RAG-2 hemizygous and five *thyr*-IFN-γ transgenic/RAG-2 knockout mice, which were analyzed for thyroid pathology. The absence of lymphocytes did not change the previously described thyroid pathology, proving that the effect is a direct consequence of the action of IFN-γ on the thyroid gland, rather than the indirect effect of an autoimmune response (Table 1). To test the requirement of the IFN-γ receptor in the production of the thyroid phenotype, we crossed the *thyr*-IFN-γ transgenic mice with IFN-γ receptor knockout mice. The F_2 intracross provided five *thyr*-IFN-γ transgenic/IFN-γ receptor hemizygous and five *thyr*-IFN-γ transgenic/IFN-γ receptor knockout mice, which were analyzed for thyroid pathology. Only the *thyr*-IFN-γ transgenic mice that have the IFN-γ receptor develop the previously described thyroid pathology. The *thyr*-IFN-γ transgenic/IFN-γ receptor knockout mice had normal thyroid morphology, thus proving the notion that, to act, IFN-γ has to bind to its plasma membrane receptor (Table 1).

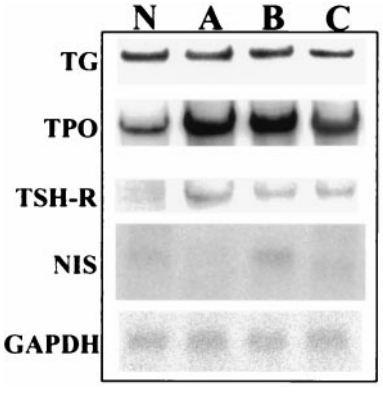

Fig. 4. Influence of transgenic IFN-γ on the expression of thyroid-specific/restricted genes. Northern analyses were performed by using 20 μg of total RNA extracted from thyroids of normal C57BL6 mice (N) and the three transgenic lines (A, B, and C). Blots were sequentially hybridized with probes for TG, TPO, TSH-R, NIS, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The ratio of binding of each probe to β-actin was calculated to correct for the amount of loading.

Discussion

In the present study we report that chronic expression of IFN-γ in the thyroid gland causes hypothyroidism via suppression of NIS gene transcription. Over the past few years, the role of cytokines in regulating thyroid function has been the focus of several studies, which have shown, in general, that cytokines such as tumor necrosis factor α, IL-1α, and IFN-γ have an inhibitory effect on thyroid function (18). Focusing on IFN-γ, it has been shown, for example, that this cytokine inhibits thyrotropin-induced transcription of TG (19), TPO (20), NIS (18), and TSH-R (21) genes, the last one mediated by a reduction of the binding of thyroid transcription factor-1 to the TSH-R promoter (22). All these studies have been performed *in vitro*, using either primary thyroid cell cultures or, more commonly, the rat thyroid cell line FRTL-5 and, thus, have looked at only the acute effects that the addition of IFN-γ to the culture medium has on the thyroid cell. This study describes targeting the expression of a cytokine to the thyroid gland, allowing us to assess the more chronic effect of IFN-γ, more closely simulating the *in vivo* pathophysiology.

We have demonstrated that IFN-γ disrupts thyroid architecture and function in a dose-dependent fashion. The two transgenic lines that express the highest thyroidal levels of INF-γ (lines A and C) show, in fact, an almost complete suppression of NIS transcription, reflected in a significantly reduced uptake of radioactive iodine. These results are in keeping with a recent report that described *in vitro* suppression of NIS transcription by IFN-γ only when high concentrations of this cytokine were used (18). In contrast, line B transgenic mice, which express low thyroidal IFN-γ levels, did not develop hypothyroidism, but had slightly greater NIS expression than

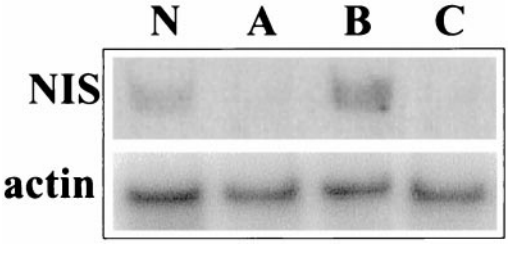

Fig. 5. Ability of transgenic IFN-γ to modulate NIS expression. Western blot analysis was performed by using 100 μg of total proteins extracted from the thyroids of normal C57BL6 mice (N) and the three transgenic lines (A, B, and C). Blots were sequentially hybridized with antibodies for NIS and actin. The ratio of binding of each probe to actin was calculated to correct for loading.

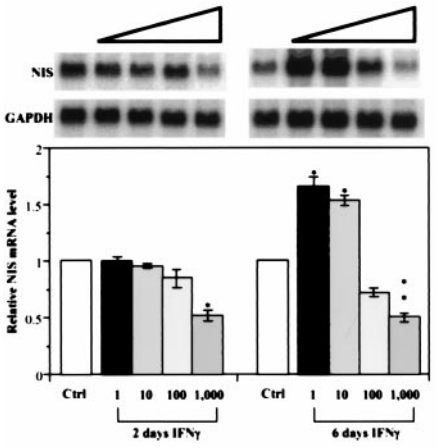

Fig. 6. The effect of IFN-γ on NIS expression is dose and time dependent. FRTL-5 cells in six hormones were washed with serum-free medium and exposed to different doses of IFN-γ for 2 or 6 days. In the 6-day experiments, the culture medium with IFN-γ was replaced every other day. At the end of the experiments, Northern blot analyses were performed by using 20 μg total of RNA. (*A*) Blots were sequentially hybridized with probes for NIS, MHC class II (as control), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (to correct for loading). (*B*) The ratio of binding of each probe to GAPDH was calculated, and this value was set at 1 for MHC class II. NIS values were compared with the corresponding MHC class II control and expressed as relative mRNA levels. Data are the mean $\pm$ SD of three different experiments, each performed in duplicate. Single or double asterisks represent a significant difference with $P < 0.05$ or < 0.01, respectively, by two-way ANOVA.

control littermates (although not statistically significant), with a corresponding slightly higher radioactive iodine uptake.

The dual effect of IFN-γ on thyroid function may explain the changes in thyroid function seen in autoimmune thyroiditis. It is, in fact, known that some patients with Hashimoto's thyroiditis develop in the early phases of disease a short-lived hyperthyroid phase (sometimes referred to as Hashitoxicosis) associated with increased, rather than decreased, iodine uptake. We suggest that in this initial phase of the disease, when the lymphocytic infiltration is forming, there are lower levels of IFN-γ and, thus, increased iodine uptake. In contrast, in the florid phase of the disease, when the thyroid has been diffusely infiltrated by lymphocytes, higher IFN-γ levels could be expected, producing the classical features of Hashimoto's thyroiditis, with low iodine uptake and hypothyroidism.

This *in vivo* model of chronic, local production of IFN-γ in the thyroid gland has shown that the expression of only one of the four thyroid-specific or -restricted genes is affected; NIS is, in fact, suppressed, whereas TG, TPO, and TSH-R do not show significant differences from the controls. As mentioned before, *in vitro* data accumulated in the past decade have shown that IFN-γ can suppress transcription of TG, TPO, and TSH-R. It is not known why *in vivo* the effect is centered mainly on NIS suppression rather than on the other three thyroid genes. The mechanisms by which NIS suppression is achieved in these transgenic models currently is under investigation. It is probably an indirect effect, mediated by a yet uncharacterized intermediate, because the promoter of the NIS gene contains neither an IFN-γ-activated site (TTNCNNNAA), typical of the primary IFN-γ-response genes, nor an IFN-stimulated response element (AGTTTCNNTTTCNC/T), typical of the secondary IFN-γ-response genes (23).

The transgenic expression of IFN-γ in the thyroid gland induces a congenital hypothyroid phenotype, with growth retardation and reduced fertility. Histological analysis of the thyroid gland showed, in addition to disruption of follicular architecture, scattered foci of mononuclear cell infiltration. These foci were limited and did not replace the entire thyroid, as can be seen in patients with florid Hashimoto's thyroiditis. With age, thyroids maintained the characteristic architectural disruption but acquired infiltration of fibrous adipose tissue, as can be seen in patients with idiopathic myxedema. The paucity of the lymphocytic infiltration can be caused by the genetic background of the mouse strain on which the transgene was maintained. Because C56BL6 mice are a relatively poor responder strain with respect to experimental autoimmune thyroiditis, developing few or no thyroid lesions after immunization with murine thyroglobulin (24), the results were not unexpected. To assess whether the morphologic and functional alterations observed in the thyroids of *thyr*-IFN-γ transgenic mice were mediated by infiltrating lymphocytes or, instead, by a direct effect of IFN-γ on thyrocytes, we crossed the transgenic mice to RAG-2 knockout mice, which fail to generate mature T and B lymphocytes because of their inability to initiate the V(D)J recombination process (25). Both *thyr*-IFN-γ transgenic/RAG-2 hemizygous and the *thyr*-IFN-γ transgenic/RAG-2 knockout mice showed the same disruption of the thyroid architecture, thus indicating that the morphologic changes in the thyroid are the result of a direct effect of the local production of IFN-γ and do not depend on lymphocytic infiltration.

In autoimmune thyroiditis the source of IFN-γ is the lymphocytes that infiltrate the thyroid gland. In contrast, in this transgenic model IFN-γ is produced ectopically and not physiologically by the thyrocytes themselves. As in all models, one should be cautious in considering that IFN-γ, when produced ectopically by the thyrocytes, exerts the same effects as when it is produced by lymphocytes that infiltrate the thyroid gland. To address this concern we have crossed the *thyr*-IFN-γ transgenic mice to IFN-γ receptor knockout mice with the following rationale. If IFN-γ produced ectopically by the thyrocyte can influence thyroid structure and function without the need to be exported outside the thyrocyte and to re-enter into the thyrocyte via its plasma membrane receptor, then crossing the *thyr*-IFN-γ transgenic to the IFN-γ receptor knockout mice should not change the phenotype. In contrast, if IFN-γ must be exported outside the thyrocyte after its synthesis and must bind to its plasma membrane receptor, located on the same thyrocytes that have produced it (autocrine effect) or on nearby thyrocytes (paracrine effect), then the same cross should abolish the phenotype. The results of this cross have shown that the hypothyroid phenotype disappears in the *thyr*-IFN-γ transgenic/IFN-γ receptor knockout mice, proving that, to act, IFN-γ must bind to its plasma membrane receptor. It further indicates that the source of IFN-γ (lymphocytes infiltrating the thyroid or thyrocytes themselves) is irrelevant to the hypothyroid phenotype.

Local production of IFN-γ has been used in several models of autoimmune diseases to investigate its pathogenetic role more deeply (26). In general, the IFN-γ transgenic models are consistent with a role of IFN-γ in inducing inflammation and, subsequently, autoimmunity, as in the case of diabetes mellitus (27, 28), hepatitis (29), retinitis (30–32), myasthenia gravis (33), and multiple sclerosis (34, 35). However, this sequence of events does not occur in other IFN-γ transgenic models, as when the promoter directs the ectopic expression of IFN-γ in skin (36, 37), lens of the eye (38, 39), or B lymphocytes (40). Thus, the same cytokine can produce dramatically different effects depending on the organ in which it is expressed, and these effects may be diametrically opposite. For example, beta cell expression of IFN-γ results in a cell-mediated immune destruction of pancreatic islets, whereas the expression at the neuromuscular junction results in a humoral immune response. These considerations should encourage further investigation of cytokine production at the target organ level in initiation or progression of autoimmune disease.

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