The Effects of Alpha Interferon on the Development of Autoimmune Thyroiditis in the NOD H2h4 Mouse

YAELOPPENHEIMA*, GRACE KIMA*, YOSHIYUKIBANB*, PAMELACUNGERB, ERLINDA CONCEPCION, TAKAONO and YARONTOMER

aDivision of Endocrinology, Diabetes, and Bone Diseases, New York, NY, USA; bDepartment of Pathology, Mount Sinai School of Medicine, New York, NY, USA

Alpha interferon (αIFN) therapy is known to induce thyroid autoimmunity in up to 40% of patients. The mechanism is unknown, but Th1 switching has been hypothesized. The aim of our study was to examine whether αIFN accelerated the development of thyroiditis in genetically susceptible mice. We took advantage of NOD-H2h4, a genetically susceptible animal model, which develops thyroiditis when fed a high iodine diet. Six to eight week old male NOD H2h4 mice were injected with mouse αIFN (200 units) or with saline three times a week for 8 weeks. All mice drank iodinated water (0.15%). Mice were sacrificed after 8 weeks of injection. Their thyroids were examined for histology and blood was tested for antithyroglobulin antibody levels. T4 and glucose levels were also assessed. In the IFN-injected group, 6/13 (46.2%) developed thyroiditis and/or thyroid antibodies while in the saline-injected group, only 4/13 (30.8%) developed thyroiditis and/or thyroid antibodies (p = 0.4). The grade of thyroiditis was not different amongst the two groups. None of the mice developed clinical thyroiditis or diabetes mellitus. Our results showed that αIFN treatment did not accelerate thyroiditis in this mouse model. This may imply that αIFN induces thyroiditis in a non-genetically dependent manner, and this would not be detected in a genetically susceptible mouse model if the effect were small. Alternatively, it is possible that αIFN did not induce thyroiditis in mice because, unlike in humans, in mice αIFN does not induce Th1 switching.

Keywords: Alpha interferon; Autoimmunity; Animal model; Thyroiditis

INTRODUCTION

Alpha interferon (αIFN) treatment for various medical conditions is known to induce thyroid autoimmunity in up to 40% of patients. The association between αIFN treatment and autoimmune thyroiditis was reported as early as 1986 by Burman et al. (1986), who studied patients receiving αIFN for carcinoid tumors. The association is most recognized in patients with hepatitis C (Watanabe et al., 1994), although it has been noted to occur in several other conditions treated by αIFN including breast cancer, carcinoid and hematologic malignancies (Fentiman et al., 1988; Ronnblo et al., 1991a,b; Gisslinger et al., 1992; Silvestri et al., 1994; Watanabe et al., 1994). The thyroid diseases associated with αIFN therapy include both autoimmune hyperthyroidism and hypothyroidism (Koh et al., 1997). In up to 40% of cases, thyroid dysfunction persists after discontinuation of the αIFN therapy (Koh et al., 1997).

Factors that predispose patients to the development of αIFN-induced thyroiditis include hepatitis C infection, positive pre-treatment antithyroid antibodies, dose and duration of αIFN therapy, genetic factors, stress, dietary iodine intake, female gender, pregnancy and HLA-A2 in the Japanese population (Preziati et al., 1995; Roti et al., 1996; Custro et al., 1997; Koh et al., 1997; Minelli et al., 1997; Fernandez-Soto et al., 1998; Dumoulin et al., 1999; Kakizaki et al., 1999, 2000). The mechanisms by which αIFN induces thyroid autoimmunity are unclear.

We hypothesized that αIFN accelerates subclinical thyroiditis by immune modulation in genetically predisposed individuals. In order to examine this hypothesis, we took advantage of the NOD H2h4 mouse model, a model in which the mice are genetically prone to develop...
thyroiditis when placed on iodinated drinking water (Rasooly et al., 1996; Braley-Mullen et al., 1999). We used the NOD H2h4 mouse model to examine the effects of αIFN on animals genetically susceptible to thyroiditis.

MATERIALS AND METHODS

Animal Protocol

Mouse interferon alpha was obtained from PBL Biomedical Laboratories (New Brunswick, NJ). Six to eight week old NOD H2h4 male mice were purchased from Taconic Farms (Germantown, NY). Male mice were used in our experiments since previous studies (Rasooly et al., 1996; Braley-Mullen et al., 1999) and our own experiments revealed that male mice are more prone to thyroiditis in this model (data not shown). Thirteen mice were injected intra peritoneally (i.p.) with αIFN (200 units, 0.4 cc) three times a week for eight weeks. As controls, 13 mice were injected with saline (0.4 cc) three times a week for eight weeks. After eight weeks of injections, mice were sacrificed, 1 ml of blood was drawn, and their thyroids were removed. All these 26 mice were given 0.15% iodinated drinking water [Sodium Iodide, Sigma Chemical Company (St. Louis, MO)]. Additional 14 mice (6 injected with saline and 8 injected with αIFN) received regular water without iodine supplementation.

Measurement of Murine Tg (mTg) Antibodies

Serum was analyzed for mTg antibodies by an ELISA assay as previously described (Imaizumi et al., 2001). Briefly, 96 well-plates (Immulon 1, Dynex Technologies Inc., Pittsburgh, PA) were coated with mouse thyroglobulin (0.1 mcg thyroglobulin/well diluted in Carbonate/Bicarbonate Buffer, pH 9.6) and incubated overnight at 4°C. The plates were washed with 0.05% PBS-Tween and nonspecific binding was blocked for 30 min with 3% BSA-PBS/Tween. After washing, the sera were diluted 1:100 in 0.5% BSA/PBS and added to the plates for 2 h at room temperature. Plates were washed again 6 times and alkaline phosphatase conjugated anti mouse IgG (Sigma Diagnostics, St. Louis, MO) was added for 30 min. Plates were washed 4 times and then developed using p-nitrophenyl phosphate as substrate. Absorbance was read at 405 nm. Samples were considered positive if the OD was 2 standard deviation (SD) above the average OD for controls.

Thyroid Histology

Mice thyroids were fixed in 10% formalin and stained by hematoxylin and eosin. The severity of thyroiditis was graded on a scale from +1 to +4.0 as follows: +1, small focal areas of lymphocytic cells; +2, focal collection of lymphocytic cells with some follicular destruction; +3, diffuse lymphocytic infiltration of thyroid follicles involving approximately 40% or less of the thyroid and +4, >40% lymphocytic infiltration of thyroid follicles (Fig. 1; Imaizumi et al., 2001).

Measurement of Total Thyroxine (T4) and Glucose Levels

T4 was measured by radioimmunoassay using the neonatal T4 Coat-A-Count kit (Diagnostics Products Corporation, Los Angeles, CA). Glucose levels were measured using the One Touch Profile Glucometer (Lifescan Inc., Milpitas, CA).
Data Analysis

Data were analyzed using Student’s, Chi-Square and Fisher Exact tests. Probability values less than 0.05 were considered significant.

RESULTS

Effects of Iodine on Development of Thyroiditis

None of the 14 mice that did not receive iodine supplementation (6 injected with saline and 8 injected with αIFN) developed thyroiditis. In contrast, 10/26 (38.5%) of the mice that received iodine supplementation developed thyroiditis \( (p = 0.007) \).

Autoantibody Levels in αIFN-injected Mice and Controls

Positive thyroglobulin antibodies were detected in 3/13 (23%) of the mice injected with αIFN compared to 0% of the control mice injected with saline \( (p = 0.07) \).

Thyroid Histology in αIFN-injected Mice and Controls

Thyroid infiltration was detected in 5/13 (38.5%) of the αIFN-injected mice and 4/13 (30.8%) in the control mice (Table I). The grade of thyroiditis was not different between the αIFN-injected versus controls. When we used positive thyroid antibodies and/or positive histology to define thyroiditis, 6/13 (46.2%) of the mice injected with αIFN developed thyroiditis compared to 4/13 (30.8%) in the control mice (Table I). This difference did not reach statistical significance \( (p = 0.4) \).

Clinical Disease in αIFN-injected Mice and Controls

None of the mice developed evidence of clinical thyroid dysfunction and/or diabetes mellitus. There were no significant differences in the T4 levels between the 4 groups mice (Fig. 2). Moreover, the levels of T4 were similar in the mice that developed thyroiditis and those that did not \( (\text{average Total T4 level} = 4.24 \text{ in mice that developed thyroiditis vs. 4.19 in mice that did not develop thyroiditis}) \) (Fig. 2).

Since it has been previously reported that insulinitis develops in 20–30% of NODH2h4 mice

<table>
<thead>
<tr>
<th>TABLE I Thyroiditis and thyroglobulin antibody frequency in mice injected with saline and αIFN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Positive histology for thyroiditis</td>
</tr>
<tr>
<td>Thyroiditis on histology</td>
</tr>
<tr>
<td>and/or elevated Tg-Ab’s</td>
</tr>
</tbody>
</table>

* NS, not significant.

DISCUSSION

Interferons are cytokines that are involved in immune modulation, possessing both antiviral and antitumoral activity. Interferons are grouped into Types I and II. Type I interferons are acid stable and include alpha, beta and omega subtypes. Type II interferons are acid labile and include the gamma subtype (Walter et al., 1998). αIFN first became available for use in humans in the form of leukocyte-derived interferon in the mid 1970’s. Subsequently, in 1986, recombinant αIFN became available for clinical use. Since that time, recombinant interferons have been used to treat a wide spectrum of medical conditions, including hepatitis, and several cancers (Walter et al., 1998).

Interferons were shown to induce and/or exacerbate several autoimmune diseases, including autoimmune thyroiditis, autoimmune hepatitis, rheumatoid and lupus like diseases, and autoimmune diabetes mellitus. Interestingly, thyroid disease is the most prevalent autoimmune disease exacerbated or induced by αIFN (Dumoulin et al., 1999). It is unclear whether this reflects the higher prevalence of thyroid autoimmunity in the general population or a thyroid specific effect of interferon.

Our hypothesis was that αIFN accelerates thyroiditis in genetically prone individuals. Evidence supporting our hypothesis is that most of the risk factors for αIFN-induced thyroiditis are genetic (e.g. positive pre-treatment antithyroid antibodies, and female gender) (Preziati et al., 1995; Roti et al., 1996; Custro et al., 1997; Minelli et al., 1997; Tunbridge et al., 1977; Koh et al., 1997; Fernandez-Soto et al., 1998; Kakizaki et al., 1999; Dumoulin et al., 1999; Kakizaki et al., 2000). In order to
test our hypothesis, we gave αIFN to mice genetically susceptible to thyroiditis.

Our study revealed that αIFN did not accelerate thyroiditis in this thyroiditis-prone mouse model. Thus our study does not support an accelerating effect of αIFN on autoimmune thyroid disease development at least in our mouse model. Therefore, it is possible that αIFN induces thyroiditis de novo and does not accelerate disease development in genetically predisposed individuals. Such a de novo effect would not be detected in our mice, which are already prone for thyroiditis, especially if this is a mild effect. Alternatively, it is possible that αIFN did accelerate thyroiditis development in the NOD H2h4 mice but that the effect was too small to be detected in our experiments. Our studies showed a higher frequency of thyroiditis in the αIFN injected group versus the controls (48.2% and 30.8%, respectively), however this difference did not reach statistical significance. Power calculations show that at least 80 mice per group would have been required to reach statistical significance for the difference that we observed.

In summary, αIFN did not accelerate thyroiditis in this mouse model. However, minor αIFN effects cannot be excluded. Additional studies are needed to examine this possibility.

**Acknowledgements**

We thank Dr Terry F Davies for his teaching, support and ever ready help in our joint studies. This work was supported in part by grants DK61659 & DK58072 from NIDDKD (to YT).

**References**


Minelli, R., Braverman, L.E., Giuberti, T., et al. (1997) “Effects of excess iodine administration on thyroid function in euthyroid patients with
a previous episode of thyroid dysfunction induced by interferon-alpha
and thyroid function in patients with chronic active hepatitis treated
with recombinant interferon alpha-2a”, Eur. J. Endocrinol. 132,
587–593.
Immunopathol. 81, 287–292.
phenomena in patients with malignant carcinoid tumors during
Ronnblom, L.E., Alm, G.V. and Oberg, K.E. (1991b) “Autoimmunity
after alpha-interferon therapy for malignant carcinoid tumors”, Ann.
Roti, E., Minelli, R., Giuberti, T., et al. (1996) “Multiple changes
in thyroid function in patients with chronic active HCV hepatitis
treated with recombinant interferon-alpha”, Am. J. Med. 101,
482–487.
autoimmune thyroid diseases during long-term treatment of
hematological malignancies with alpha-interferons”, Haematologica
79, 367–370.
thyroid disease in a community: the Whickham survey”,
recent developments in the molecular characterization of recombi-
nant alfa interferons on the 40th anniversary of the discovery of
Watanabe, U., Hashimoto, E., Hisamitsu, T., Obata, H. and Hayashi, N.
(1994) “The risk factor for development of thyroid disease
during interferon-alpha therapy for chronic hepatitis C”,
Am. J. Gastroenterol. 89, 399–403.
Submit your manuscripts at http://www.hindawi.com