Eur J Cancer Clin Oncol, Vol. 24, No. 8, pp. 1299–1303, 1988.
Printed in Great Britain

0277–5379/88$3.00 + 0.00

An Autoimmune Aetiology for Hypothyroidism Following Interferon Therapy for Breast Cancer

I.S. FENTIMAN,* F.R. BALKWILL,† B.S. THOMAS,† M.J. RUSSELL,† I. TODD‡ and G.F. BOTTAZZO‡

**Imperial Cancer Research Fund, Clinical Oncology Unit, Guy's Hospital, London SE1 9RT, U.K., †Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K. and Department of Immunology, Middlesex Hospital Medical School, London W1P 9PG, U.K.*

Abstract—*Alpha-interferon has been administered as an adjuvant treatment for women with loco-regional relapse of breast cancer. During the course of treatment* 5/10 (50%) *of women receiving interferon developed* de novo *thyroid autoantibodies. Three patients became clinically myxoedematous, with biochemical evidence of hypothyroidism which responded to thyroxine replacement therapy. The leucocyte alpha-interferon preparations used in the trial enhanced Class I but not Class II MHC antigens on thyrocytes* in vitro. *These data strongly suggest that patients receiving alpha-interferon therapy should be closely monitored for the possible development of thyroid dysfunction and that thyroid antibody determination can greatly help to predict overt thyroid clinical abnormalities.*

INTRODUCTION

INTERFERONS have been shown to be of value in the treatment of certain cancers including hairy cell leukaemia [1], renal carcinoma [2] and endocrine pancreatic tumours [3]. However, these biological response modifiers have not been found to be efficacious for patients with more common solid tumours [4, 5]. This may partly result from the large tumour burden in such cases and therefore we decided to carry out an adjuvant study on patients with small tumour burden [6]. The group chosen were women with loco-regional recurrence of breast cancer but no other evidence of metastases. These patients, despite apparent local control by irradiation, are at high risk of subsequent local or distant relapse [7, 8].

In this controlled trial patients were either observed or given leucocyte alpha-interferon at a dose of 3 megaunits subcutaneously daily for 1 year or until relapse. In a preliminary communication we reported that three patients developed clinical and biochemical evidence of hypothyroidism [9]. All responded well to replacement thyroxine therapy with abolition of symptoms including lethargy and hair loss.

Interferons have been shown to alter surface MHC molecule expression on a wide variety of cells including thyroid cells, and recent experiments have linked aberrant MHC expression with the development of destructive autoimmunity [10, 11]. We therefore investigated in detail the occurrence of autoantibodies in a group of 10 women who received adjuvant interferon.

Accepted 24 March 1988.

PATIENTS AND METHODS

Measurement of TSH

Thyroid stimulating hormone was measured by radioimmunoassay (100 μl serum) using the TSH-Irma MoAb Kit (^{125}I) obtained from Diagnostic Products (U.K.) Limited.

Autoantibody tests

The patients and controls were tested for gastric parietal cell (PCA) and islet cell (ICA) antibodies by the indirect immunofluorescence (IFL) technique on sections of human blood group O tissues with undiluted serum [12].

Antinuclear (ANA), antimitochondrial (AMA) and smooth muscle (SMA) antibodies were measured by indirect IFL on rat liver and kidney sections with serum diluted 1:10 [13]. Thyroid microsomal (TMAb) and thyroglobulin (TgAB) antibodies were tested by passive haemagglutination with commercial kits (Wellcome Thymune Reagents). All sera were titrated to end point.

Investigation of HLA expression by thyroid cells

Thyroid tissue was obtained from patients

undergoing partial thyroidectomy because of non-autoimmune thyroid diseases or laryngeal carcinoma. The proportion of thyroid epithelial cells (thyrocytes) initially expressing HLA Class II at the time of isolation in the specimens employed was usually less than 5% (in one case it was 10–20%). Without stimulation such expression was lost over several days in culture.

The preparation, culturing and staining of the thyrocytes was performed essentially as described previously [10, 14]. Monolayers of the thyrocytes were cultured with the leucocyte-derived alpha-interferon and various other cytokines and hormones, as appropriate. Recombinant human gamma-interferon [specific activity about 3×10^7 U/mg protein, endotoxin contamination 0.125 ng/mg protein and recombinant tumour necrosis factor alpha (TNF-alpha) specific activity 5×10^7 U/mg protein, endotoxin contamination <0.125 ng/mg protein] were products of Genentech Inc. (California) and were kindly provided by Dr. G.R. Adolf (Boehringer-Ingelheim, Vienna). Bovine thyroid stimulating hormone (TSH, specific activity 0.4–0.5 U/mg) was obtained from Armour Pharmaceutical (Eastbourne, U.K.). After several days of culture the thyrocyte monolayers were stained by indirect immunofluorescence using monoclonal antibodies followed by fluoresceinated rabbit anti-mouse immunoglobulins (RaM-F, Dakopatts, Denmark). Surface expression of HLA Class II molecules was detected with monoclonal antibody (MoAb) MID-3 (from Dr. P. Lydyard) and HLA Class I molecules with MoAb W6/32 (from Drs. J. and W. Bodmer): both of these antibodies are directed against non-polymorphic HLA determinants. The anti-B2-microglobulin MoAb BBMI was also employed (obtained from Drs. J. and W. Bodmer). The stained cells were examined microscopically under ultraviolet and phase illumination (Zeiss photomicroscope III). Non-specific staining was checked for by using the MoAb against an irrelevant specificity (clone P11, anti-mouse thyroglobulin).

RESULTS

The number of cases with autoantibodies in their pre-treatment serum are shown in Table 1. Thyroid microsomal antibodies were detected in 4/15 (27%) patients in the control group (titre ranging between 10^2 and 40^2) and TgAb were also present in two of these at a titre of 1:20. The two cases (13%) with low titre ANA (1:10) were negative for thyroid antibodies. In the interferon group prior to treatment 2/10 (20%) had TMAb and only one had TgAb (titre 1:20). One case (10%) had AMA at a titre of 1:80 in the absence of thyroid antibodies.

In the serial samples taken during the course of interferon therapy, thyroid antibodies appeared in three other patients. Serial blood samples were not taken from the control group, but there is no prior reason for any change in the autoantibody status of these women. Clinically, none of the control group developed symptoms or signs of hypothyroidism, although this occurred in 3/10 (30%) of the interferon-treated group. Using Fisher's exact test the difference between the control and interferon-treated groups just failed to reach statistical significance ($P = 0.055$).

Table 1. Detection of autoantibodies in control and interferon treated patients

	TGAb	TMAb	PCA	ICA	ANA	AMA
Control (pre-treatment) $n = 15$	2	4	—	—	2	0
Interferon (pre-treatment) $n = 10$	1	2	2	—	0	1
Interferon (on-treatment) $n = 10$	5	5	2	—	1	1

TGAb = thyroglobulin antibodies; TMAb = thyroid microsomal antibodies; PCA = parietal cell antibodies; ICA = islet cell antibodies; ANA = anti-nuclear antibodies; AMA = anti-mitochondrial antibodies.

Sequential measurements of TMAb, and TSH on the five patients who showed an increase or induction of thyroid autoimmunity during interferon therapy, as shown in Fig. 1.

In the first patient (S.D.), TMAb, which were detectable prior to interferon, rose in titre during therapy. Thyroglobulin antibodies appeared after 2 months of treatment and progressively rose to a titre of 1:640 over a period of 6 months. There was a parallel rise in TSH and fall in free T_4 (FT_4). Clinical hypothyoidism was noted 6 months after starting therapy and responded to thyroxine replacement with a fall in TSH level, and amelioration of symptoms.

For case 2 (S.M.), low levels of TMAb were present before interferon treatment was started. There was no change for 1 month but a gradual increase in titre occurred reaching a maximum at 5 months. Thyroglobulin antibodies fluctuated in titre becoming negative during the subsequent months and reappearing at a titre of 1:80 at 8 months. This patient was never clinically hypothyroid and interferon therapy was discontinued at 6 months because of progressive metastatic disease. TSH levels fell but elevated titres of TMAb persisted.

Case 3 (L.L.) developed low titre TMAb by 4 months and at this time she became clinically hypothyroid. The serum FT_4 levels were found to

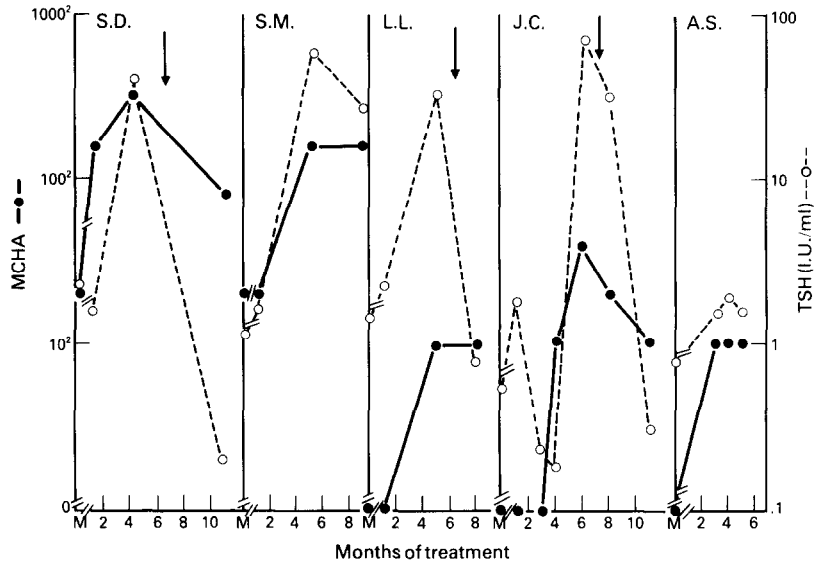

Fig. 1. Serial measurements of thyroid microsomal antibodies (MCHA), closed circles, and thyroid stimulating hormone (TSH), open circles.

be subnormal with elevation of TSH. She was given replacement thyroxine with resolution of symptoms and signs. At 10 months she lost both thyroid antibody specificities.

Case 4 (J.C.) developed TMAb and TGHA between 3 and 4 months of therapy. Hypothyroidism became manifest and thyroxine therapy was given. TSH was elevated and FT_4 was low. Thyroid antibodies fluctuated but remained positive after 10 months. At 5 months she developed ANA at a titre of 1:80 which slightly decreased throughout the rest of the follow-up period.

Case 5 (A.S.) developed both thyroid antibodies at 3 months. At this time interferon therapy was stopped because of progressive malignant disease. Although the autoantibodies persisted in her serum and TSH was slightly elevated the patient did not have signs of hypothyroidism. Mitochondrial antibodies which were present at a titre of 1:80 at mastectomy decreased during therapy.

Investigation of direct effects of leucocyte IFN-α preparations on thyrocyte HLA expression

The alpha-interferon used in the trial was added to thyrocyte cultures and 5–8 days later these were stained for expression of HLA Class I and Class II molecules. Three batches of alpha-interferon were used in the different experiments. Stimulation with alpha-interferon in the dose range 10^2 to 10^5 U/ml caused enhancement of the basal level of Class I expression seen in untreated thyrocytes, but did not cause significant induction of Class II expression. We have previously shown that thyrocyte Class II expression induced by gamma interferon is enhanced by stimulation with TSH [15] or TNF [16]. However, in preliminary studies the alpha-interferon did not synergise in the induction of thyrocyte Class II expression with a suboptimal dose of gamma interferon, nor with TSH or TNF at doses which we previously found to synergise with gamma-interferon.

DISCUSSION

This study has shown that patients who became hypothyroid on interferon therapy developed autoantibodies to thyroid tissue, both TGAb and TMAb. The appearance or a rise in titre of autoantibody ran parallel with an increase in serum TSH. The antibody persisted during interferon administration, but because of the poor prognosis of these patients, long-term follow-up blood tests have not been possible and it is not known whether the process is reversible.

A recent study, in which 49 patients received long-term alpha-interferon therapy, showed that seven patients developed hypothyroidism after the start of treatment [17].

It seems likely that the leucocyte interferon preparation used in these studies was responsible for the development or exacerbation of thyroid autoimmunity. Recent experiments have shown that epithelia that do not normally express class II HLA are found to be positive when they are the target of autoimmune attack. This positivity can be induced by gamma interferon [10, 11] or combinations of gamma interferon and tumour necrosis factor [16, 18]. Bottazzo *et al.* [11] suggested that the aberrant induction of Class II HLA expression by such lymphokines would allow presentation of autoantigen to the host and may therefore be central to the disease process. However, alpha-interferon derived from the Namalwa B cell line does not induce HLA Class II expression by thyrocytes although it does enhance expression of HLA Class

I [10]. Our present results with leucocyte-derived alpha-interferon are consistent with this: this preparation increased thyrocyte Class I expression but did not induce Class II in thyrocyte monolayers, nor was it found to synergise with gamma interferon, TSH or TNF to induce Class II. It thus appears unlikely that the thyroid disease observed in some of the patients resulted from direct induction of Class II expression in the thyroid by the alpha-interferon preparations, although these findings do not exclude some form of indirect effect on such expression. Our results are in contrast to those of Burman *et al.* who showed that their leucocyte interferon preparations could induce Class II MHC on thyroid cells *in vitro*.

The *in vitro* effects of alpha-interferon on thyrocyte Class I expression may be relevant as this could facilitate direct activation of cytotoxic T cells [19]. The destructive process might then lead to the generation of autoantibodies.

The possibility that contaminants of the leucocyte alpha-interferon preparations are responsible for the side-effects on the thyroid is supported by our recent finding that treatment of patients with highly purified Namalwa-derived alpha-interferon has not resulted in thyroid autoimmunity. In a preliminary study of 19 patients receiving long-term low dose 'Wellferon', no thyroid autoantibodies were found. Although gamma-interferon is a possible contaminant of leucocyte alpha-interferon which could induce thyrocyte Class II expression [15], our present findings do not indicate the presence of such an activity. This differs from the report of Burman *et al.* [17], whose results suggested their preparations had very low, but measurable, contamination with gamma interferon. However, according to their data, those patients would have received only 5–10 U/day of gamma-interferon systemically, which would seem unlikely to be sufficient to have a dramatic effect on the thyroid. In a study in which mice were injected with 5×10^4 U/day of gamma interferon for 6–9 days Class II expression was induced in the thyroid, but also in many other tissues [20].

Although there is no evidence, as yet, that patients receiving more purified preparation of alpha-interferons will develop thyroid autoimmunity, it is important that patients receiving long-term low dose alpha-interferon should be regularly monitored for thyroid function.

Acknowledgements—We are grateful to Dr. G.R. Adolf (Boehringer-Ingelheim) for providing recombinant human IFN-α and TNF produced by Genentech, and to Drs. P. Lydyard, J. Bodmer and W. Bodmer for monoclonal antibodies. Mrs. L.J. Hammond gave excellent technical assistance.

REFERENCES

1. Quesada Jr, Hersh EM, Gutterman JU. Hairy cell leukaemia. Induction of remission with alpha interferon. *Blood* 1983, **62**, 207a (Abstr.).
2. Quesada JR, Swanson DA, Trindade A *et al.* Renal cell carcinoma: anti-tumour effects of leucocyte interferon. *Cancer Res* 1983, **43**, 940–947.
3. Eriksson B, Oberg K, Alm G *et al.* Treatment of malignant endocrine pancreatic tumours with human leucocyte interferon. *Lancet* 1986, **2**, 1307–1308.
4. Krown SE, Stoopler MB, Cunningham-Rundles S *et al.* Phase II trial of human leukocyte interferon in small cell lung cancer. *Proc Am Assoc Cancer Res* 1980, **21**, 179.
5. Padmanabhan N, Balkwill FR, Bodmer JG, Rubens RD. Recombinant DNA human interferon alpha 2 in advanced breast cancer. *Br J Cancer* 1985, **51**, 55–60.
6. Fentiman IS, Balkwill FR, Cuzick J *et al.* A trial of human alpha interferon as an adjuvant agent in breast cancer after loco-regional recurrence. *Eur J Surg Oncol* 1987, **13**, 425–428.
7. Fentiman IS, Matthews PN, Davison OW *et al.* Survival following local skin recurrence after mastectomy. *Br J Surg* 1985, **92**, 14–16.
8. Fentiman IS, Lavelle MA, Kaplan D *et al.* The significance of supraclavicular fossa node recurrence after radical mastectomy. *Cancer* 1986, **57**, 908–910.
9. Fentiman IS, Thomas BS, Balkwill FR *et al.* Primary hypothyroidism associated with interferon therapy of breast cancer. *Lancet* 1985, **1**, 1166.
10. Todd I, Pujol-Borrel R, Hammond LJ *et al.* Interferon-gamma induces HLA-DR expression by thyroid epithelium. *Clin Exp Immunol* 1985, **61**, 265–273.
11. Bottazzo GF, Pujol-Borrell R, Hanafusa T, Feldmann M. Role of aberrant HLA-DR expressions and antigen presentation in induction of endocrine autoimmunity. *Lancet* 1983, **2**, 1115–1118.
12. Scherbaum WA, Mirhkian R, Pujo-Borrell R, Dean BM, Bottazzo GF. Immunocytochemistry in the study and diagnosis of organ-specific autoimmune disease in immunocytochemistry: modern methods and applications. In: Polak JM, Van Norden S, eds. Bristol, J. Wright. 1986, 456–476.
13. Scherbaum WA, Blaschek M, Berg PA, Doniach D, Bottazzo GF. Spectrum and profiles of non-organ-specific autoantibodies in autoimmune diseases in immunocytochemistry: modern methods and applications. In: Polak JM, Van Norden S, eds. Bristol, J. Wright. 1986, 477–491.
14. Pujol-Borrell R, Hanausa T, Chiovato L, Bottazzo GF. Lectin-induced expression of DR antigen on human cultured follicular thyroid cells. *Nature* 1983, **303**, 71–73.

15. Todd I, Pujol-Borrell R, Hammond LJ *et al.* Enhancement of thyrocyte HLA Class II expression by thyroid stimulating hormone. *Clin Exp Immunol* 1987, **69**, 524–531.
16. Todd I, Pujol-Borrell R, Belfiore A, Bottazzo GF. Thyrocyte HLA Class II expression and regulation in relation to thyroid autoimmunity. *Acta Endocrinol* 1987, **15** (Suppl. 281), 27–34.
17. Burman P, Totteman TH, Oberg K, Karlsson FA. Thyroid autoimmunity in patients on long-term therapy with leucocyte derived interferon. *J Clin Endocrinol Metab* 1986, **63**, 1086–1090.
18. Pujol-Borrell R, Todd I, Doshi M *et al.* HLA Class II induction in human islet cells by interferon-plus tumour necrosis factor or lymphotoxin. *Nature* 1987, **326**, 304–306.
19. Buller RML, Holmes KL, Hugin A *et al.* Induction of cytotoxic T cell responses *in vivo* in the absence of CD4 helper cells. *Nature* 1987, **328**, 77–79.
20. Skoskiewicz MJ, Colvin RB, Schneeberger EE, Russell PS. Widespread and selective induction of major histo-compatability complex-determined antigens *in vivo* by interferon. *J Exp Med* 1985, **162**, 1645–1664.