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Suppression of HLA class II expression on thyrocytes by interferon-alpha 1

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SUMMARY

Inappropriate expression of HLA class II molecules by human thyroid epithelial cells (thyrocytes) is commonly associated with autoimmune thyroid disease. HLA class II expression can be modulated in thyrocytes *in vitro* by a variety of substances: in particular, it is readily induced by interferon-gamma (IFN-γ). Here we show that recombinant IFN-α1 (rIFN-α1) does not induce HLA class II expression by thyrocytes, but rather it suppresses the induction of such expression by rIFN-γ. Similar effects were observed with IFN-α derived from a lymphoblastoid cell line. The effect of rIFN-α1 on thyrocytes differs from its effect on human monocytes, reported by others, in which it was found to enhance the expression of HLA class II. Thus, rIFN-α1 appears to have a differential effect on HLA class II expression, depending on the cell type involved.

Keywords thyroid autoimmunity thyroid epithelium HLA class II interferon-alpha 1 interferon-gamma

INTRODUCTION

Class II molecules of the major histocompatibility complex (MHC; HLA in humans) are normally expressed on various immunocompetent cells. Recently, it has been shown that thyroid epithelial cells (thyrocytes) of patients with autoimmune thyroid diseases express HLA class II molecules, whereas normal thyrocytes do not (Hanafusa *et al.*, 1983; Jansson, Karlsson & Forsum 1985; Aichinger, Fill & Wick, 1985). Inappropriate HLA class II expression has similarly been demonstrated on target epithelial cells in a variety of other autoimmune diseases (reviewed by Pujol-Borrell & Todd, 1987). It has also been shown that HLA class II$^+$ thyrocytes can act as antigen-presenting cells for both exogenous antigens (Londei *et al.*, 1984) and intrinsic autoantigens (Londei, Bottazzo & Feldmann, 1985; Weetman *et al.*, 1985; MacKenzie *et al.*, 1987) in an antigen specific, MHC class II restricted fashion. These findings support the hypothesis that such inappropriate MHC class II expression by epithelial cells may enable them to promote an autoimmune response against themselves by presenting their own constituents to autoreactive CD4$^+$helper T cells (Bottazzo *et al.*, 1983).

In vitro studies have shown that interferon (IFN) gamma (IFN-γ) is a potent inducer of HLA class II expression in human thyrocytes (Todd *et al.*, 1985; Weetman *et al.*, 1985) and this induction is enhanced by thyroid-stimulating hormone (TSH) (Todd *et al.*, 1987a,b) and tumour necrosis factor (TNF-α) (Buscema *et al.*, 1989). In contrast, epidermal growth factor (EGF) and transforming growth factor-α (TGF-α) suppress induction of thyrocyte class II expression by IFN-γ (Todd *et al.*, 1987a, 1990).

We reported previously that IFN-α derived from the Namalva cell line (containing a mixture of several IFN-α subspecies) did not induce HLA class II expression by thyrocytes, although it did enhance expression of HLA class I (Todd *et al.*, 1985). By contrast, Rhodes, Ivanyi & Cozens (1986) found that the IFN-α1 subspecies enhanced class II expression by human monocytes. We have therefore investigated the effects of recombinant IFN-α1 on class II expression by thyrocytes.

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MATERIALS AND METHODS

Antibodies and cytokines

HLA class II molecules were detected with the mouse monoclonal antibody (MoAb) MID-3, which reacts with a non-polymorphic determinant of HLA-DR. MoAb P11 (anti-mouse thyroglobulin) served as a control antibody of irrelevant specifity. Both MoAb are IgG1 isotype and were employed in immunofluorescence as supernatants derived from the hybridoma cultures (kindly provided by Dr P. Lydyard, University College and Middlesex School of Medicine, London).

Purified human IFN-α (Wellferon, Wellcome Biotechnology, Beckenham, UK) was derived from the Namalva lymphoblastoid cell line and contained more than ten subspecies: its range of specific activity was $81–213 \times 10^6$ U/mg protein. Human IFN-α1 was purified from a bacterial recombinant DNA source; it had a specifity of $3{\cdot}6 \times 10^7$ U/mg protein. Both the above preparations were kindly provided by the Wellcome

Foundation. Human IFN-γ was also purified from a bacterial recombinant DNA source and was kindly provided by Boehringer-Ingelheim (produced by Genentech): it had a specific activity > 10^7 U/mg of protein and endotoxin contamination was < 0·125 ng/mg protein.

Thyroid specimens and cultures

Two sources of thyroid cells were employed: thyroid tissue from patients who underwent laryngectomy for carcinoma of the larynx (HT-79, HT-168, HT-175) with low spontaneous expression of HLA class II by thyrocytes (less than 2%); and thyroid tissue from patients who underwent subtotal thyroidectomy for Graves' disease (HT-153, HT-173, HT-177 with high spontaneous class II expression by thyrocytes of 35%, 40%, and 35%, respectively). The thyrocytes were prepared and cultured as previously described (Pujol-Borrell *et al.*, 1983; Todd *et al.*, 1987b) with lymphoblastoid IFN-α, IFN-α1, IFN-γ and TSH being added to the cultures as appropriate, as a single dose following the initial washing of the cultures. Surface or cytoplasmic immunofluorescence staining for HLA-DR on the cultured thyrocyte monolayers was performed 6 days after the addition of the reagents as previously described (Pujol-Borrell *et al.*, 1983) with a test or control murine MoAb as the first layer followed by FITC-conjugated rabbit anti-mouse immunoglobulin (Dako, High Wycombe, UK). The proportion of thyrocytes, usually in duplicate cultures, expressing HLA class II was calculated by scoring at least 400 thyrocytes of each culture by phase and fluorescence microscopy using a Zeiss Photomicroscope III. Fluorescent and non-fluorescent cells were readily distinguished, and the degree of subjectivity in the scoring was minimal. We have previously assessed thyrocyte HLA class II expression by flow cytometry (in an EPICS-C), and found this to give results comparable with those obtained by differential counting under the fluorescence microscope (Todd *et al.*, 1987b). However, the technical difficulties associated with clumping of the highly adherent thyrocytes pose difficulties in applying flow cytometric analysis routinely. The mean variability of class II expression within the duplicates was 3·26% (±3·8). Prior to staining, the viability of the cultured thyrocytes was assessed.

RESULTS

Induction of HLA class II expression on thyrocytes

Thyrocytes from either normal or Graves' disease glands showed very little or no expression of HLA class II molecules (assessed by indirect immunofluorescence) following 7 days in culture (Fig. 1). The thyrocytes from individuals with Graves' disease largely lost their spontaneous class II expression during this culture period. However, as we have previously reported (Todd *et al.*, 1985), culturing the thyrocytes with recombinant IFN-γ (rIFN-γ) induced surface expression of HLA class II (Fig. 1). This was apparent on thyrocytes from both normal and Graves' disease glands. In contrast, no expression of class II was detected with either IFN-α or rIFN-α1 alone over a range of concentrations (10^2, 10^3 or 10^4 U/ml) (Fig. 1).

Suppression of rIFN-γ-induced HLA class II expression

Addition of rIFN-γ plus rIFN-α1 to thyrocytes cultures resulted in reduced class II expression compared with the effect of rIFN-γ alone (Figs 1–3). This inhibitory effect of rIFN-α1 increased

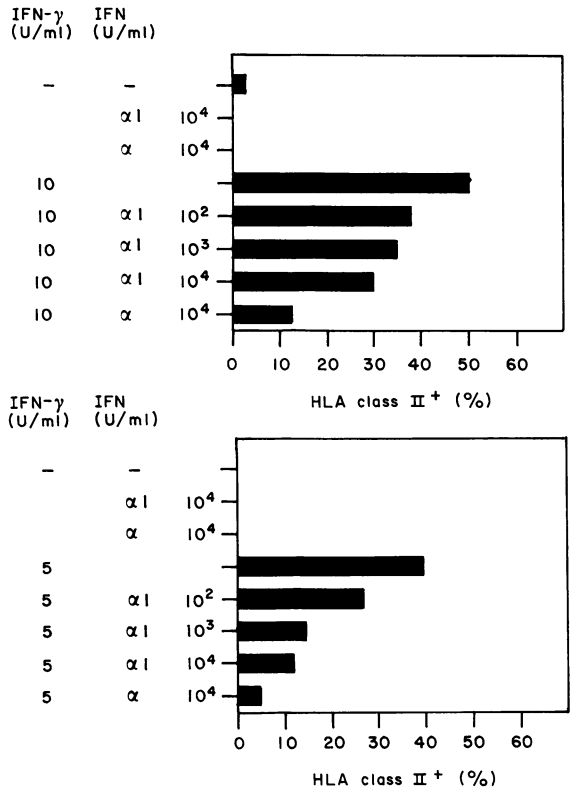

Fig. 1. Modulation of cell surface HLA class II expression in thyrocytes monolayers treated with IFN-γ, IFN-α and IFN-α1. Results are shown using thyrocytes from one patient with Graves' disease (HT 153, top) and one with carcinoma of the larynx (bottom) (HT 168, i.e. 'normal' thyrocytes).

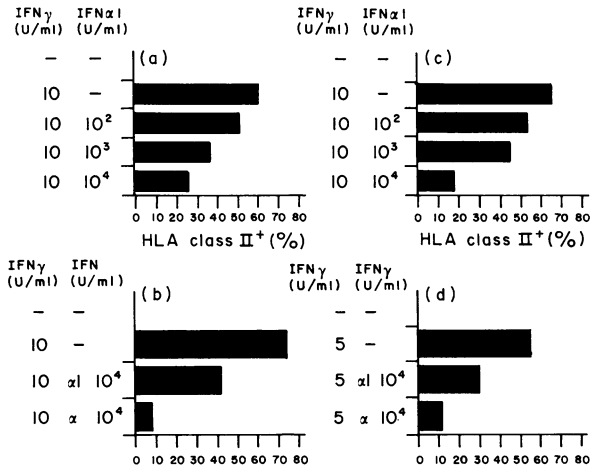

Fig. 2. Inhibition by IFN-α1 and lymphoblastoid IFN-α of IFN-γ-induced cell surface HLA class II expression on normal thyrocytes (HT 79, c; and HT 175, d) and thyrocytes from patients with Graves' disease (HT 173, a; and HT 177, b).

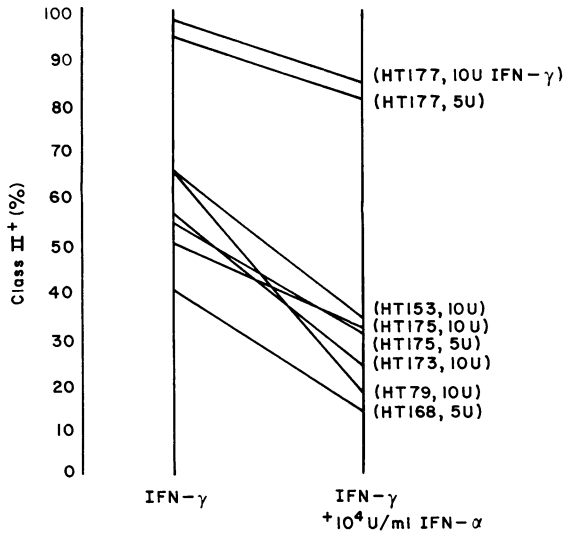

Fig. 3. Inhibition of IFN-γ-induced class II expression by 10^4 U/ml rIFN-α1 on normal and Graves' thyrocytes.

over a range of concentrations employed (10^2–10^4 U/ml); the highest level of inhibition observed in any of the experiments was 80–90% using 10^4 U rIFN-α1/ml. The ability of IFN-α1 (at 10^4 U/ml) to inhibit HLA class II induction was observed consistently on all thyroid specimens employed (Fig. 3).

Lymphoblastoid IFN-α was also found to suppress class II induction in thyrocytes by rIFN-γ. Indeed, the lymphoblastoid IFN-α appeared to be more inhibitory than rIFN-α1 when the same doses of these two preparations were employed in terms of U/ml (Fig. 2).

DISCUSSION

Investigations of both human (Londei *et al.*, 1985; Weetman *et al.*, 1986; MacKenzie *et al.*, 1987) and animal (Salamero & Charreire, 1983) systems support the hypothesis that thyrocytes expressing MHC class II molecules can present intrinsic autoantigens to autoreactive T cells, and in this way contribute to the pathogenesis of autoimmune thyroid disease (Bottazzo *et al.*, 1983). It is therefore important to understand the processes which can modulate such inappropriate class II expression. We have previously demonstrated that IFN-γ is a potent inducer of HLA class II in human thyrocytes (Todd *et al.*, 1985), and that TSH (Todd *et al.*, 1987a,b) and TNF-α (Buscema *et al.*, 1989) synergize with IFN-γ in this effect. Activation of leucocytes and/or destruction of thyrocytes in autoimmune thyroiditis could itself lead to increased levels of these modulators in the thyroid, and so exacerbate the pathogenesis (Pujol-Borrell & Todd, 1987). However, EGF and TGF-α suppress class II induction in thyrocytes by IFN-γ (Todd *et al.*, 1987a, 1990). We report here that rIFN-α1 as well as lymphoblastoid IFN-α also suppress the induction of thyrocyte HLA class II expression by IFN-γ. In this respect, lymphoblastoid IFN-α appears to be the more effective preparation; since this contains at least ten subspecies of IFN-α, it is possible that some of these may be more potent in this particular activity than is the IFN-α1 subspecies *per se*. The most clear-cut effects were obtained with relatively very high concentrations of IFN-α (10^3 or 10^4 U/ml). However, it is feasible that such concentrations may occur in pathological situations *in vivo*; for example, levels of type I IFNs up to 1000 U/ml have been detected in brain tissue of mice infected with neurotropic Semliki forest virus (Morris & Tomkins, 1989). Consistent with our own findings, Morris & Tomkins (1989) suggest that high levels of type I IFNs induced during viral infections may inhibit inappropriate induction of MHC class II by IFN-γ derived from T cells responding to the virus, thus possibly inhibiting the development of autoimmune responses.

There is a general agreement that type I IFNs (i.e. IFN-α and -β) enhance MHC class I expression by a variety of cell types, including thyrocytes (Todd *et al.*, 1985). However, contrasting results have been presented for the effects of type I IFNs on MHC class II expression. Several reports indicate that type I IFNs do not induce class II expression by, for example, monocytes and melanomas (Houghton *et al.*, 1984; Kelley, Fiers & Strom, 1984; Basham & Merigan, 1983) as well as epithelial cells like thyrocytes (Todd *et al.*, 1985; Weetman *et al.*, 1985) Furthermore, consistent with our studies reported here, it has been found that type I IFNs inhibit IFN-γ-mediated enhancement of class II expression in murine macrophages (Ling, Warren & Vogel, 1985; Inaba *et al.*, 1986), fibroblasts and glial cells (Morris & Tomkins, 1989), and in human endothelial cells (Lapierre, Fiers & Pober, 1988). By contrast, others have reported type I IFNs to stimulate class II expression by melanoma and lymphoblastoid cell lines (Dolei, Capobiancho & Ameglio, 1983; Giacomini *et al.*, 1984), but that this effect was slight, and certainly much less than that of IFN-γ. Of particular relevance in the present context are the findings of Rhodes *et al.* (1986) who showed that whereas rIFN-α2 has no effect on HLA class II by human monocytes, rIFN-α1 increases class II expression by these cells. However, Morris & Tomkins (1989) found that rIFN-α2 (unlike natural IFN-α, or IFN-β) weakly induced MHC class II expression by murine fibroblasts or glial cells. All these findings suggest that type I IFNs can have different modulatory effects on class II expression, ranging from stimulatory to inhibitory, depending on the experimental circumstances. Factors which might affect this could include the subspecies of IFN involved (e.g. IFN-α1 compared with IFN-α2), and the responding cell type (e.g. thyrocytes compared with monocytes), as well as tissue source and animal species. One possible explanation of these differences could be provided by the observations of Collins *et al.* (1986) who reported that type I IFNs induce the synthesis of a protein inhibitor of class II gene expression in human umbilical vein endothelial cells, and that when the synthesis of the inhibitor is blocked by cycloheximide, type I IFNs induce class II gene transcription. If the induction of such an inhibitor varies with the cell type and/or subspecies of type I IFN involved, this could help to explain the differential modulatory effects of type I IFNs on class II expression described above. This explanation could clearly indicate an intracellular mechanism for the inhibitory effect of IFN-α, rather than IFN-α and IFN-γ merely competing at the cell surface. In any case, the latter possibility would seem unlikely based on various lines of evidence that the cell surface receptors for IFN-γ and IFN-α are distinct (Petska *et al.*, 1987).

The observations that rIFN-α1 has the capacity to promote HLA class II expression by human monocytes (Rhodes *et al.*,

1986), but inhibits such expression in thyrocytes is reminiscent of findings with EGF. As mentioned above, EGF and TGF-α suppress IFN-γ-mediated class II induction in thyrocytes (Todd *et al.*, 1987a, 1990). In contrast, Acres, Lamb & Feldmann (1985) reported EGF to enhance class II expression by peripheral blood antigen-presenting cells. In this latter study, antigen presenting ability was simultaneously enhanced. However, this does not necessarily follow, as shown by the studies of Rhodes *et al.* (1986) where rIFN-α1 actually inhibited antigen presentation by monocytes although it enhanced their HLA class II expression.

In general terms, however, these various studies raise the interesting possibility that certain modulators act to inhibit class II expression by epithelial cells like thyrocytes where this could have pathological consequences without causing generalized immunosuppression by simultaneously suppressing class II expression by 'conventional' antigen-presenting cells. There have been several reports of thyroid autoimmunity following treatment for cancer with leucocyte-derived IFN-α (Burman *et al.*, 1986; Fentiman *et al.*, 1988; Gisslinger, Gilly & Weissel, 1989). Such preparations may possibly contain low levels of other cytokines, including IFN-γ. However, it would seem unlikely that in these cancer patients the induction of thyroid disease is facilitated by any contaminating IFN-γ inducing thyrocyte HLA class II expression, in view of the inhibition of such an effect by the IFN-α which would be expected from the studies reported here. This is consistent with the more recent observation that even cancer patients treated with IFN-α from a recombinant DNA source have a tendency to develop autoimmune thyroid dysfunction (presented at the workshop 'Autoimmune Thyroiditis—Approaches Towards its Aetiological Differentiation' Homburg/Saar, FRG, 11 October 1989, by F.A. Karlssen *et al.*). At present, however, the mechanism of such *in vivo* effects of IFN-α are not understood, and it is clear from other studies that care must be taken when comparing the *in vivo* and *in vitro* effects of cytokines. For example, TNF-α has been reported to stimulate angiogenesis *in vivo* (Leibovich *et al.*, 1987) whereas our group has found TNF-α to depress endothelial cell proliferation *in vitro* (T. Mauerhoff *et al.*, submitted for publication).

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