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Interferon Gamma Regulates HLA-D Expression on Solid Tumors *in vivo*

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Abstract—In vivo *administration of recombinant human interferon-γ, rIFN-γ, to nude mice bearing human tumor xenografts resulted in a strong induction of HLA-DR expression on the tumor cells in 2 of 3 lines tested. The induction was dose-dependent and declined rapidly after cessation of therapy. IFN-γ also switched on DQ and DP products when the xenograft cells were treated* in vitro. *The* in vivo *alteration of tumor surface properties by rIFN-γ may have therapeutic implications.*

INTRODUCTION

HLA-D ANTIGENS are key elements in the control of immune responses functioning in the recognition of antigens by T-lymphocytes [1], and their expression is restricted to cells of the mononuclear phagocyte system, activated T-lymphocytes, B-lymphocytes and a subset of other tissues [2–4]. An important and much-studied property of interferon-gamma, (IFN-γ), is its ability to induce, or enhance, HLA-D expression on a variety of normal and malignant cells *in vitro* [5]. Alteration of HLA-D levels results in altered biological behaviour; for instance IFN-γ induced surface expression of HLA-D antigens on thyroid epithelial cells and allowed these cells to present antigen to cloned human T-cells [6].

Some tumor cells have also been shown to express *de novo* HLA-D *in vivo* [7], although it is not known whether this is due to the local presence of IFN-γ or an inherent part of the malignant or metastatic change. However, it is possible that if HLA-D antigens could be induced or enhanced on tumor cells *in vivo*, this might promote cellular or humoral host immune response to the tumor. In this paper we show that systemically administered IFN-γ can induce or enhance HLA-DR antigen expression *in vivo* on 2/3 human tumors growing as xenografts in nude mice. We also show that when cells from these tumors are cultured *in vitro* with IFN-γ, HLA DP and DQ antigens are induced.

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MATERIALS AND METHODS

Mice

Female specific pathogen free outbred nu/nu mice of mixed genetic background were bred and maintained as previously described [8].

Tumors

All tumor lines were derived from primary untreated human carcinomas implanted into the mice no more than 4 hr after removal from the patient and maintained by serial passage in nude mice. Tumor 1068, derived from a mucoid carcinoma of the breast was used between passages 39 and 41. Tumors GFC and GFW, derived from adenocarcinomas of the caecum, were used at passages 6–7, and 10 respectively. For experiments, tumors were implanted and grown for 2 weeks until approx. 0.5 cm in size [8].

Interferons

Recombinant human IFN-γ (Immuneron), kindly supplied by Biogen S.A., Geneva, Switzerland, had a specific activity of 2×10^7 U/mg. Lymphoblastoid IFN (Wellferon) (IFN-α Ly), kindly supplied by Wellcome Research Laboratories, Kent, U.K., had a specific activity of 2×10^8 U/mg. The IFNs were titred in our laboratory against the relevant reference standards as described in [8] and stored at −70° C in single dose aliquots diluted in PBS with 3 mg/ml bovine serum albumin (Sigma Chemical Co, U.K.), (PBS,BSA).

IFNs were administered intraperitoneally (i.p.) in 0.2 ml daily for 4 days.

HLA-D region monoclonal antibodies

Class I antibody W6/32 [9] was an HLA-A,B,C, monomorphic antibody. 1B5 was directed against the DR alpha chain and can be used to stain paraffin fixed sections [10, 11]. The antibody DA2 [12] was directed at the DR beta chain. B7/21 [13] identified the HLA/DP product [14]. TU 22 reacted with a monomorphic determinant of DQ [15].

Immunoperoxidase techniques

Tumors were removed from mice and immediately fixed in Methacarne (methanol : chloroform : acetic acid 60 : 30 : 10). Paraffin embedded sections were stained using the immunoperoxidase method as described in Reference [16], with peroxidase-conjugated rabbit anti-mouse immunoglobulin (DAKO, Dnemark) at 1/100 dilution. At least 400 cells were counted in each of 5 different areas of the section. No mouse stromal areas were scored.

Dissagregation and culture of 1068 *tumors*

Four-to-five-week-old 1068 tumors were excised and minced finely in pancreatin (Gibco Biocult, Paisley, U.K.) : versene 1 : 4 and stirred at room temperature in this solution for 75 min. The cells were filtered through a 75 μm nylon mesh and cultured in RPMI 1640 medium (Gibco Biocult, Paisley, U.K.) + 10% Foetal Bovine Serum FBS (Sera Laboratories, Sussex, U.K.).

Immunofluorescence

Trypsinized 1068 cells were washed and suspended in the appropriate antibody solution for 1 hr at room temperature. Cells were washed 3 times in PBS + BSA 1% + azide 0.2% and then incubated for 30 min at 4° C in FITC Rabbit anti mouse IgG (Cappel Laboratories, Cochranville PA 19330, U.S.A.) diluted 1 + 4 in PBS/BSA/Azide. After 3 washes cells were analysed by flow cytometry using a FACS II.

RESULTS

rIFN-γ at a dose of 2×10^5 U/day was given i.p. to mice bearing small (approx. 0.5 cm) subcutaneous tumors. After 4 days therapy tumors were removed and fixed. The levels of HLA-D antigen expression in paraffin embedded sections of tumor were studied by immunoperoxidase techniques using an anti-HLA-DR monoclonal that reacts on fixed sections, 1B5. As shown in Table 1, rIFN-γ increased expression of HLA-DR on the 1068 breast cancer xenograft from 1% to a mean of 81% cells positive, and on the GFC bowel cancer xenograft from 19% to a mean of 69% cells positive. Immunoperoxidase sections of control and IFN-γ treated GFC tumors are shown in Fig. 1. The effects seen were dose-related. Mice bearing 1068 tumors and treated with 5×10^4 U or 2×10^4 U of rIFN-γ showed 25% and 2% HLA-DR positivity of their tumor cells respectively. Mouse stromal cells were uniformly negative (see Fig. 1) and areas of stromal cells were not counted. No effect was seen on HLA-DR expression if the same dose of IFN-α was given (data not shown). IFN-γ therapy failed to induce HLA–D in a third xenograft, GFW. The induction of HLA-DR was rapidly switched off in the absence of rIFN-γ as shown in Fig. 2. In this experiment using the 1068 xenograft a mean of 73% cells was HLA-DR positive after 4 days therapy, but this declined to 22% 2 days post-treatment and 4% 4 days post-treatment.

Table 1. *The effect of rIFN-γ therapy on tumor HLA-DR expression* in vivo

	% tumor cells positive with 1B5 monoclonal antibody	
Tumor	GFC	1068
Control	24*,19,22,13	1,1,0,0
IFN-γ treated	76,82,67,50	79,87,90,70

Mice with approx. 0.5 cm tumors growing s.c. were treated daily for 4 days with 2×10^5 U rIFN-γ given i.p.
*Cells from 2 different tumors in 2 different experiments were scored as described above.

We further investigated the effect of IFN-γ on different HLA-D region products on live cells of 1068 which were dissagregated and incubated in short-term culture with rIFN-γ or IFN-α(Ly) (1×10^3 U/ml) and analysed for antigen expression using the FACS.

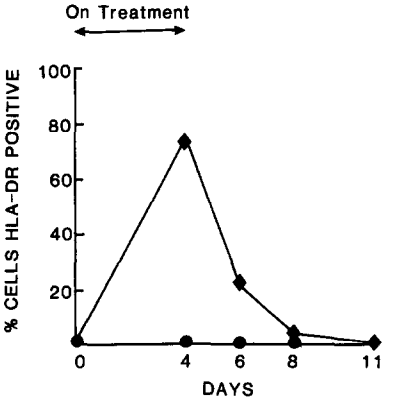

Fig. 2. *The levels of HLA-DR expression in* 1068 *breast cancer xenograft in the presence and absence of IFN-γ therapy.*
–●– *control mice.*
–◆– *mice treated with* 2×10^5 *U rIFN-γ i.p. daily for* 4 *days. Each value represents a mean of values obtained from sections of* 3 *different tumors. Approx.* 400 *cells counted per section in* 5 *different areas.*

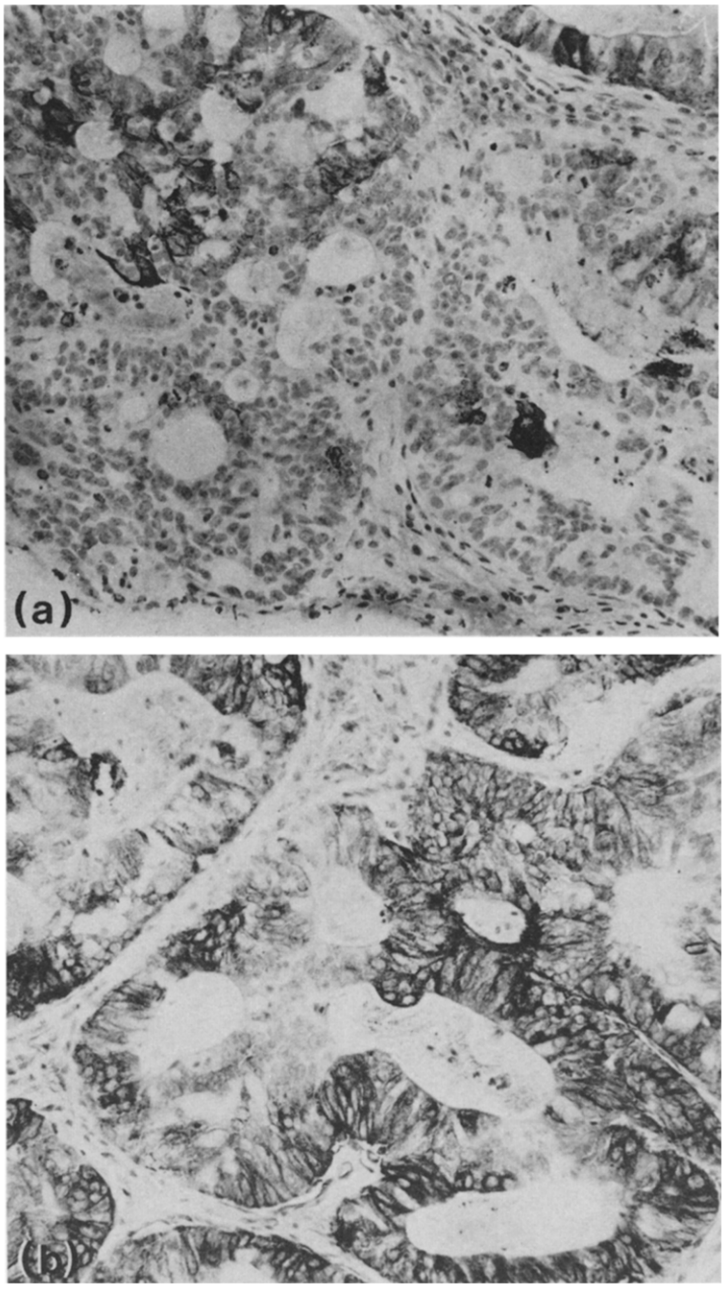

Fig. 1. Immunoperoxidase staining GFC bowel cancer xenografts using 1B5 anti HLA DR monoclonal antibody. A control B mice treated with 2×10^5 *U rIFN-γ i.p. daily for 4 days.*

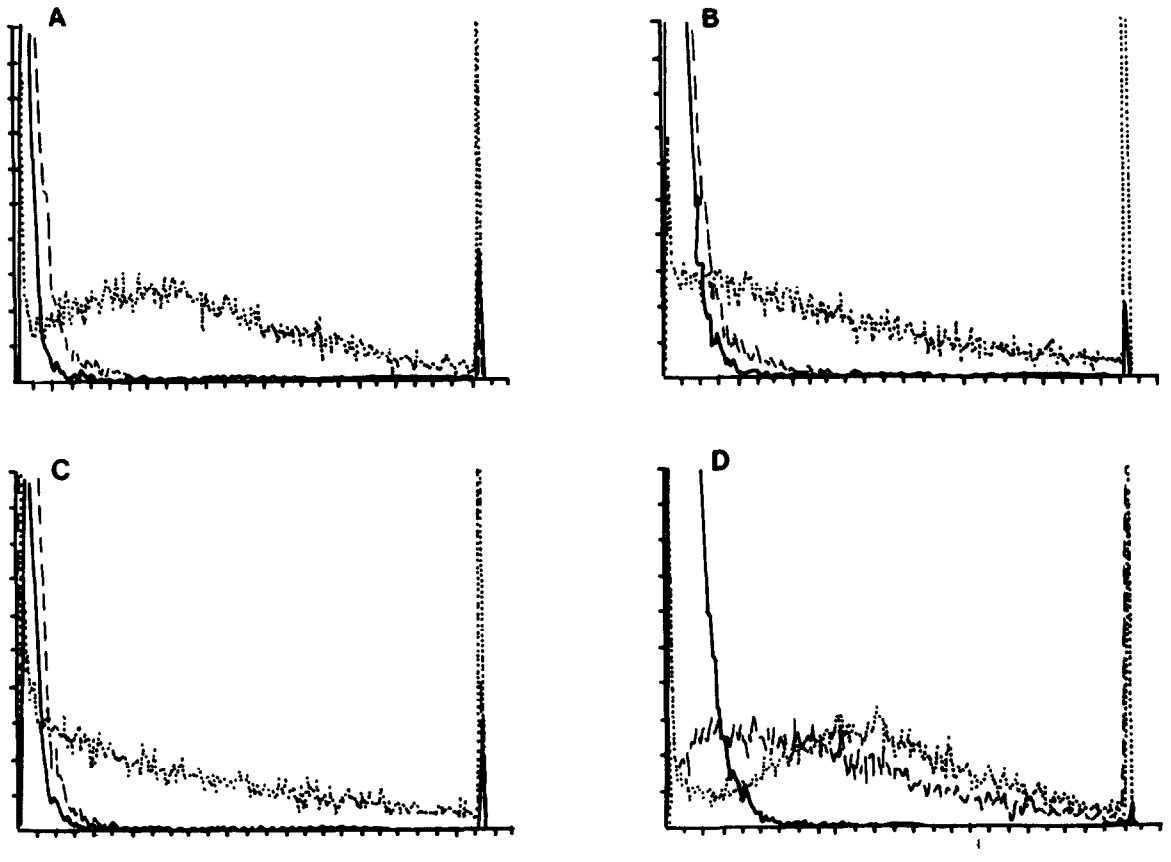

Fig. 3. FACS analysis of 1068 *cultured cells stained with a range of antibodies to HLA region products.*
———— = *control untreated cells.*
—— = 10^3 *U/ml IFN-α(Ly) for 4 days.*
. . . . = 10^3 *U/ml IFN-γ for 4 days.*
A = *cells stained with B21.1 HLA DP monomorphic antibodies.*
B = *cells stained with DA2 HLA DR monomorphic antibodies.*
C = *cells stained with TU22 HLA DQ monomorphic antibodies.*
*D = *cells stained with W6/32 HLA Class I monomorphic antibodies.*
**In D, due to the brightness of staining in all groups the voltage setting on the Fluorescence photomultiplier tube was lowered.*

As can be seen in Fig. 3 our *in vivo* findings were confirmed. 1068 cells were essentially negative for all HLA-D region products on the cell surface, but after IFN-γ treatment strong induction of DR antigens (86% cells positive) was seen. DP region products (84% cells positive) and DQ (74% cells positive) were also induced. IFN-α had no effect on any D region antigens. Greater than 95% cells expressed Class 1 HLA A,B,C, and this number was not affected by treatment with either IFN. However, both IFNs significantly increased the intensity of staining with antibody against Class I HLA A,B,C, with IFN-γ having the stronger effect (Fig. 3D). The 50% window (the channel No: at which 50% of the cells showed positivity) changed from window 16 in control cells to 73 for IFN-α(Ly) treated cells and 113 for rIFN-γ treated cells.

DISCUSSION

In this paper we show that induction of HLA-D can occur *in vivo* when rIFN-γ is given systematically to animals bearing tumor xenografts, even though this IFN is rapidly cleared from the circulation. (In a previous paper [8] we reported that 2×10^5 U rIFN-γ given i.p. to mice resulted in a peak serum level of greater than 10^4 U/ml which declined rapidly with a half life of approx. 8 hr). The implication of this finding is that tumor therapy with rIFN-γ may promote host humoral and cellular reactions to the tumor by allowing presentation of tumor-associated antigens in association with HLA-D.

Therapy with human IFN-γ in man could also result in switch on of HLA-DR in normal tissues. Recently, Skoskiewicz *et al.* reported that systemic administration of murine IFN-γ to mice caused strong and selective increases in H-2K and 1A antigens in many organs [17]. Botazzo *et al.* [18] have suggested that inappropriate induction of HLA-D may promote or initiate autoimmunity. However, the induction of HLA-DR was only transient in our model system and declined rapidly after the end of therapy, whereas a long-term local

production of IFN-γ associated with chronic infection may be necessary for an autoimmune response.

In spite of the membrane changes seen, the growth of all three xenografts described in this paper was unaffected by daily therapy with rIFN-γ [8, and Balkwill, unpublished results). However, it is probable that growth inhibitory effects of IFN-γ and its modulation of HLA-D antigens can be dissociated. Although, because it has different pharmacokinetics from IFN-α, it is possible that insufficient IFN-γ is reaching the tumor to inhibit cell growth.

One of the 3 xenograft cell lines expressed HLA-D in the absence of rIFN-γ therapy. Tumor cell expression of HLA-D could be an inherent part of the malignant or metastatic change, or due to local presence of IFN-γ, but in this case the human tumor cells were growing in a nude mouse with no possibility of exposure to IFN-γ.

While there is experimental evidence for both host-mediated and direct antitumor effects of IFNs there is very little evidence concerning tumor surface modulatory effects of IFNs *in vivo*. This, to our knowledge, is the first report of *in vivo* tumor surface antigenic modulation by systemic IFN therapy, and such alterations of tumor surface properties may have important therapeutic implications.

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