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# Expression of high-affinity IL-4 receptors on human melanoma, ovarian and breast carcinoma cells

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## SUMMARY

It has previously been shown that murine sarcoma cells express high-affinity IL-4 receptors (IL-4R) which are internalized after binding to the ligand (Puri *et al.*, Cancer Res 1991; **51**:3011–7). We have also reported that human renal cell carcinoma cells express high-affinity IL-4R, and IL-4 inhibits tumour growth *in vitro* (Obiri *et al.*, J Clin Invest 1993; **91**:88). In this study we investigated the expression and function of IL-4R on other human solid tumours. Human melanoma, ovarian carcinoma and breast carcinoma cell lines were assessed for the cell surface expression of IL-4R by radio-ligand receptor binding and for IL-4R gene expression by Northern blot analysis. Primary cultures of mesothelioma and neurofibrosarcoma cells were similarly investigated. Human melanoma, ovarian carcinoma and breast carcinoma cell lines expressed IL-4R on their cell surface with a dissociation constant ($K_d$) of 140–549 pM. These tumour lines expressed a single 4 kb species of mRNA for IL-4R. Similarly, primary cultures of mesothelioma and neurofibrosarcoma cells were positive for the IL-4R mRNA by Northern blot analysis. Fresh, non-cultured mesothelioma and neurofibrosarcoma tumour sections were also positive for the presence of IL-4R as determined by immunohistochemistry of frozen sections using anti-IL-4R antibody. In order to study possible functions of IL-4R, we evaluated the effects of IL-4 on cell growth and its effect on MHC antigen expression in the presence or absence of interferon-gamma (IFN-$\gamma$). In tissue culture, IL-4 reduced the growth of tumour cell lines and primary cell cultures studied. IL-4 had very little effect on MHC class I antigen expression on ovarian, breast and melanoma cell lines; however, MHC class II (HLA-DR) expression was enhanced on melanoma and breast carcinoma cells. IL-4 also enhanced the IFN-$\gamma$-induced class II expression on melanoma and breast carcinoma cells. Taken together, our observations indicate that IL-4R are expressed on a variety of human solid tumours and these receptors may be functional. IL-4 alone and in combination with IFN-$\gamma$ may play a role in host immune response against cancers.

**Keywords** IL-4 receptors solid human tumours tumour growth MHC regulation

## INTRODUCTION

IL-4 is a pleiotropic cytokine primarily produced by T helper cells [1–4], and mast cells [5]. IL-4 can exert numerous effects on various haematopoietic cell types. On B cells, IL-4 can promote immunological class switching to IgE and IgG1 isotypes and up-regulate MHC class II [6–8] and CD23 expression [9]. It can promote survival, growth and differentiation of both B and T lymphocytes [10–13], mast cells [14,15] and endothelial cells [16]. On macrophages, it can inhibit the production of tumour necrosis factor (TNF), IL-1 and IL-6 [17,18]. It is believed that IL-4 exerts its effects by interacting with cell surface receptors [19–21]. IL-4R have been shown to be present on haematopoietic and some non-haematopoietic cells [22]. On both human and murine T and B cells, they are present in low numbers, which are reported to be up-regulated by IL-2 and IL-4 [23–25]. Recently, Puri *et al.* [26] showed that murine solid tumours express high-affinity IL-4R which appear to be internalized after binding to ligand. Others, using MR6, a MoAb that recognizes an uncharacterized IL-4R-associated protein, have suggested that human lung carcinoma may also express IL-4R [27]. In this study, we present data from equilibrium binding studies and Northern analysis indicating a widespread distribution of IL-4R on human solid tumours. The direct effects of IL-4 on tumour cell growth and HLA class I and class II expression are also evaluated.

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## MATERIALS AND METHODS

*Tumour cell lines*

The ovarian tumour cell lines IGROV-1, SKOV-3, OVCAR 4, OVCA 420 and OVCA 429; melanoma cell lines A375 and

RPMI 7951; normal breast (Hs578Bst) and tumour cell lines HSc-57.8T, MCF-7, BT-20 and ZR-75-1; and human umbilical vein endothelial cells (HUVEC) were obtained from American Type Cell Culture Collection (ATCC; Rockville, MD). The melanoma cell line UMS 297 was a kind gift from Dr B. Fox (University of Michigan, Ann Arbor, MI). The Ramos.G6.C10 was derived from the Ramos Burkitt lymphoma cell line as previously described [28]. These cells were maintained in RPMI 1640 containing 10% heat-inactivated fetal calf serum (FCS) supplemented with glutamine (2 mM), sodium pyruvate (1 mM), essential amino acids (1 mM) and gentamicin (1 mM).

Fresh tumour specimens were obtained from the Surgery Branch, National Cancer Institute. Tumour pieces were digested with triple enzymes (0·002% deoxyribonuclease, type 1; 0·01% hyaluronidase, type V, and 0·1% collagenase, type IV (Sigma Chemical Co., St Louis, MO)) and used for total RNA extraction or used to establish primary cultures. These cells were maintained in Eagle's modified minimum essential medium with 4·5 g/*l* glucose supplemented with glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 μg/ml).

*Cytokines and reagents*

Recombinant human IL-4 (specific activity, $10^7$ U/mg protein), rat anti-human IL-4R MoAb and an 836 base pair cDNA probe for the human IL-4 receptor were a kind gift from Immunex Corporation (Seattle, WA). Recombinant human interferon-gamma (IFN-γ) (specific activity $2–4 \times 10^7$ U/mg) was a kind gift from Genentech, Inc. (San Francisco, CA).

Radiolabelled iodine ($^{125}$I) and $^{125}$I-labelled sheep anti-mouse F(ab′)$_2$ antibody were obtained from Amersham Research Products (Arlington Heights, IL). $^{32}$P-deoxycytidine was obtained from ICN Immunobiologicals (Costa Mesa, CA). The MoAb against human HLA-DR and an FITC-conjugated goat anti-mouse IgG antibody were obtained from Becton Dickinson (San Jose, CA). Human anti-HLA class 1 antibody, W6/32, was a kind gift from Dr T. Gerrard (Division of Cytokine Biology, FDA, Bethesda, MD).

*Radioreceptor binding assay*

Recombinant human IL-4 was enzymatically labelled with $^{125}$I by the enzymobead method according to manufacturer's instructions (Bio-Rad, Richmond, CA). The specific activity of the radiolabelled IL-4 was determined by the competition of binding sites by unlabelled IL-4 on Ramos.G6.C10 or MLA 144 cells. The specific activity was estimated to range from 1·4 to $4·7 \times 10^{10}$ ct/min per mg protein in numerous labellings.

For IL-4R assays, equilibrium binding studies were performed by the method previously described [25,26]. Briefly, $2·5 \times 10^6$ cells in 126 μl binding buffer (RPMI 1640 plus 0·2% human serum albumin) were incubated with various concentrations of $^{125}$I-IL-4 at 4°C in polypropylene tubes in a shaking water bath. For each concentration of $^{125}$I-IL-4, non-specific binding was determined by including 100–200 molar excess of unlabelled IL-4 in duplicate tubes. Cell-bound $^{125}$I-IL-4 was separated by centrifugation through a cushion of phthalate oils, which was prepared by mixing 48% Bis (2-ethylhexyl) phthalate obtained from Fisher Scientific (Fair Lawn, NJ) and 52% *n*-butyl phthalate obtained from Eastman Kodak (Rochester, NY) and counted in a gamma counter. The number of IL-4 molecules bound/cell and binding affinities were determined from Scatchard plot analysis [29] of the binding data.

*Northern blot analysis*

Adherent tumour cells were harvested by brief exposure to 0·25% trypsin (Sigma). In some instances adherent cells were dislodged with Versene (Whittaker Bioproducts, Walkersville, MD). After several washes, total RNA was extracted with RNAzol (Cinna/Biotecx Laboratories, Friendswood, TX) according to the manufacturer's instructions. Ten micrograms of total RNA were electrophoresed through 1% agarose/formaldehyde denaturing gel and transferred to a nylon membrane (S and S Nytran; Schleicher and Schuell, Keene, NH) by capillary action [30]. The nucleic acid was bound to the membrane by UV crosslinking (Stratagene, La Jolla, CA) and allowed to hybridize overnight with a $^{32}$P-labelled human IL-4R cDNA following a 12-h prehybridization. Filters were exposed to autoradiographic film for 3–15 days.

*Radiometric assays for HLA antigen expression*

The binding of antibodies to tumour cell surface was examined using a sandwich-type antibody-binding radiometric assay [31]. Briefly, tumour cells ($1 \times 10^6$ cells) were incubated at 37°C with IL-4 (20 ng/ml), IFN-γ (5 ng/ml) or both in a total volume of 10 ml in 6-well tissue culture plates (Costar). After the appropriate incubation period, cells were washed three times in Hanks' balanced salt solution (HBSS) and $2 \times 10^5$ cells incubated at 4°C in duplicate or triplicate wells of a U-bottomed 96-well microtitre plate for 1 h with one of the following primary antibodies: normal mouse IgG2a; anti-HLA class I (W6/32) or anti-HLA-DR. Cells were washed twice in binding buffer (PBS, 3% FCS and 0·01% sodium azide) and incubated with $^{125}$I-labelled sheep anti-mouse F(ab′)$_2$ fragment as the second antibody. After a 1-h incubation and three washings with binding buffer, cell bound radioactivity was counted with a gamma counter. Control samples from each treatment group were treated with binding buffer or second antibody alone.

*Assay for tumour cell growth*

The effect of IL-4 on tumour cell growth was evaluated by quantifying the reduction of 3(4,5-dimethyl-thiazoyl-2-yl)2,5 diphenyltetrazolium bromide (MTT) as previously described [32,33] with slight modifications. Briefly, tumour cells ($5 \times 10^4$–$4 \times 10^5$/ml) in 100 μl culture medium were plated in a 96-well microtitre plate and incubated at 37°C in a 5% $CO_2$ incubator overnight. One hundred microlitres of IL-4 (3–100 ng/ml, final concentration) were then added to triplicate wells. Some of the cells received culture medium alone and were used as untreated controls. After an appropriate incubation period (72–96 h), 100 μl culture medium were removed from each well and 10 μl of a 5·0 mg/ml solution of MTT in PBS were added to each well. The cultures were incubated for an additional 4–5 h and 150 μl of acidified isopropyl alcohol (0·04 N HCl in isopropanol) were added to each well. The formazan produced was solubilized and optical density readings at 570 nm were taken with an MR600 microplate spectrophotometer (Dynatech). The equipment was standardized with the wells containing MTT and medium only. Cell viability in treatment cultures was expressed as a percentage of the viability observed in control cultures. Student's *t*-test was used to analyse the results for statistically significant differences.

In some experiments, cell proliferation was assessed by the incorporation of $^3$H-thymidine. Normal breast (Hs578Bst) or HUVEC were cultured with various concentrations of IL-4 for

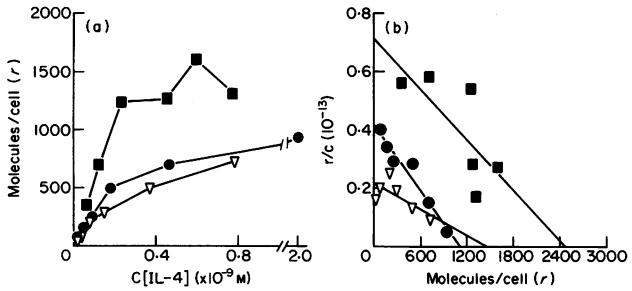


**Fig. 1.** Binding of $^{125}$I-IL-4 to human tumour cells. Human ovarian, melanoma and breast tumour cells were incubated with 50 pM to 2 nM $^{125}$I-IL-4 for 2 h at 4°C. In duplicate tubes, 100–200-fold molar excess unlabelled IL-4 was included to determine non-specific binding. The number of specifically bound $^{125}$I-IL-4/cell was determined and used to plot the binding curve shown in a, while the Scatchard plot is shown in b. ●, IGROV-1; ▽, RPMI 7951; ■, ZR-75-1.

2–3 days and cells were then pulsed with 1·0 $\mu$Ci of $^{3}$H-thymidine for an additional 18 h.

*Immunohistochemistry and immunocytochemistry*

For immunohistochemistry of fresh, non-cultured tumours, tissue sections were snap frozen in tissue-tek OCT compound (Miles Inc., Elkhart, IN) immediately after surgical resection of the tumours. Frozen sections were prepared and slides kept frozen at −70°C until staining with anti-IL-4R antibody was performed. For immunocytochemistry, cytospin slides were prepared from cells in culture. Immunohistochemistry was performed by the peroxidase-antiperoxidase technique according to the manufacturer's instructions (Zymed Laboratories, South San Francisco, CA). Anti-IL-4R antibody (M-57) which is a neutralizing MoAb was generated in a rat by immunizing it against human IL-4R, and was obtained from Immunex. Tissue sections or cytospin preparations were incubated with anti-IL-4R antibody at 4°C overnight at a concentration of 8·5 $\mu$g/ml. Normal rat IgG served as the negative control.

## RESULTS

*Cell surface expression of IL-4R*

To investigate the expression and binding affinity of IL-4R, tumour cells were incubated at 4°C with increasing concentrations of $^{125}$I-IL-4 for 2 h. As seen in Fig. 1a, ovarian (IGROV-1), melanoma (RPMI 7951) and breast (ZR-75-1) tumour cells bound IL-4 in a concentration-dependent manner. Scatchard plot analysis (Fig. 1b) of the binding data indicated that a single class of high affinity (ovarian, $K_d = 331 \pm 64$ pM; breast, $K_d = 294 \pm 54$ pM; melanoma, $K_d = 549 \pm 133$ pM) receptors was expressed on these cells. In multiple experiments, the number of IL-4 molecules bound/cell on the ovarian tumour cells, IGROV-1 ($n = 3$) was $1365 \pm 115$ (mean ± s.e.m.); on the breast tumour cells, ZR-75-1 ($n = 2$) $2307 \pm 132$, and on the melanoma cells, RPMI 7951 ($n = 2$) $1442 \pm 12$. The binding data for additional tumour lines are shown in Table 1. In some cases IL-4R expression was examined by utilizing a single concentration of radiolabelled IL-4. For positive control and comparative purposes, Ramos.G6.C10 and MLA 144 cells which have been shown to express high-affinity IL-4R [19] were also evaluated. The receptor numbers varied from 271 to 1365 sites/cell on ovarian carcinoma lines, 1237 to 1442 sites/cell on melanoma cells and 724 to 2289 sites/cell on breast carcinoma lines. The $K_d$ values of IL-4R on breast and ovarian carcinoma (217–331 pM) appeared to be similar to that on MLA 144 cells (264 pM), while on melanoma cells (364–549 pM) it appeared to be slightly higher.

*IL-4R gene expression*

As seen in Fig. 2a, a single species (size 4·0 kb) mRNA for IL-4R was observed in all tumour cell lines examined (lanes 3–10). RNA from CTLL-2 cells, a mouse cytotoxic T cell line, was included as a negative control and RNA from CTLL-2 cells transfected with the human IL-4R cDNA (CTLL-T22-8) [19] was used as a positive control. RNA from CTLL-2 cells did not hybridize with the hIL-4R cDNA probe (lane 1), while RNA from the transfected cell line did (lane 2).

In Fig. 2b, RNA samples isolated from fresh tumour cultures after two or three passages were examined for expression of the human IL-4R message. Fresh tumour cultures expressed IL-4R mRNA of similar size to that in cultured cell lines (lanes 2, 3).

*Immunohistochemical expression of IL-4R*

To rule out that the expression of IL-4R on cultured solid tumour cells is a result of culture artefact, we performed immunohistochemistry on fresh, non-cultured solid tumour tissues from malignant mesothelioma and neurofibrosarcoma samples obtained from patients undergoing surgical resection. By utilizing a neutralizing MoAb to human IL-4R, we found that mesothelioma and neurofibrosarcoma tumour samples stained intensely with anti-IL-4R antibody. As seen in Fig. 3a, islands of malignant mesothelial tumour cells were stained intensely with anti-IL-4R antibody. No staining was observed by using IgG control antibody or with no primary antibody (not shown). Similarly, neurofibrosarcoma tissue sections also stained very well with anti-IL-4R antibody (Fig. 3b). A solid sheet of small tumour cells was stained intensely. This tumour section showed very little stroma, and the tumour cells possessed very little cytoplasm. Again, no staining was observed when normal rat IgG was used as control or when no primary antibody was used (not shown). These data further confirm mRNA and binding data from primary and long term cultured tumour cells, and indicate that the expression of IL-4R on solid human tumours is not a culture artefact.

To investigate possible expression of IL-4R on normal breast epithelial cells, we also stained cytospin preparation of cells from normal breast (Hs578Bst) and tumour cells (ZR-75-1). Normal breast cells did not stain with anti-IL-4R antibody; however, tumour cells stained well (not shown). When normal control IgG was used as primary antibody, breast tumour cells did not stain at all (not shown).

*Effect of IL-4 on tumour cell growth*

To assess the function of IL-4R on solid tumours, we examined the effect of IL-4 on tumour cell growth in tissue culture. We utilized the MTT assay to assess the number of viable tumour cells at the end of various culture periods. As depicted in Fig. 4a, a small but statistically significant reduction in the number of viable cells was observed when A375, BT-20 and IGROV-1 cells were cultured with various concentrations of IL-4 (3–100 ng/ml) for 72 h.

**Table 1.** Expression of IL-4R on human and gibbon tumour lines

| Cell line | Tumour type | IL-4 molecules* bound/cell (mean ± s.e.m.) | $K_d$ ($\times 10^{-12}$ M) (mean + s.e.m.) |
|---|---|---|---|
| 1. Ramos.G6.C10 | Burkitt lymphoma | 2107 ± 679 | 140 |
| 2. RPMI 7951 | Melanoma | 1442 ± 12 | 549 ± 133 |
| 3. A375 | Melanoma | 1237 ± 237 | 364 ± 54 |
| 4. ZR-75-1 | Breast carcinoma | 2289 ± 182 | 294 ± 54 |
| 5. MC-7 | Breast carcinoma | 931† | 217 |
| 6. HSc 57-8-T | Breast carcinoma | 724† | ND |
| 7. IGROV-1 | Ovarian carcinoma | 1365 ± 115 | 331 ± 64 |
| 8. SKOV-3 | Ovarian carcinoma | 995† | ND |
| 9. OVCA 429 | Ovarian carcinoma | 433† | ND |
| 10. OVCA 420 | Ovarian carcinoma | 271† | ND |
| 11. MLA 144 | Gibbon lymphoma | 3848 ± 705 | 264 ± 5 |

* IL-4 molecules bound/cell was determined by equilibrium binding analysis in the presence of various concentrations of $^{125}$IL-4. Specific binding was calculated by subtracting non-specific binding (determined in the presence of 100–200 molar excess of unlabelled IL-4) from total binding. $K_d$ was calculated from Scatchard plot analysis of the binding data.

† A single concentration of $^{125}$I-IL-4 was used to determine the presence of IL-4R on these cells.
ND, Not determined.

Primary cultures of fresh tumours, e.g. mesothelioma and neurofibrosarcoma, were also used in the MTT assay to assess their growth in response to IL-4. As seen in Fig. 4b, IL-4 at the concentration of 6–100 ng/ml caused a maximum reduction in the number of viable neurofibrosarcoma cells. In mesothelioma cells, IL-4 also caused a small but significant reduction in cell viability (Fig. 4b) at two highest concentrations of IL-4.

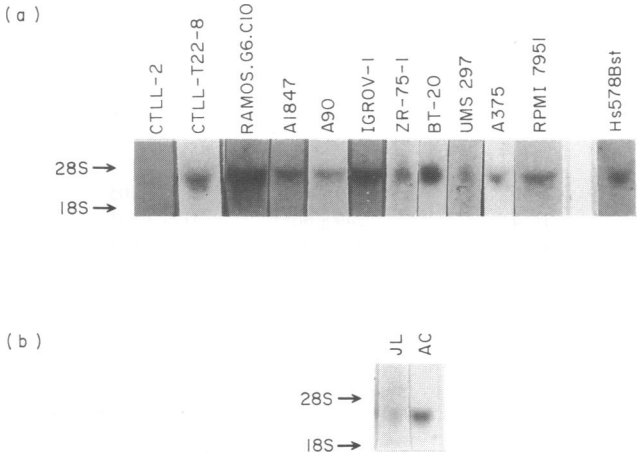


**Fig. 2.** (a) Expression of IL-4R mRNA by human tumour cell lines. Total RNA was isolated from human tumour cell lines and a 10-$\mu$g sample from each was electrophoresed and transferred to a nylon filter. The immobilized RNA was analysed with a $^{32}$P-labelled cDNA probe for human IL-4R. RNA from a mouse T cell line before (CTLL-2) and after transfection with the human IL-4R gene (CTLL-T22-8) were included as controls (lanes 1 and 2 respectively). Lane 3, human B lymphoma, Ramos.G6.C10; lanes 4–6, ovarian carcinoma: A-1847, A90, IGROV-1; lanes 7, 8, breast carcinoma: ZR-75-1, BT-20; lanes 9–11, melanoma: UMS 297, A375, RPMI 7951; lane 12, normal breast: Hs578Bst. (b) Expression of IL-4R by primary human tumour cultures. Total RNA (10 $\mu$g) isolated from primary human tumour cultures was immobilized on a nylon filter and probed with a $^{32}$P-labelled cDNA for IL-4R. Lane 1, mesothelioma (JL); lane 2, neurofibrosarcoma (AC).

In other experiments, we also examined the effect of IL-4 on the growth of normal cells and some tumour cell lines by assessing its effect on the incorporation of $^3$H-thymidine in the cells. IL-4 had no effect on the normal breast cell line (Hs578Bst). However, IL-4 caused a higher incorporation in the HUVEC endothelial cell line compared with untreated cells (data not shown). On the other hand, in IL-4-treated cultures of the breast tumour cell lines MCF-7 and ZR-75-1 and the melanoma cell line A375, $^3$H-thymidine incorporation was significantly lower than untreated cultures. This growth-inhibiting effect was reversed when purified antibody to recombinant human IL-4 (0·05–5·0 $\mu$g/ml) was included in the cultures (data not shown). Thus, the modest growth-inhibitory effects of IL-4 observed in the MTT assay (Fig. 4) were not due to non-specific cytotoxicity of IL-4, because (i) anti IL-4 antibody neutralized the growth inhibitory effect of IL-4; (ii) IL-4 did not inhibit normal breast cell growth; and (iii) IL-4 caused a modest growth-stimulatory effect on HUVEC (not shown). IL-4 has been shown to be mitogenic for HUVEC and human adrenal capillary endothelial cells *in vitro* [16].

*Effect of IL-4 on the expression of HLA class I and class II (HLA-DR) antigen*

We also examined the effect of IL-4 on the expression of HLA antigens using a quantitative radiometric binding assay. Tumour cells were cultured with or without IL-4 (20 ng/ml) for 72 h and the radiometric assay was performed to determine the effect of IL-4 on the expression of MHC class I or class II antigen. Breast carcinoma, ovarian carcinoma and melanoma cells expressed MHC class I antigen which was not altered by the treatment of IL-4 (data not shown). The expression of HLA-DR antigen on melanoma cells was enhanced by IL-4 (Table 2). This increase was small but statistically significant in two of three experiments. HLA-DR expression was also up-regulated by IFN-$\gamma$. This increase was further enhanced (in all three experi-

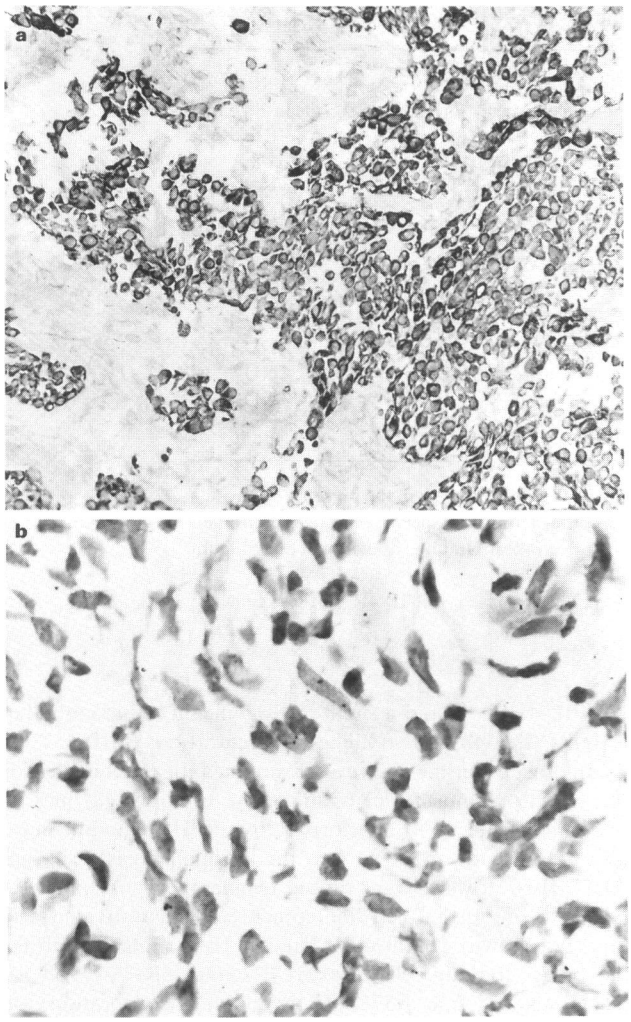

**Fig. 3.** Immunohistochemical identification of IL-4R on fresh malignant mesothelioma and fresh neurofibrosarcoma tumours. Frozen sections of tumours were stained with anti-IL-4R MoAb overnight at 4°C. Staining was performed as described in Materials and Methods. (a) Fresh malignant mesothelioma (magnification ×20) stained with anti-IL-4R antibody. (b) Fresh neurofibrosarcoma tumour section stained with anti-IL-4R antibody.

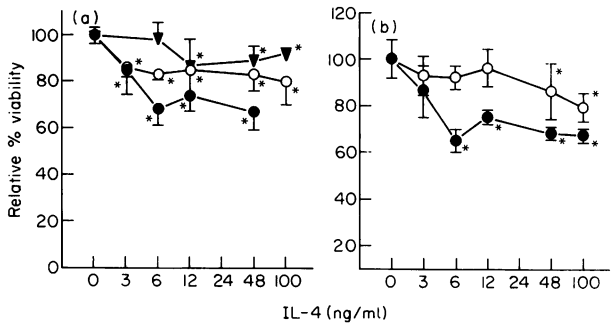


**Fig. 4.** (a) Effect of IL-4 on tumour cell growth. Melanoma cells (A375) (●) at $5 \times 10^4$ cells/ml, breast tumour cells (BT-20) (○) at $4 \times 10^5$ cells/ml, and ovarian carcinoma cells (IGROV-1) (▼) at $5 \times 10^4$/ml were incubated with varying concentrations of IL-4. After 72 h (BT-20 and A375) or 96 h (IGROV-1), cell viability was determined by the 3(4,5-dimethyl-thiazoyl-2-yl)2,5 diphenyltetrazolium bromide (MTT) assay as described in Materials and Methods. Per cent viability, relative to viability in control cultures consisting of cells and culture medium alone, is shown. * Significant at $P < 0.05$ from control untreated group. (b) Effect of IL-4 on primary cultures of human tumours. Primary cultures of neurofibrosarcoma cells at $5 \times 10^3$/ml (●) or mesothelioma cells at $1 \times 10^4$ cells/ml (○) were incubated with varying concentrations of IL-4 for 96 h. Cell viability was evaluated with the MTT assay as described in Materials and Methods. * Significant at $P < 0.05$ from control untreated group.

ments) in the presence of IL-4, indicating an additive effect of the two cytokines. A similar pattern was observed in breast carcinoma cells in which IL-4 caused a small increase in HLA-DR expression, IFN-γ alone had a profound effect, and an additive effect was observed in the presence of both cytokines.

## DISCUSSION

This study provides direct evidence indicating that a variety of human solid tumours express high-affinity IL-4R at the cell surface and at the mRNA level. The receptor numbers and their affinity to IL-4 on solid tumour cells appear to be similar to those observed by us and others [34] on human B and gibbon T (MLA 144) lymphoma cell lines. Additionally, human tumours expressed a single 4 kb mRNA as observed in B and T cell lines.

By immunohistochemistry of frozen sections of fresh solid tumours, we also demonstrate that solid tumours express immunoreactive surface IL-4R. We utilized a neutralizing anti-

**Table 2.** Effects of IL-4 and IFN-γ on HLA-DR antigen expression on human melanoma and breast carcinoma cell lines

| | Bound ct/min ± s.e.m. | |
|---|---|---|
| Treatment* | Isotype† control | HLA-DR |
| Melanoma (A375) | | |
| CM | 279 ± 48 | 2897 ± 105 |
| IL-4 | 249 ± 60** | 4171 ± 54‡ |
| IFN-γ | 317 ± 21 | 8264 ± 6‡§ |
| IL-4 + IFN-γ | 418 ± 68** | 8873 ± 20‡§ |
| Breast carcinoma (BT-20) | | |
| CM | 321 ± 81 | 470 ± 2 |
| IL-4 | 199 ± 91 | 546 ± 33‡ |
| IFN-γ | 244 ± 6 | 2025 ± 63‡¶ |
| IL-4 + IFN-γ | 275 ± 12 | 2717 ± 80‡¶ |

* Tumour cells incubated with culture medium (CM) or cytokines for 72 h at 37°C in 5% $CO_2$ environment were washed and $2 \times 10^5$ cells from each group incubated with the anti-human HLA-DR antibody. The cells were subsequently incubated with an $^{125}$I-labelled sheep anti-mouse F(ab')$_2$ fragment second antibody. Cell bound radioactivity was quantified with a gamma counter.

† Tumour cells cultured in CM or cytokine, but incubated with isotype control antibody.

‡ Different from CM controls ($P < 0.05$).

§ Different from each other ($P < 0.05$).

¶ Different from each other ($P < 0.05$).

** Not different from each other ($P < 0.05$).

human IL-4R MoAb for this purpose. This observation led us to conclude that expressed IL-4 receptors on solid tumours are not due to long term culture of tumour cells.

The expressed IL-4R on human solid tumours appear to be functional, as IL-4 reduced tumour cell growth *in vitro*. The growth-suppressive effects of IL-4 on most tumour cells were modest, but statistically significant and reproducible (Fig. 4a, b). However, in some tumours, very little effect was observed (data not shown). Since IL-4 did not alter the growth rate of normal breast epithelial cells, and modestly stimulated the growth of HUVEC, our data suggest that the modest growth-inhibitory effects observed in our study are not due to non-specific cytotoxicity of IL-4. IL-4 has been shown to stimulate the growth of normal HUVEC and adrenal capillary endothelial cells [16]. Thus our data agree with the published report. Recently IL-4 was reported to inhibit human melanoma and renal carcinoma cell growth *in vitro* [35,36], and it was also shown to suppress the spontaneous growth of chronic myelomonocytic leukaemia cells [37]. Although the growth-inhibitory effects of IL-4 in the current study were small compared with that observed in human renal cell carcinoma cells, the potential significance of this growth inhibition is not known. However, our data agree with published observations, and further suggest that these effects of IL-4 are probably mediated through its receptors. Further studies are underway to characterize and examine the regulation of IL-4R on solid human tumours.

We also examined the expression of IL-4R on normal breast tissue cells by immunocytochemistry. Using the experimental conditions described above, we did not see staining of these cells. However, IL-4R mRNA was identified by Northern analysis. This suggests that normal breast tissue cells may express IL-4R, but at a level below the detectable limit of immunocytochemistry.

Our data are also consistent with a recent report suggesting that human lung cancers express IL-4R [27,38]. That study used a poorly characterized MoAb, MR-6, which reportedly recognizes an IL-4R-associated protein [39]. This antibody was raised by the immunization of mice with human thymic cortex [40]. Although MR-6 has been shown to block the IL-4-dependent proliferation of T cells as well as antigen-induced production of IgE by B cells [39,41], it was also able to inhibit antigen and IL-2-induced proliferation of cloned T cells, indicating that MR-6 effects may not be specific for IL-4. Furthermore, MR-6 was not able to block the binding of IL-4 to its receptors [27], and there is no direct evidence that it binds to IL-4R. Our data, however, provide direct evidence that human solid tumours express functional high-affinity IL-4R, and also demonstrate that human lung mesothelioma, reported to be negative for IL-4R by MR-6 staining [38], do express functional IL-4R, as IL-4 was able to modestly inhibit their growth in tissue culture.

Our observations suggest that IL-4R may be a useful tumour-associated protein for the screening of certain human malignancies by radionuclide scans. IL-4 or MoAbs to human IL-4R can be radiolabelled and utilized for nuclear medicine scans. IL-4R may also be a useful target for the specific delivery of toxins to the tumour. Puri *et al.* [26] have recently demonstrated that IL4-PE40 chimeric protein is toxic to IL-4R-bearing tumour cells *in vitro*. In that study they found that IL4-PE40 was cytotoxic to tumour cells bearing more than a few hundred IL-4 binding sites/cell. If this is true for human tumours, it should be possible to deliver the exotoxin to tumour targets, sparing normal non-activated immune cells which express only about 200 receptors on their cell surface [34]. These possibilities are currently being investigated. Although normal cells that may express high numbers of IL-4R would become targets for IL-4/toxin proteins, several normal cell lines including fibroblasts, hepatocytes, epithelial cells and capillary endothelial cells have so far been shown to express only several hundred receptors/cell [16,21,34].

It was recently reported that IL-4 plays a role in the regulation of MHC class I and II antigens on human melanoma [42]. In that report, IL-4 appeared to up-regulate MHC class I, class II, $\beta$-microglobulin and tumour-associated antigens on the melanoma cells. In the present study we found that IL-4 had little effect on the expression of class I antigen on melanoma breast and ovarian carcinoma cells. The reason for this discrepancy is not clear. Aside from differences in tumours, it is possible that the expression of class I antigen on solid tumours is unstable, which may explain some of the discrepancies in our results and those of others.

Melanoma and breast carcinoma cells expressed varying levels of HLA-DR antigen, and IL-4 treatment appeared to enhance its expression in both cell lines (Table 2). IFN-$\gamma$ also enhanced the expression of HLA-DR antigen in melanoma and breast carcinoma cells, and IL-4 further enhanced this expression. This observation indicates an additive effect of IL-4 and IFN-$\gamma$ similar to that observed on melanoma cells by Hoon *et al.* [42]. The enhancement of HLA-DR expression by IL-4 was small and the significance of this increase is not clear. It is possible that IL-4 may modulate tumour immunogenicity through small up-regulation of MHC expression. It is of interest to note that in our study we observed a combined effect of IL-4 and IFN-$\gamma$ on HLA-DR expression, while in immune cells these cytokines are shown to cause antagonistic effects. The combined effects of IL-4 and IFN-$\gamma$ on HLA-DR expression observed in our study are novel, but combined effects of these cytokines on other cell types have been previously reported. For example, IL-4 has been shown to synergize with IFN-$\gamma$ in the regulation of polymeric immunoglobulin receptor and IgA binding by human HT-29 epithelial cell lines (human colon adenocarcinoma) [43]. IL-4 and IFN-$\gamma$ have also been shown to augment anti-leishmanial potential of macrophages [44]. More recently, IL-4 has been shown not to inhibit IFN-$\gamma$-induced activation of human colostral macrophages [45].

IL-4 has recently been shown to cause the regression of established murine tumours, and host immune cells appear to play a significant role in the observed effect [46]. In another study, tumour establishment in mice was prevented by the injection of tumour cells transfected with the IL-4 gene [47,48]. These findings suggest that IL-4 might have an anti-tumour effect. However, constitutive production of IL-4 by the transduced tumour cells had very little effect on their growth *in vitro*. In the *in vivo* experiments host monocytes and eosinophils infiltrated the solid tumours, indicating their involvement in the observed effects. The exact mechanisms of the anti-tumour effects of IL-4 are still not clear. However, our data and data from other laboratories [35,37,42,49,50] suggest that in addition to its effects on host defences, IL-4 may also have direct antiproliferative effect on human tumour cells, and in some instances may modulate tumour immunogenicity through up-regulation of MHC antigen expression. These effects may be enhanced when IL-4 is present along with IFN-$\gamma$. Taken

together, our observations and those of others [35,42,49,50] suggest that IL-4R on human solid tumours may be functional, and IL-4 may play a role directly and/or indirectly in host anti-tumour responses.

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