Comparison of the potential therapeutic effects of interleukin 2 or interleukin 4 secretion by a single tumour

P.M. Patel1, C.L. Flemming1, S.J. Russell1, I.A. McKay4, K.A. MacLennan2, G.M. Box3, S.A. Eccles3 & M.K.L. Collins1

1Section of Cell and Molecular Biology, 2Department of Histopathology and 3Section of Immunology, Institute of Cancer Research and Royal Marsden Hospital, London; 4Department of Experimental Dermatology, London Hospital Medical College, London, UK.

Summary  Engineering of a variety of rodent tumour cells to secrete either interleukin 2 (IL-2), or interleukin 4 (IL-4), has been demonstrated to reduce their tumorigenicity. However the mechanisms of action of secreted IL-2 and IL-4 have not been compared in a single rodent tumour. Here we demonstrate that the weakly immunogenic murine fibrosarcoma FS29 had reduced growth rate and in some cases was rejected by syngeneic animals, when modified to secrete either IL-2 or IL-4, but not IL-5. Immunohistochemical analysis of tumour nodules undergoing regression showed stimulation of a largely lymphocytic infiltrate by IL-2 and a macrophage and granulocyte infiltrate, with a small number of lymphocytes by IL-4. Indeed, secretion of low levels of IL-2 and IL-4 in combination resulted in optimal rejection, suggesting that the two cytokines might mobilise different and complementary effector cell mechanisms. Both IL-2 and IL-4-secreting cells failed to induce regression of FS29 tumours. Therapy of animals bearing tumours from such admixtures occurred more rapidly for IL-2-secreting cells. Injection of IL-4-secreting, but not IL-2-secreting FS29 cells could protect mice from a delayed challenge with unmodified FS29 cells. These data suggest that IL-4 secretion stimulates the better long-term host anti-tumour response.

Observations that rodents from which a primary, syngeneic tumour had been excised could be resistant to a secondary tumour challenge (Prehn & Main, 1957; Klein et al., 1960) led to the proposition that a specific host anti-tumour immune response could be stimulated. Subsequent studies have demonstrated that the stimulation of tumour specific cytotoxic T lymphocytes (CTLs) (Boon et al., 1980; Brunner et al., 1981) is important in eliciting tumour rejection in rodent models (Uyttenhove et al., 1983). Several approaches to the treatment of human tumours have aimed to boost what may be a sub-optimal patient anti-tumour immune response. The systemic administration of IL-2, a stimulatory factor for many cells of the immune system (Smith, 1988), to patients with metastatic melanoma and renal cell carcinoma, showed some therapeutic effect (Rosenberg et al., 1989). However, toxic side-effects were observed at the high concentrations required (Rosenberg et al., 1989). A further strategy has been the use of irradiated patient tumour cells, rendered more antigenic by viral infection, as a potential vaccine to protect against metastases (Bohle et al., 1990). Viral infection (Lindemann & Klein, 1967; Kobayashi et al., 1969; Boone & Blackman, 1972; Ito et al., 1990) or the expression of recombinant viral antigens (Fearon et al., 1988; Sugiura et al., 1988), or MHC molecules (Hui et al., 1984; Tanaka et al., 1985; Wallich et al., 1985), has proved successful in the enhancement of immunogenicity of rodent tumours.

To overcome the toxicity of systemic cytokine administration, the effect of engineering rodent tumour cells to secrete cytokines locally has been examined. Secretion of IL-2 (Fearon et al., 1990; Gansbacher et al., 1990; Russell et al., 1991; Ley et al., 1991), IL-4 (Tepper et al., 1989; Golumbek et al., 1991), γ-interferon (γ-IFN) (Watanabe et al., 1989; Gansbacher et al., 1990; Esumi et al., 1991), tumour necrosis factor-α (TNF-α) (Ascher et al., 1991; Blankenstein et al., 1991), granulocyte colony stimulating factor (Colombo et al., 1991), or interleukin 7 (Hock et al., 1991; McBride et al., 1992) by a variety of rodent tumour cells has been demonstrated to reduce, or eliminate, their tumorigenicity in syngeneic animals. For this observation to be exploited in the treatment of human malignant disease, two approaches could be attempted. Firstly, in vivo cytokine gene delivery to established tumours might lead to local cytokine secretion and ultimate tumour rejection. Direct delivery of a foreign MHC gene to tumour cells in situ is the basis of a currently approved human gene therapy trial (Miller, 1992). As gene delivery to all cells within a tumour will probably not be feasible, cytokine secretion by some cells within a tumour would have to lead to the rejection of adjacent, unmodified cells for direct cytokine gene delivery to be effective. An effect on the rejection of admixed, unmodified cells has been reported for IL-2 (Gansbacher et al., 1990; Ley et al., 1991), IL-4 (Tepper et al., 1989; Golumbek et al., 1991) and TNF-α (Asher et al., 1991)-secreting rodent tumour cells.

The second therapeutic use of cytokine-secreting tumour cells would be as an injection post primary tumour excision, to enhance the elimination of minimal residual disease. This would require the culture of cells from resected tumour material and their in vitro modification to secrete cytokines, followed by re-injection. Another human gene therapy trial is employing this approach with IL-2 and TNF-α-secreting tumour cells (Rosenberg et al., 1992a; Rosenberg, 1992b). In rodent tumour studies IL-2 (Gansbacher et al., 1990; Ley et al., 1991), IL-4 (Golumbek et al., 1991) and γ-IFN (Watanabe et al., 1989; Gansbacher et al., 1990)-secreting tumour cells have each been shown to induce protection against parental tumour cell challenge. In order to determine which of these therapeutic routes might be the more feasible, we have compared the properties of IL-2 and IL-4-secreting tumour cells in a single murine tumour, the transplantable fibrosarcoma, FS29 (Eccles et al., 1980). We have demonstrated that either IL-2, or IL-4-secreting cells show a poor ability to induce the rejection of admixed parental cells. This failure can be ascribed to a rapid loss of the secreting cells from tumours. However, IL-4-secreting FS29 cells showed a greatly enhanced ability to protect animals against subsequent parental tumour challenge, when compared with IL-2-secreting, or parental cell injections.

Methods and materials

Cell lines

FS29 is a benzpyrene induced murine sarcoma cell line (Eccles et al., 1980) that was grown in vitro as an adherent monolayer in DMEM with 10% fetal calf serum (FCS).
PA317 (Miller & Buttimore, 1986) and GP + envAM12 (Markowitz et al., 1988) amphotropic retroviral packaging cell lines were grown as adherent monolayers in DMEM and 10% newborn calf serum. IL-2-dependent CTLL-2 (Gillis & Smith, 1977) and IL-2-dependent, IL-4-responsive HT-2 (Lichtman et al., 1987) cells were grown in suspension in RPMI with 10% FCS, 5 x 10^{-3} M mercaetoethanol, 2 mM glutamine and 50 U ml^{-1} recombinant human IL-2 (Eurocetus).

Tumorigenicity assays

Exponentially growing tumour cells were trypsinised and counted, then washed, resuspended in phosphate buffered saline (PBS) and injected subcutaneously in 100 μl into each flank of syngeneic C57Bl or athymic (nu/nu) mice. Tumour diameter was measured with calipers X2/week. Animals were maintained under a barrier system in accordance with Institutional guidelines. Mice were killed if they showed signs of widespread malignancy, or when tumours were greater than 1 cm diameter or ulcerating. For explantation, tumour nodules were resected, macerated with crossed scalpels and incubated on a magnetic stirrer in 0.1% trypsin and 0.02% DNAse for 1 h. The cell suspension was washed, plated and the adherent monolayer expanded. 10^6 cells were replated and after 48 h, supernatant was harvested for cytokine assays and DNA prepared from cells for Southern blot analysis.

Plasmids and recombinant retroviruses

The plasmid pZipNeoSV(X) (Cepko et al., 1984), pZip-NeoSVIL-2 (Yamada et al., 1987), containing the human IL-2 cDNA, pZipNeoSVIL-4, containing a murine IL-4 cDNA (which was constructed by ligating a series of synthetic oligonucleotides to give an identical amino-acid sequence to that produced by the published murine IL-4 cDNA (Lee et al., 1986)) and pZipNeoSVIL-5 containing the murine IL-5 cDNA (Campbell et al., 1987) were transfected by calcium phosphate precipitation into PA317 or GP + envAM12 packaging cell lines. G418 resistant colonies were picked and assayed for recombinant retrovirus production and absence of helper virus as described previously (Danos, 1991). Clones producing the highest titre of helper free, recombinant virus were used to infect FS29 cells by overnight incubation with the packaging cell supernatant, in the presence of 8 μg ml^{-1} polybrene. The plasmid pZipNeoMuIL-2 was constructed by ligating a blunt ended, BamHI linked PstI/SspI fragment from pCDMuIL-2, encoding murine IL-2, to pZipNeoSV(X); this was used to transfect FS29 cells. G418 resistant FS29 colonies were picked and assayed for cytokine secretion. Stability of cytokine secretion was established by re-assay after at least 6 weeks of in vitro culture. The in vitro growth rate for cytokine-secreting clones was tested by plating 5 x 10^3 cells into 6 well plates and counting wells daily for 7 days. Plating efficiencies were assessed by plating 100 cells in an 80 cm^2 flask and counting colonies after 7 days. All clones were tested for absence of helper virus (Danos, 1991).

Cytokine bioassays

Supernatants were harvested and passed through a 0.2 μm filter 48 h after plating 10^4 tumour cells. Serial dilutions, and recombinant human IL-2 standards (Eurocetus) were added to 96 well microtitre plates containing 5,000 CTLL cells per well in a final volume of 200 μl. After 16 h, the cells were pulsed with 0.5 μCi ^3H-thymidine and incorporation was measured 4 h later. One U human IL-2 (Gillis & Smith, 1977) gives half maximal thymidine incorporation under these conditions. Results are expressed as units of IL-2 produced by 10^6 cells per 48 h. Supernatants also containing IL-4 were incubated with Hb188 (5% hybridoma supernatant), an IL-4-blocking antibody (Ohara & Paul, 1985) for 2 h prior to testing. For IL-4 assays, serial dilutions and recombinant murine IL-4 standards (Genzyme, UK) were added to 96 well microtitre plates containing 5,000 HT-2 cells per well in a final volume of 200 μl. After 16 h, the cells were pulsed with 0.5 μCi ^3H-thymidine and incorporation was measured 4 h later. Under these conditions 0.3 U IL-4 gives half maximal thymidine incorporation. Results are expressed as units of IL-4 produced by 10^6 cells per 48 h. Supernatants also containing IL-2 were incubated with EP100 (12.5 μg ml^{-1}), an anti-IL-2 blocking antiserum (New Brunswick Scientific, UK) for 2 h prior to testing. IL-5 was measured as previously described (Strath et al., 1985).

| Clone       | Cytokine secretion units/10^6 cells/48 h | Tumour growth | Explant secretion |
|-------------|-----------------------------------------|---------------|------------------|
| FS29Neo     | 0                                       | 38/38         |                  |
| FS29IL2.1   | 54.000                                  | 2/12†         | 0                |
| FS29IL2.2   | 18.100                                  | 18/22*        | 7 (0-46)         |
| FS29muIL2.7 | 3.000                                   | 8/8           | 87 (15-164)      |
| FS29IL4.1   | 9.9200                                  | 26/26         | 68900 (57200-75300) |
| FS29IL2.4   | 7.4500                                  | 4/24†         | 70150 (62200-87200) |
| FS29IL4.5   | 9.4500                                  | 8/10          | nd               |
| FS29IL4.1IL2.b | 7100(IL2)                            | 0/10†         | nd               |
| FS29IL5.1   | 6567                                    | 6/6           | nd               |

Cumulative data on tumour formation by FS29 clones, following the injection of 10^6 cells in C57Bl mice, are presented. Cytokine secretion by FS29 clones before injection (first column), and after explantation of tumours greater than 0.5 cm in diameter from C57Bl mice between 16 and 73 days after injection (third column), was measured as described in Materials and methods. FS29IL2.1 and 2.2 secrete human IL-2, FS29muIL2.7 secrete murine IL-2, FS29IL-4.1, 4.2 and 4.5 secrete murine IL-4, FS29IL-4.1IL2.b secrete murine IL-4 and human IL-2, FS29IL5.1 secretes murine IL-5, -6, *P < 0.001; †P < 0.05 compared to the growth of FS29Neo using a two-tailed Fisher’s exact probability test.
THERAPEUTIC EFFECTS OF IL-2 OR IL-4 SECRETING TUMOURS

Immunohistochemistry

Exponentially growing tumour cells were injected subcutaneously into each flank of syngeneic mice, as for tumorigenicity studies. Tumour nodules were removed at 4, 7, 11 and 14 days. Haematoxylin and eosin sections were fixed in 10% formalin and embedded in paraffin wax. Sections from frozen tissue blocks were fixed in acetone, then incubated for 1 h with primary rat monoclonal antibodies; anti-CD4 GK1.5 (gift from Dr R. Zamoyska), anti-CD8 53-6.7 (Becton Dickinson), anti-IL-2 receptor 7D4 (gift from Prof. T. Malek), anti-IL-4 receptor MI (gift from Immunex), anti-CD45RA (B cell specific) 14.8 (gift from Dr J. Marvel), washed with PBS, then incubated for 1 h with biotinylated anti-rat IgG (Dako). The antigen antibody complexes were detected by incubation with ABC complex conjugated HRP (Dako) and development with DAB.

Results

Growth of cytokine-secreting FS29 tumours

In order to compare the effect of secretion of either IL-2 or IL-4 on tumour growth in vivo, a panel of cell lines was generated from the murine transplantable sarcoma FS29, by infection with recombinant retroviruses carrying cytokine cDNAs. To generate a control cell line, FS29Neo, FS29 cells were infected with a retrovirus lacking a cDNA insert. Table I details the level of human IL-2, murine IL-2, murine IL-4, or murine IL-5 produced in vitro by cell clones, selected as secretors of the highest levels of cytokine. Cytokine secretion did not affect the growth rate of the clones in vitro and the levels of cytokine secreted were unchanged following prolonged passage of the cell clones in culture (data not shown). All clones expressed very low or undetectable levels of MHC.

![Figure 1](image-url)

Figure 1 Growth of cytokine secreting tumour cells. 10⁶ exponentially growing tumour cells were injected subcutaneously into C57bl mice. Tumour diameter was measured twice weekly. Growth for each tumour in a typical experiment is shown. *P<0.005 when tumour size at 16 days compared to FS29Neo by calculating the standard error of the difference between the means.)
class I antigens, in comparison to transfected HeLa cells expressing K	extsuperscript{b}, when analysed by staining with the Y3 anti-K	extsuperscript{b} antibody (data not shown).

The growth rate of cytokine secreting tumours in vivo, following the subcutaneous injection of 10	extsuperscript{6} cells in syngeneic mice, was then monitored. Figure 1 shows a typical experiment for each clone and a compilation of all experiments is presented in Table I. FS29IL-2.1 which secreted the highest level of IL-2, formed an initial tumour nodule which then regressed completely in most animals. Those clones secreting less IL-2, FS29IL-2.2 and FS29muIL-2.7 showed some delay in growth compared to FS29Neo cells, but ultimately formed tumours in the majority of animals. Tumours which arose from the IL-2-secreting cell clones were explanted, cultured and assessed for their ability to secrete IL-2. In every case the explanted tumour cells secreted considerably reduced, or no, IL-2 (Table I). This could be attributed to a decrease in the IL-2-encoding retroviral integrant in each case (Figure 5, upper panel, track 4 and data not shown). Thus, a strong selective pressure against both human and murine IL-2 secretion in vivo resulted in rapid selection of cells within the tumour which had lost the retroviral integrant. This selection was not observed upon growth of FS29IL-2.1 in athymic mice (Figure 5, upper panel, track 3).

The three IL-4-secreting FS29 clones also displayed slower growth rates and reduced tumorigenicity in syngeneic mice (Figure 1 and Table I). One clone, FS29IL-4.2, formed small nodules and then regressed in most animals. Two further clones, FS29IL-4.1 and FS29IL-4.5, formed small tumours which persisted for prolonged periods, in contrast to the IL-2 secreting clones. When such persistent tumours were explanted and their IL-4-secretion was measured, it was found that they secreted unreduced levels of IL-4 (Table I) and retained the IL-4 encoding retroviral integrant (Figure 5, lower panel, track 5). In contrast to previous reports (Tepper et al., 1989; Golumbek et al., 1991), IL-4 secretion did not affect the growth rate of FS29 cells in athymic mice (data not shown). IL-4 secretion, by murine plasmacytoma, adenocarcinoma or renal cell carcinoma cells had previously been reported to induce an eosinophilic infiltrate in vivo (Tepper et al., 1989; Golumbek et al., 1991), and anti-IL-5 antibodies had been shown to partially restore the tumorigenicity of IL-4-secreting cells (Tepper et al., 1992). Therefore, we monitored the effect of secretion of IL-5, the major growth factor for cells of the eosinophil lineage, upon FS29 tumour formation. Figure 1 shows that IL-5 secretion did not cause any slowing of tumour growth in syngeneic animals; no eosinophil infiltrate was observed in IL-5-secreting tumours (data not shown). To observe the effect of simultaneous IL-2 and IL-4 secretion, a doubly-transfected clone secreting sub-optimal levels of both cytokines was isolated. Additional secretion of IL-2, by the non-rejecting IL-4 secreting clone FS29IL-4.1, was able to induce tumour rejection (Table I). This cooperative effect suggested that the two cytokines might act by mobilisation of different effector mechanisms.

**Host cells infiltrating cytokine-secreting FS29 tumours**

To examine the host cells involved in the response to cytokine-secreting FS29 cells, small tumour nodules were excised and infiltrating host cells were examined morphologically and by immunohistochemical staining. While few host cells were present in the unmodified FS29 tumour nodule, a pronounced lymphocytic infiltrate was present in

![Figure 2](image-url)  
**Figure 2** Histopathology of cytokine secreting tumours. Tumour nodules were excised 7 to 10 days after injection of: 10	extsuperscript{6} FS29Neo in C57bl mice (a), FS29IL-2.1 in C57bl mice (b), FS29IL-4.2 in C57bl mice (c), FS29IL-4.2 in nu/nu mice (d). Sections were stained with haematoxylin and eosin and photographed at a magnification of 500 x.
the IL-2-secreting tumours (Figure 2). The majority of these cells were CD8+, with some CD4+ cells and B cells (Table II). In contrast, the IL-4-secreting tumours were characterised by a macrophage and granulocyte infiltrate, with a small number of CD8+ and CD4+ lymphocytes (Figure 2, Table II). This inflammatory cell infiltrate was not observed in IL-4-secreting tumour nodules from athymic animals (Figure 2) and thus appears to depend on a T lymphocyte response.

Cytokine-secreting cells are selectively lost from tumours

To assess the potential of cytokine gene delivery to existing tumours in vivo as a tumour therapy, the ability of cytokine-secreting FS29 cells to induce rejection of admixed, unmodified cells was measured. Figure 3 shows that neither IL-2, nor IL-4-secreting FS29 cells caused any slowing of growth of an equal number of admixed non-secreting FS29 cells. When the IL-2-, or IL-4-secreting cells were present in a 10-fold excess, initial delay in the growth of the unmodified cells was observed, but rapidly growing tumours eventually formed in all animals (Figure 3). These data are in contrast to several previous studies, which have demonstrated effective induction of parental tumour rejection by admixture of 50% IL-2 (Gansbacher et al., 1990; Ley et al., 1991), or IL-4-secreting cells (Tepper et al., 1989; Golumbek et al., 1991).

To investigate the reason for this lack of effect of cytokine secretion on adjacent unmodified FS29 cells, tumour nodules were explanted soon after injection of IL-2 and parental cell admixtures. When the level of IL-2 secretion from such explanted cells was analysed, admixtures of IL-2-secreting and parental cells were found still to secrete IL-2 after 7 days in vivo growth (Figure 4). However, following 14 days of tumour development, IL-2 secretion was lost (Figure 4). This correlated with loss of IL-2-encoding retroviral DNA sequences, which could be detected after 7 days but not 14 days tumour growth, in the explanted cells (Figure 5, upper panel, tracks 3 and 6). Similar analysis of admixtures of IL-4-secreting and parental cells demonstrated greatly reduced IL-4 secretion after 14 days growth in vivo (Figure 4) and undetectable IL-4-encoding retroviral DNA (Figure 5, lower panel, track 8). A more rapid loss of IL-2-secreting cells could be clearly observed in an admixture of IL-2 and IL-4-secreting cells. IL-2 and IL-4 rates of secretion by tumours explanted after 7 days were similar to the injected cells (Figure 4); both IL-2 and IL-4 retroviral DNA could be detected (Figure 5, upper panel, track 2 and lower panel, track 9). However, following 14 days growth of the admixture IL-2 secretion (Figure 4) and retroviral DNA sequence (Figure 5, upper panel, track 8) were reduced, while IL-4 secretion (Figure 4) and retroviral DNA (Figure 5, lower panel, track 11) were maintained. These data show that a strong selective pressure against IL-2-secreting FS29 cells and a slightly weaker selective pressure against IL-4-secreting FS29 cells, results in their loss from cell admixtures. This provides an explanation for the inability of such cytokine secreting FS29 cells to induce rejection of admixed parental cells. These data suggest that direct retroviral-mediated cytokine gene delivery to tumour cells in vivo will not be able to induce tumour rejection, even if delivery to over 50% of cells within a tumour were feasible.

IL-4-secreting cells can protect animals from parental tumour challenge.

An alternative application of retroviral cytokine gene delivery in cancer therapy would be the use of tumour cells, cultured following primary lesion excision and infected with cytokine-encoding retroviruses, as an injection to enhance eradication of minimal residual disease. The efficacies of

**Table II** Characterisation of host cell infiltrate in FS29 tumour nodules

| Table II | Characterisation of host cell infiltrate in FS29 tumour nodules |
| --- | --- |
| **PMN** | +/− | + | ++ | + |
| **macrophages** | +/− | + | ++ | + |
| **lymphocytes** | +/− | ++ | ++ | + |

A summary of morphological and immunohistochemical analysis of tumour nodules, explanted between 7 and 14 days after injection of 10⁶ FS29Neo, FS29IL-2.1 and FS29IL-4.2 cells in C37bl mice.

**Figure 3** Growth of admixtures of cytokine secreting and non-secreting tumour cells. FS29IL-2.1 cells (a) or FS29IL-4.1 cells (b) were mixed with FS29Neo at ratios of 1:1 (10⁵ cytokine secretors + 10⁵ FS29Neo), and 10:1 (10⁶ cytokine secretors + 10⁵ FS29Neo), and injected into each flank of C37bl mice. As controls, mice were injected with 10⁶ or 10⁷ FS29Neo cells.
parental, IL-2 and IL-4-secreting FS29 cells in the induction of lasting protection of syngeneic mice against FS29 tumour challenge were therefore compared. Figure 6 shows that animals which had rejected the IL-4-secreting FS29 cell primary tumour showed considerably delayed tumour incidence, when challenged after 48 days with FS29Neo cells. Five out of 13 of these animals survived tumour-free for over 30 days. In contrast, parental cell injection afforded no lasting protection, confirming the weak basal immunogenicity of this tumour (Figure 6). IL-2-secreting tumour cells also did not protect animals against delayed parental cell challenge (Figure 6). These data suggest that IL-4-secreting tumour cells, while unable to induce a rapid enough immune response to cause the rejection of admixed parental cells, can stimulate an effective long-term anti-tumour response.

Discussion

This study represents a detailed comparison of the effects of IL-2 and IL-4 secreted by the same tumour. Secretion of a sufficiently high level of IL-2 by FS29 cells resulted in tumour rejection, whereas lower levels of secretion slowed initial growth but resulted in the later appearance of rapidly growing tumours. This could be explained by the loss of the IL-2 transgene and thus IL-2 secretion by these outgrowing cells, which suggests that a strong selective pressure against IL-2 secretion occurred in syngeneic animals. Loss of cytokine was observed with tumour cells secreting either human, or murine IL-2. A similar selection against IL-2 secretion was previously observed in the rat tumour HSN when passaged in syngeneic immunocompetent, but not athymic, animals (Russell et al., 1991). In contrast, IL-4 secretion by FS29 cells resulted in either tumour rejection or the appearance of slow growing tumours which retained the IL-4 transgene and secreted IL-4. Thus, IL-4 secretion was not subject to such a strong immune selection.

However, when either IL-2- or IL-4-secreting tumour cells were admixed with unmodified tumour cells, the parental tumours rapidly emerged. By analysing explanted tumour nodules, it was determined that the IL-2-secreting cells were lost from such admixtures after 14 days, and that IL-4-secreting cells were greatly reduced in proportion at this time. Thus, a weak immune selection against IL-4-secreting cells does occur when they are admixed with unmodified cells. This lack of rejection of admixed, unmodified cells is in contrast to previous studies with other IL-2-secreting (Gansbacher et al., 1990; Ley et al., 1991) or IL-4-secreting (Golumbek et al., 1991; Tepper et al., 1989) tumour cells. The level of cytokine secretion that we have achieved is similar, or higher than that reported in these studies; perhaps the difference can be attributed to a lower intrinsic immunogenicity of the FS29 tumour. The strong selection of cytokine secreting cells from an admixture implies specific, local immune stimulation by such cells. Furthermore, these data would argue against direct cytokine gene delivery to tumours in situ as an effective therapy, with the efficiency of currently available gene therapy techniques.

A more feasible therapeutic approach appears to be the injection of cytokine secreting tumour cells. Animals which had rejected IL-4-secreting FS29 cells were protected against parental tumour when challenged after 48 days. IL-2-
Figure 6 Growth of secondary challenge tumours. Mice were inoculated with 10^6 FS29Neo, FS29II-L2.1 or FS29II-L4.2 cells. Any tumours growing at 18 days were excised; all surviving mice, and a control group, were rechallenged after 48 days, on the opposite flank, with 10^6 FS29Neo and tumour growth monitored. Animals with tumour of greater than 0.4 cm which was increasing in size were considered tumour positive. Percentage of tumour free mice is shown as a function of time. *P < 0.05 when tumour incidence compared with naive controls on day 30 using a two-tailed Fisher's exact probability test.

...may be possible. The effects of IL-4 in the FS29 model depend on the presence of T lymphocytes as we did not observe any slowing of tumour growth, or cell infiltrate, in athymic animals. Thus, IL-4 stimulation of T cells recruits inflammatory cells, perhaps by the stimulation of further cytokine production. While induction of protective immunity clearly requires T cell stimulation, the inflammatory cell infiltrate may also be crucial in the generation of an optimal response. A previous study has demonstrated that IL-4-secreting renal carcinoma cells protect against subsequent tumour challenge and can cure animals pre-injected with a small number of parental cells (Golumbek et al., 1991). However, another report describes a complete absence of induction of protection by IL-4-secreting plasmacytoma cells (Tepper et al., 1992). Two reports described lasting protection induced by IL-2-secreting cells (Gansbacher et al., 1990; Ley et al., 1991), the work of Ley et al. (Ley et al., 1991) demonstrated an enhanced protection compared to parental mastocytoma cells. The study of Fearon et al. described short-term protection induced by IL-2-secreting colon tumour cells which is greatly diminished after 28 days (Fearon et al., 1990). Such differences observed in previous studies might be ascribed to differential intrinsic immunogenicity of the various tumours. Our direct comparison, in the FS29 sarcoma model, suggests that IL-4-secreting cells provide the better protection. The greater protective response induced by IL-4 may be partly explained by two of our observations. Firstly, IL-4 recruits different subsets of host immune cells, as demonstrated by the different infiltrate observed in IL-4 compared with IL-2- secreting tumours. Some of the cells recruited by IL-4, but not by IL-2, may be important to the establishment of a long-term anti-tumour response. Secondly, as IL-4-secreting cells suffer a less stringent host immune selection, IL-4-secreting tumour cells are maintained for longer than their IL-2 counterparts. This longer period of immune stimulation may also be important in the generation of a greater response.

We would like to thank Janine Salter for immunohistochemical analysis. pZipNeoSV(X) was provided by Prof. R.C. Mulligan, pZipNeoSVII-2 by Prof. T. Taniguchi, a murine IL-2 cDNA by DNAx (Palo Alto) and a murine IL-5 cDNA by Dr C. Sanderson (NIMR, London) to whom we are also indebted for performing the IL-5 bioassays. The anti murine IL-4 receptor antibody M1 was a gift from Immunex (Seattle) and the anti murine IL-2 receptor antibody 7D4 from Prof. T. Malek.

This work was supported by the Cancer Research Campaign. I.A.M. acknowledges the support of the Restoration of Appearance and Function Trust (RAFT) and the Emmandjay Trust.

Abbreviations: IL-2, interleukin 2; IL-4, interleukin-4; IL-5, interleukin 5; γIFN, gamma interferon; TNF-α, tumour necrosis factor alpha.

References

ASHER, A.; MULE, J.; KASID, A.; RESTIFO, N.; SALO, J.; REICHERT, C.; JAFFE, G.; FENDLY, B.; KRIEGLER, M. & ROSENBERG, S. (1991). Murine tumour cells transduced with the gene for tumour necrosis factor-α. J. Immunol., 146, 3227–3234.

BLANKENSTEIN, T.; QIN, Z.; UBERLA, K.; MÜLLER, W.; ROSEN, H.; VÖLK, H.-D. & DIAMANTSTEIN, T. (1991). Tumour suppression after tumour cell-targeted tumour necrosis factor α gene transfer. J. Exp. Med., 173, 1047–1052.

BOHLE, W.; SCHLAG, P.; LIEBICH, W.; HOHENBERGER, P.; MANASTERSKI, M.; MOLLER, P. & SCHIRRMACHER, V. (1990). Postoperative active specific immunisation in colorectal cancer patients with virus-modified autologous tumour-cell vaccine. Cancer, 66, 1517–1523.

BOON, T.; SNICK, J.V.; PEL, A.V.; UYTTENHOVE, C. & MARCHAND, M. (1980). Immunogenic variants obtained by mutagenesis of mouse mastocytoma P815. II. T lymphocyte-mediated cytosis. J. Exp. Med., 152, 1184–1193.

BOONE, C. & BLACKMAN, K. (1972). Augmented immunogenicity of tumour cell homogenates infected with influenza virus. Cancer Res., 32, 1018–1022.

BRUNNER, K., MACDONALD, R. & CEROTTINI, J. (1981). Quantitation and clonal isolation of cytolytic T lymphocyte precursors selectively infiltrating murine sarcoma virus-induced tumours. J. Exp. Med., 154, 362–373.

CAMPBELL, H.; TUCKER, W.; HORT, Y.; MARTINSON, M.; MAYO, G.; CLUTTERBUCK, E.; SANDERSON, C. & YOUNG, I. (1987). Molecular cloning, nucleotide sequence, and expression of the gene encoding human eosinophil differentiation factor (interleukin 5). Proc. Natl Acad. Sci. USA, 84, 6629–6633.

CEPKO, C.; ROBERTS, B. & MULLIGAN, R. (1984). Construction and applications of a highly transmissible murine retrovirus shuttle vector. Cell, 1053–1062.
302P.M.

ECCLES, S., HECKFORD, S., ALEXANDER, P. (1980). Effect of cyclosporin A on the growth and spontaneous metastasis of syngeneic animal tumours. Br. J. Cancer, 42, 252–259.

ESUMI, N., HUNT, B., ITAYA, T., & FROST, P. (1991). Reduced tumourogenicity of murine tumour secreting γ-Interferon is due to nonspecific host responses and is unrelated to class I major histocompatibility complex expression. Cancer Res., 51, 1185–1189.

FEARON, E., ITAYA, T., HUNT, B., VOGELSTEN, B. & FROST, P. (1988). Induction in a murine tumour of immunogenic variant transfected with a foreign gene. Cancer Res., 48, 2975–2980.

FEARON, E., PARDOLE, D., ITAYA, T., GOLUMBEK, P., KARASUYAMA, H., VOGELSTEN, B. & FROST, P. (1990). Interleukin-2 production by tumour cell bypasses T helper function in the generation of an antitumor response. Cell, 60, 397–403.

GANSBACHER, B., BANNERJEE, R., DANIELS, B., ZIER, K., CRONIN, K. & GILBOA, E. (1990). Retroviral vector-mediated γ-Interferon gene transfer into tumour cells generates potent and long lasting antitumor immunity. Cancer Res., 50, 7820–7825.

GANSBACHER, B., ZIER, K., DANIELS, B., CRONIN, K., BANNERRJI, R. & GILBOA, E. (1990). Interleukin 2 gene transfer into tumour cells abrogates tumorigenicity and induces protective immunity. J. Exp. Med., 172, 1217–1224.

GILLIS, S. & SMITH, K. (1977). Long term culture of tumour-specific cytotoxic T cells. Nature, 268, 154–156.

GOLUMBEK, P., LAZENBY, A., LEVITSKY, H., JAFFEE, L., KARASUYAMA, H., BAKER, M. & PARDOLE, D. (1991). Treatment of established nonspecific cancer by tumour cells engineered to secrete interleukin-4. Science, 254, 713–717.

HOCK, H., DORSCI, M., DIAMANTSSTEIN, T. & BLANKENSTEIN, T. (1991). Interleukin 7 induces CD4+ T cell-dependent tumor rejection. J. Exp. Med., 174, 1291–1298.

HUI, K., GROSVELD, F. & FESTENSTEIN, H. (1984). Rejection of transplantable AKR leukemia cells following MH C DNA-mediated cell transformation. Nature, 311, 750–752.

ITO, T., WANG, D.-Q., MARU, M., NAKAJIMA, K., KATO, S., KURIMURA, T. & WAKAMIYA, N. (1990). Antitumor efficacy of virus-modified tumour cells vaccine. Cancer Res., 50, 6915–6918.

KLEIN, G., SJOGREN, H., KLEIN, E. & HELLSTROM, K. (1960). Demonstration of resistance against methylcholanthrene-induced sarcomas in the primary autochthonous host. Cancer Res., 20, 1561–1572.

KOBAYASHI, H., SENDO, F., SHIRAI, T., KAJI, H., KODAMA, T. & SAITO, H. (1969). Modification in growth of transplantable rat tumours exposed to Friend virus. J. Natl Cancer Inst., 42, 413–419.

LEE, F., YOKOTA, T., OTSUKA, T., MEYERSON, P., VILLARET, D., COFFMAN, R., MOSMANN, T., RENNICK, D., ROEHM, N., SMITH, C., ZLOTNIK, A. & ARAI, K. (1986). Isolation and characterization of a mouse interleukin cDNA clone that expresses B-cell stimulatory factor 1 and T-cell and mast-cell-stimulating activities. Proc. Natl Acad. Sci. USA, 83, 2061–2065.

LEY, V., LANGLADE-DEMOYEN, P., KOURILSKY, P. & LARSSON-SCIIARD, E. (1991). Interleukin 2-dependent activation of tumor-specific cytotoxic T lymphocytes in vivo. Eur. J. Immunol., 21, 851–854.

LICHTMEN, A., KURT-JONES, E. & ABBAS, A. (1987). B cell stimulatory factor 1 and not interleukin 2 is the autocrine growth factor for some helper T lymphocytes. Proc. Natl Acad. Sci. USA, 84, 824.

LINDENMANN, J. & KLEIN, P. (1967). Viral oncology: increased immunogenicity of host cell antigen associated with influenza virus. J. Exp. Med., 126, 93–108.

MARKOWITZ, D., GOFF, S. & BANK, A. (1988). Construction and use of a safe and efficient amphotropic packaging cell line. Virology, 167, 400–406.

MCBRIDE, W., THACKER, J., COMORA, S., ECONOMOU, J., KELLEY, D., HOGGE, D., DUBINETT, S. & DOUGHERTY, G. (1992). Genetic modification of a murine fibrosarcoma to produce interleukin 7 stimulates host cell infiltration and tumour immunity. Cancer Res., 52, 3931–3937.

MILLER, A. (1992). Human gene therapy comes of age. Nature, 375, 455–460.

MILLER, A. & BUTTIMORE, C. (1986). Redesign of retrovirus packaging cell lines to avoid recombination leading to helper virus production. Mol. & Cell. Biol., 6, 2895–2902.

OHARA, J. & PAUL, W. (1985). B cell stimulatory factor BSF-1: production of a monoclonal antibody and molecular characterisation. Nature, 315, 333.

PAUL, W. (1991). Interleukin-4: a prototypic immunoregulatory lymphokine. Blood, 77, 1859–1870.

PREHN, R. & MAIN, J. (1957). Immunity to methyl-cholanthrene-induced sarcomas. J. Natl Cancer Inst., 18, 769–778.

ROSENBERG, S. (1992a). Immunisation of cancer patients using autologous cancer cells modified by insertion of the gene for Interleukin-2. Human Gene Therapy, 3, 75–90.

ROSENBERG, S. (1992b). Immunisation of cancer patients using autologous cancer cells modified by insertion of the gene for tumour necrosis factor. Human Gene Therapy, 3, 57–73.

ROSENBERG, S., LOTZE, M., YANG, J., ABERGOLD, P., LINEHAN, W., SEIPP, C. & WHITE, D. (1989). Experience with the use of high-dose interleukin-2 in the treatment of 652 cancer patients. Ann. Surg., 210, 474–485.

RUSSELL, S., ECCLES, S., FLEMMING, C., JOHNSEN, C. & COLLINS, M. (1991). Decreased tumorigenicity of a transplantable rat sarcoma following transfer and expression of an IL-2 cDNA. Int. J. Cancer, 47, 244–251.

SMITH, K. (1988). Interleukin-2: infection, impact, and implications. Science, 240, 1169–1176.

STRATH, M., WARREN, D. & SANDERSON, C. (1985). Detection of cosinophilis using an eosinophil peroxidase assay. J. Immunol. Meth., 33, 209.

SUGIURA, C., ITAYA, T., KONDO, N., OIKAWA, T., KUZUMAKI, N., TAKEI, N., HOSOKAWA, M. & KOBAYASHI, H. (1988). Xenogenization of tumor cells by transfection with plasmid containing env gene of Friend leukaemia virus. Jpn. J. Cancer Res., 79, 1259–1263.

TANAKA, K., ISELBACHK, K., KHOURY, G. & JAY, G. (1985). Reversal of oncogenesis by the expression of a major histocompatibility complex class I gene. Science, 228, 26–30.

TEPPER, R., COFFMAN, R. & LEDER, P. (1992). An eosinophil-dependent mechanism for the antitumour effect of interleukin-4. Science, 257, 548–551.

THOR, R., PATTENGLE, P. & LEDER, P. (1989). Murine Interleukin-4 displays potent anti-tumor activity in vivo. Cell, 77, 503–512.

UYTTENHOVE, C., MARYANSKI, J. & BOON, T. (1983). Escape of mouse mastocytoma P815 after nearly complete rejection is due to antigen-loss variants rather than immunosuppression. J. Exp. Med., 157, 1040–1052.

WALLICH, R., BULBUC, N., HAMMERLING, G., KATZAV, S., SEGAL, S. & FELDMAN, M. (1985). Abrogation of metastatic properties of tumor cells by de novo expression of H-2K antigens following H-2 gene transfection. Nature, 315, 301–305.

WATANABE, Y., KURIBAYASHI, H., MIYATAKE, S., NISHIHIRA, K., NAKAYAMA, E.-I., TANAYAMA, T. & SAKATA, T.-A. (1989). Exogenous expression of mouse interferon γ-cDNA in mouse neuroblastoma C1300 cells results in reduced tumorigenicity by augmented anti-tumor immunity. Proc. Natl Acad. Sci. USA, 86, 9456–9460.

YAMADA, G., KIMURA, Y., SONODA, H., HARADA, H., TAKI, S., MULLIGAN, R., OSAWA, H., DIAMANSSTEIN, T., YOKOYAMA, S. & TANIGUCHI, T. (1987). Retroviral expression of the human IL-2 gene in a murine T cell line results in cell growth autonomy and tumorigenicity. EMBO J., 6, 2705–2709.