A Novel Approach to Tumor Suppression with Microencapsulated Recombinant Cells

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ABSTRACT

A novel approach to cancer gene therapy is to implant microcapsules containing nonautologous cells engineered to secrete molecules with antineoplastic properties. The efficacy of this treatment is now tested in a mouse model bearing HER-2/neu-positive tumors. Nonautologous mouse myoblasts (C2C12) were genetically modified to secrete interleukin-2 linked to the Fv region of a humanized antibody with affinity to HER-2/neu. The resulting fusion protein, sFvIL-2, would encompass immune-stimulatory cytokine activity now targeted to the HER-2/neu—expressing tumor. These recombinant cells were then immunoprotected with alginate-poly-L-lysine-alginate microcapsules before implantation into tumor-bearing mice. Treatment with these encapsulated cells led to a delay in tumor progression and prolonged survival of the animals. The long-term efficacy was limited by an inflammatory reaction against the implanted microcapsules probably because of the secreted cytokine and antigenic response against the xenogeneic fusion protein itself. However, over the short term (initial 2 weeks), efficacy was confirmed when a significant amount of biologically active interleukin-2 was detected systemically, and targeting of the fusion protein to the HER-2/neu—expressing tumor was shown immunohistochemically. The tumor suppression in the treated animals was associated with increased apoptosis and necrosis in the tumor tissue, thus demonstrating successful targeting of the antiproliferative effect to the tumors by this delivery paradigm. In conclusion, this new approach to systemic cancer gene therapy needs to be modified to provide long-term delivery, but has demonstrated short-term efficacy and potential to become a cost-effective, benign, and non-viral-based adjunct to the current armory of anticancer strategies.

OVERVIEW SUMMARY

Immunosolation provides a potentially safe and effective method of delivering recombinant therapeutic molecules. Its application as a drug-delivery platform for the treatment of cancer has shown promising developments recently. In this approach, a nonautologous cell line is genetically modified to secrete a recombinant product with potential for tumor suppression. Such a cell line may be implanted without graft rejection into all patients with similar neoplastic disease. We use a mouse model of human breast cancer overexpressing the HER-2/neu receptor to test the potential efficacy of delivering a targeted interleukin-2 for the immunotherapy of tumors. This has now been shown to be effective in tumor suppression and prolonged survival.

INTRODUCTION

Immunosolation technology using nonautologous cells is a novel alternative approach to the treatment of cancer. It involves the implantation of genetically modified, nonautologous cells secreting a gene product with tumor suppression potential from within immunoprotected microcapsules. The cell lines are rendered immunologically tolerant to nonautologous recipients by protection within microcapsules so that a single cell line may be implanted into different nonautologous individuals. The microcapsules used would be sufficiently permeable to allow the passage of recombinant products and nutrients while the entry of the host’s immune mediators would be prohibited because of their larger size (Chang, 1995; Chang et al., 1999). Once implanted, these microcapsules should function as “microreactors”
to deliver a constitutive and continuous source of the antineo-
plastic recombinant protein, thus circumventing the problems
of toxicity, limited half-lives, variation in circulating levels, and
repetitive administration of bolus protein injection.

The concept of gene therapy with immunoinosolation targeting
mendelian inherited diseases is relatively straightforward. A
previously missing protein product caused by the genetic mu-
tation is now provided continuously with the “microreactors,”
which are the encapsulated cells genetically modified to secrete
the required protein. The efficacy of this approach has now been
demonstrated in several genetic diseases in murine models, e.g.,
growth-hormone deficiency in dwarfism (Al-Hendy et al., 1995,
1996), β-glucuronidase deficiency in the lysosomal storage dis-
ease MPSVII (Ross et al., 2000a,b), and factor IX in hemo-
philia B (Van Raamsdonk et al., 2002). However, the treatment
of cancer with this approach is highly dependent on the nature
of the cancer and type of molecules chosen for the tumor sup-
pression. In this study, we chose a tumor expressing the sur-
face antigen HER-2/neu, and an antitumor recombinant mole-
ecule encoded by the single chain Fv region (sFv) of a humanized
monoclonal antibody recognizing human HER-2 fused to the
cytokine, interleukin-2 (IL-2). HER-2/neu is a proto-oncogene
encoding a 185-kd transmembrane receptor tyrosine kinase
(Hynes et al., 1994). Because the amplification and overexpres-
sion of this gene has been described in several human can-
cers including mammary and ovarian carcinomas (Cirisano et
al., 1996; Baselga et al., 1997; Meden et al., 1997), it serves as
a practical target for specific cancer therapeutic strategies
(Baselga et al., 1996; Pegram et al., 1998). Fusion of anti-HER2
sFv to IL-2 could then potentially allow targeting of a potent
immune stimulatory cytokine (Gillis et al., 1978) to HER-2/neu-
expressing tumors in vivo, and provoke an IL-2–driven immune
reaction against the tumor cells (Xiang et al., 1994). Such tar-
geted fusion proteins have been shown to stimulate the cell-me-
diated killing activity of cytotoxic T lymphocytes (CTLs)
(Gillies et al., 1992; Xiang et al., 1994; Becker et al., 1996),
in addition to lymphokine activated killer (LAK) cells. Im-
munotherapy via the administration of recombinant IL-2 has
been shown to be of value in the treatment of patients with ad-
vanced melanoma or renal cell carcinoma (Rosenberg, 1997;
Hoffman and Figlin, 2000) but in vivo efficacy of recombinant
IL-2 treatment has been limited by its associated severe sys-
temic toxicity and difficulties in maintaining prolonged high
concentration of the cytokine in the tumor microenvironment
(Rosenstein et al., 1986; Rosenberg et al., 1989). Because
IL-2 is known to have a short half-life in serum, it is required
in high doses to induce a therapeutic effect, thus further con-
tributing to its toxicity (Siegel et al., 1991; Addison et al.,
1995).

We hypothesize that the application of microencapsulation
to deliver systemically and continuously a low level of the
sFv-IL-2 immunoconjugate targeted to the tumor may over-
come some of the above problems and serves as a novel par-
adigm for cancer treatment. This fusion protein was shown
to retain both IL-2 bioactivity as well as HER-2/neu binding
affinity (Li et al., 1999). Hence, the delivery of this bifunc-
tional fusion protein would provide an effective method of
targeting therapeutic doses of the cytokine IL-2 to HER-
2/neu–positive tumors.

MATERIALS AND METHODS

Cell lines

Mammalian expression plasmid-encoding sFvIL-2 (pcDNA-
H520C9sFv-hIL-2) was constructed as described previously (Li
et al., 1999) by fusing the sFv antibody cDNA versus human
HER-2 from a baculovirus expression plasmid pACHis-
5209sFv to the wild-type human IL-2 cDNA from plasmid
pLW46. pcDNA-H520C9sFv-hIL-2 was transfected into a
mouse myoblast cell line derived from CH3 mice, C2C12
(ATCC, Rockville, MD; catalog #CRL-1772) by calcium phos-
phate precipitation (Chang, 1994), selected with G418 and
screened with enzyme-linked immunosorbent assay (ELISA)
for human interleukin-2 (hIL-2) as described previously (Li
et al., 1999), using a secondary antibody of goat anti-rabbit
immunoglobulin G (IgG)/alkaline phosphatase conjugate (Gibco
BRL, Ontario, Canada). Human 293 cells stably transfected
with the H520C9sFvIL-2–expressing plasmid were generated
as described (Li et al., 1999) and used as a positive control for
sFvIL-2 expression.

B16/neu mouse cells genetically modified from a B16/F0
parental cell line to express human HER-2/neu on its cell surface
was a generous gift from Dr. Louis Weiner, Fox Chase Cancer
Center, Philadelphia, PA. The B16/neu and the 293 cells were
maintained in Dulbecco’s minimal media supplemented with 10%
fetal bovine serum (FBS), 1% penicillin/streptomycin and 1.2
mg/ml of G418. CTLL2 cells (ATCC, Manassas, VA) used for
the IL-2 bioassay (Gillis et al., 1978) were maintained in supple-
mented RPMI media with 100 IU IL-2 to allow for rapid growth.

Animals

Five- to 6-week old C57BL/6 (Charles River, Ontario,
Canada) female mice were used in accordance with Canadian
Institutional Animal Care guidelines. Serum from orbital bleeds
or cardiac punctures was stored at −70°C until use.

The tumor model was developed by injecting 5 × 106
B16/neu cells into the left flank of the mice. Tumor dimen-
sions were measured with calipers to estimate the volume ac-
cording to \( V = \frac{a \times b^2}{2} \), where \( a \) is the longest diameter and \( b \) the short-
est (Heike et al., 1997). Mice were killed when the tumor vol-
ume reached 1 cm³ or 28 days posttumor cell injection. All mice
arriving at the end point were killed with isoflurane followed by
rapid cervical dislocation.

Microencapsulation

Alginate-Poly-4-Lysine-Alginate (APA) microcapsules
containing either parental C2C12 cells or the derivative
C2C12sFvIL-2 expressing the immunocompetent were con-
structed as previously described (Peirone et al., 1998; Van
Raamsdonk et al., 2000). Briefly, cells were resuspended in
filtered 1.5% alginate (Improved Kelmar, a gift from the Nut-
raSweet Kelco Company, San Diego, CA) at a concentration of
2 million cells per milliliter of alginate solution, extruded
through a 27-gauge needle under constant rate and pressure into
1.1% CaCl₂, followed by a series of washes whereby coatings of
poly-4-lysine and alginate were added. The resulting APA-mi-
roencapsulated cells were used immediately for implantation.
Western blot to detect sFvIL-2 and tissue preparation

Western blots were performed according to standard procedures (Sambrook et al., 1989). Tissue extracts were prepared by sonicating (at 30-sec intervals) in homogenization buffer (25 mM Tris, 0.1 M NaCl, 0.2% Triton X-100, pH 7.6) using 100 mg of wet weight tissue per 100 μl of buffer and centrifuging at 14,000 revolutions per minute (rpm) for 30 min (in an Eppendorf centrifuge kept at 10°C). The supernatant was stored at −70°C until use. For the detection of sFvIL-2, the primary and secondary antibodies were as used in the ELISA described above. Bands were visualized with BCIP and NBT according to the manufacturer’s protocols (Gibco BRL, Ontario, Canada).

CTLL2 assay

IL-2 biologic activity was determined by proliferation of IL-2–dependent CTLL-2 (Gillis et al., 1978) using alamarBlue (AccuMed, Westlake, OH) according to the supplier’s instruction at the end of an overnight incubation to measure proliferative activity of IL-2.

Anti-sFvIL-2 antibody assay

The titer of antibodies produced by the mice against the transgene product was quantified with a competition assay against 293sFvIL-2–treated media. One microliter serum samples from mice were added in the antigen-binding step of the ELISA for IL-2 (Li et al., 1999). The loss of detectable IL-2 was taken as neutralizing activity of the given sample.

Immunohistochemistry and histology

Tissues were fixed and processed for paraffin sectioning and hematoxylin and eosin (H&E) staining by standard protocols (Histology Laboratory, McMaster University Hospital, Hamilton, Ontario, Canada). Mitotic and apoptotic indices were obtained by counting the corresponding cells in 10 high-power fields (400×) per given slide. This was repeated for a total of 5 slides (mean value ± range). Necrotic index was determined by the percent of necrosis over entire area of tumor [10 high-power fields (400×) per slide for each of 5 slides].

For immunohistochemical analysis, paraffin sections, after removal of paraffin, were permeabilized and reacted with the primary antibody (mouse anti-human-IL-2 MAB202, R&D Systems, Minneapolis, MN) at 1:100 dilution in phosphate buffer supplemented with 5% goat serum in a moist chamber for 1 hr at 37°C. After washing, the slides were incubated similarly with the secondary antibody (goat anti-mouse IgG-FITC, Jackson Laboratories, Bar Harbor, ME) at a dilution of 1:1000 before washing and mounting for viewing immediately with fluorescence microscopy. True-positive signals were assessed qualitatively for presence or absence of sFvIL-2 by use of Image Pro 6.0 Plus software (Media Cybernetics, Silver Spring, MD).

RESULTS

Development of microencapsulated cells secreting sFvIL2

A mouse myoblast cell line, C2C12, was transfected via calcium phosphate precipitation with the plasmid pcDNA-H520C9sFv-hIL-2 encoding the fusion protein sFvIL-2 (Li et al., 1999). Stable clones (n = 87) were screened by an ELISA directed against human IL-2, and the clone secreting the highest level of the fusion protein (0.41 ng per 10^5 cells per day) was chosen for encapsulation. The media from this cell line (C2C12sFvIL-2) was examined with Western blot analysis (Fig. 1) to confirm the molecular mass (45 kd) of the fusion protein, consistent with the previously reported value (Li et al., 1999). As expected, this band was not observed in the media from the nontransfected parental cell line.

With a cell line stably expressing sFvIL-2, we further examined how effective this cell line was in expressing the transgene in the microcapsular environment and if the product could diffuse freely from the microcapsule membrane (Table 1). Hence, sFvIL-2 content was assayed in the media incubated with encapsulated sFvIL-2–secreting cells. The secretion of sFvIL-2 from encapsulated cells was examined weekly and compared to that of the nonencapsulated cells (Table 1, in vitro studies). Although transgene expression was transiently diminished immediately after the encapsulation process (Table 1, day 0), this was increased to approximately half that of the cells on a monolayer culture (Table 1, caption) within a week (Table 1, day 7). In fact, by the following week, the level of fusion protein detected in the media (Table 1, day 14) was increased to 170% compared to that of the naked cells (Table 1, caption), and maintained at least until day 21. To test if this enhanced secretion in vitro was replicated in vivo, we implanted mice with the microencapsulated cells for varying times before retrieving them to assay for the secretion of the fusion protein.
(Table 1, in vivo studies). A similar pattern was observed: on day 0 (1 day after implantation), the retrieved microcapsules were unable to deliver any transgene product, but by day 7 post-implantation, approximately 70% more fusion protein was detected in the media from the retrieved microcapsules compared to that of the nonencapsulated cells. However, this enhanced delivery was not sustained. By day 14 postimplantation, the rate of fusion protein secretion was reduced to approximately 50% of that of the nonencapsulated cells. By day 21 postimplantation, no fusion protein was produced from the retrieved capsules.

The viability of the encapsulated cells from the retrieved microcapsules provided some insight into the reason for the diminishing level of fusion protein delivery in vivo (Table 1, in vivo studies). Within the first week of implantation, the viability of the encapsulated cells was maintained close to the preimplantation level, thus accounting for the high level of fusion protein production on day 7. By day 14 postimplantation, however, the number of viable cells per microcapsule increased by approximately sevenfold, this applied only to the approximately 10% of the microcapsules that remained free in the intraperitoneal cavity. The remainder of the microcapsules were found embedded in fibrotic tissue (Fig. 2A-I) and no longer contained any viable cells (data not shown). By day 21, no free microcapsule could be recovered in the intraperitoneal cavity, thus accounting for the lack of fusion protein secretion and viable cells in the in vivo samples (Table 1, day 21). This is also consistent with the decreased level of fusion protein observed in the seral compartment by day 21 (Fig. 3) as described below.

**Delivery of sFvIL-2–secreting microcapsules in vivo**

The efficacy of fusion protein delivery was first characterized in normal C57BL6 mice with no tumor burden (Fig. 3). The amount of IL-2 that could be measured in circulation was quantified at weekly intervals with a standard CTLL-2 assay. In these mice, the delivery of IL-2 to the circulatory compartment was at its highest level within the first week, decreased slightly by day 14, and dramatically to almost background level by day 21 postimplantation. The time course of delivery is correlated qualitatively with the profile of fusion protein secretion from the recovered microcapsules in vivo (Table 1). This gradual loss of circulating IL-2 was also associated with a concurrent development of antibody against the fusion protein. This was deduced from the titer of antibodies present in sera that could neutralize sFvIL-2 in an ELISA assay (Fig. 4). Such antibodies were not present at day 7, when the circulating level of sFvIL-2 was the highest in the serum. After day 14, the antibody titer continued to increase until the end of the experiment on day 28, at which time the circulating level of sFvIL-2 was reduced to background level.

The efficacy of this system in delivering the cytokine to the various intraperitoneal organs was measured by implanting tumor-bearing mice with encapsulated sFvIL-2–secreting cells. The bioactivity associated with IL-2 was measured in various intraperitoneal organs and the tumor (Fig. 5). All the abdominal organs measured, i.e., kidney, liver, and spleen, showed elevated levels of IL-2 bioactivity while the tumor showed only approximately 20% of the organ levels. In the mock-treated controls implanted with encapsulated but nontransfected cells, the level of detectable IL-2 bioactivity was at least threefold lower in the intraperitoneal organs and twofold lower in the tumors, indicating that the enhanced IL-2 activity was associated with the implantation of encapsulated sFvIL-2–secreting cells.

**Efficacy in treating B16/neu tumors**

Mice injected with the experimentally determined minimal tumor dose (data not shown) of $5 \times 10^6$ B16/neu tumor cells developed palpable tumor after the first week of injection. The tumor volume increased gradually during the following week but escalated to an exponential phase of expansion from day 14 to day 18, at which point the tumor volume reached the end

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**Table 1. Characterization of Microencapsulated Cells for Implantation In Vitro and In Vivo**

<table>
<thead>
<tr>
<th>Days Postimplantation</th>
<th>0</th>
<th>7</th>
<th>14</th>
<th>21</th>
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<tr>
<td><strong>sFvIL-2 secretion</strong></td>
<td></td>
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<tr>
<td>(ng/1000 cells per day)</td>
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<tr>
<td>In vitro</td>
<td>0.05 ± 0.00</td>
<td>0.21 ± 0.15</td>
<td>0.71 ± 0.15</td>
<td>0.67 ± 0.13</td>
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<tr>
<td>In vivo</td>
<td>0.00 ± 0.00</td>
<td>0.75 ± 0.28</td>
<td>0.24 ± 0.08</td>
<td>0.00</td>
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<tr>
<td><strong>Viability</strong></td>
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<tr>
<td>(cell number per capsule)</td>
<td></td>
<td></td>
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<tr>
<td>In vitro</td>
<td>603 ± 66</td>
<td>207 ± 41</td>
<td>678 ± 7</td>
<td>856 ± 125</td>
</tr>
<tr>
<td>In vivo</td>
<td>603 ± 66</td>
<td>550 ± 38</td>
<td>4261 ± 43</td>
<td>0.00</td>
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</table>

Transfected C5C12 cells secreting 0.41 ± 0.02 ng sFvIL-2/1000 cells per day were encapsulated and kept either in vitro under normal tissue culture conditions or in vivo after implantation on day 0 into the mouse intraperitoneal cavities. Media were collected over a 24-hr period from either the microcapsules kept in vitro or those retrieved from the intraperitoneal implantation and returned to normal tissue culture conditions. The rates of sFvIL-2 secretion were determined by assaying for the fusion protein sFvIL-2 in the media with enzyme-linked immunosorbent assay (ELISA). The viabilities of the encapsulated cells were estimated from alamarBlue.

*a* Value applied to those microcapsules that were found free within the intraperitoneal cavity (~10% of the implanted capsule load).
point of larger than 1 cm³ so that the mice had to be killed according to institutional animal care guidelines (Fig. 6A). During this course of tumor development, the mice were implanted on day 14 with either encapsulated C2C12-sFvIL-2 cells or encapsulated nontransfected parental cells as the mock control. In the mock-control series, there was no difference in tumor progression from the untreated series and the animals also had to be killed soon after day 18 because of tumor progression. In contrast, the group treated with the encapsulated recombinant cells showed a dramatic delay in the tumor expansion. Instead of entering an exponential phase of growth after day 14, the tumors only increased in volume gradually from day 15 to day 24 (corresponding to day 7 postimplantation of microcapsules). After this point, the tumor suppression effect was no longer observed in the implanted animals, and the tumor expansion entered into the exponential phase in spite of the microcapsule treatment.

The suppression of tumor expansion by the encapsulated cells was corroborated with the improved survival of the treated animals (Fig. 6B). Hence, while only 25% of the control or
mock-treated animals survived after day 21 of tumor cell injection, 60% of the treated animals were still alive by day 25, and 10% survived until the end of the experiment on day 28.

Histologic examination of liver, kidney, and spleen sections from mice treated with encapsulated C2C12sFvIL-2 cells had shown some acute immune responses that were not observed in the mock-treated animals. In the liver, hepatocytes showed signs of vacuolation and ballooning consistent with an acute inflammatory response. Similar staining of spleen sections from these mice had shown a marked increase in the volume of the lymphocyte-containing white pulp, which showed activation by follicle formation. Gross anatomic examination of euthanized mice 7–10 days postimplantation of encapsulated C2C12sFvIL-2 cells showed some fibrous tissue development around the microcapsules that were not seen with encapsulated parental C2C12 cells. This tissue and the mesenteric embedded capsules were processed for H&E staining to show the surrounding inflammatory exudate including fibrin, neutrophils, histocytes, and a zone of necrotic cellular debris around the microcapsules. This caused some local destruction of the mesenteric adipose tissue (Fig. 2A-I), in contrast to untreated, healthy mouse mesentery (Fig. 2A-II).

The tumor deposits were examined histologically 7 days after microcapsule implantation (Fig. 2B, 2C). Untreated (Fig. 2B-I) and mock-treated (Fig. 2B-II) tumors examined with H&E staining showed no obvious histologic differences in terms of size, mitotic activities, or areas of necrosis. However, tumors from mice that were treated with the encapsulated C2C12sFvIL-2 cells (Fig. 2B-III) showed a dramatic inflammatory response. Apoptotic cells as well as necrotic debris were significantly increased (p < 0.001) in addition to a decrease in mitotic index (p < 0.01). Comparing the untreated and mock-

![Graph](image)

**FIG. 3.** Delivery of sFvIL-2 to the seral compartment. Normal mice (n = 3) were implanted on day 0 with encapsulated recombinant C2C12 cells secreting the fusion protein. At various days postimplantation, serum samples were obtained to assay for IL-2 bioactivity. Sera from mock-treated controls (mice implanted with microcapsules containing nontransfected C2C12 cells) or normal untreated mice showed no IL-2 bioactivity under the same conditions (data not shown).

![Graph](image)

**FIG. 4.** Host immune reaction against secreted sFvIL-2 and implanted microcapsules. Mice were implanted intraperitoneally on day 0 with microcapsules containing either transfected C2C12 cells secreting sFvIL-2 or nontransfected C2C12 cells (n = 3 in each group). Development of antibody in the sera against the fusion protein sFvIL-2 was followed from day 0 to day 28. The antibody titer is expressed as micrograms (µg) of sFvIL-2 neutralized per microliter (µl) of serum.
FIG. 5. Interleukin-2 (IL-2) levels in various tissues. Tumor-bearing mice were implanted with microcapsules containing either nontransfected C2C12 cells (mock-treated) or fusion-protein-secreting C2C12-sFvIL-2 cells (treated) on day 0 (n = 3 in each group). On day 7 postimplantation, the mice were killed and various organs and the tumors were removed to assay for IL-2 biologic activity. The units are expressed as average international unit (IU) per milligram (mg) of protein ± standard deviation (SD).

FIG. 6. Tumor suppression and life-span extension with immunoisolation. A: Time course of tumor progression. Mice were injected with tumor cells on day 0 and were either left untreated (○, n = 10), mock-treated with encapsulated parental C2C12 cells (◆, n = 10) or treated with encapsulated C2C12-sFvIL-2 cells secreting sFvIL-2 (■, n = 10). The size of the palpable tumors was measured at various days postinjection. Error bars represent standard errors of the mean for each value plotted. B: Time course of mice survival. Survival plot of the mice depicted in (A) when they were considered to have reached their end point on their tumors arriving at a volume of 1 cm³.
treated tumors, the treatment with encapsulated C2C12sFvIL-2 cells led to an increase of twofold to threefold increase in apoptotic (Fig. 7-I) and necrotic (Fig. 7-II) indices in the tumor tissue. In addition, immunohistochemical analysis for the humanized sFvIL-2 at the tumor confirmed the presence of the immunoconjugate at the B16/neu cells, which were not present in either the untreated or the mock-treated controls (Fig. 2C). The presence of the positive signal in the cytoplasmic compartment of the tumor cells indicated the internalization of the transgene product delivered (Fig. 2C-I).

DISCUSSION

The feasibility of delivering a fusion protein sFvIL-2 of the correct molecular weight (Fig. 1) with IL-2 bioactivity has now been shown both in vitro and in vivo from encapsulated cells (Table 1, Fig. 3). Media from the microcapsules kept under normal tissue culture condition or retrieved from the mouse intraperitoneal cavity after implantation showed continued fusion protein secretion (Table 1). However, after 21 days of implantation in mice, the microcapsules were no longer capable of delivering the fusion protein. The reason for this is likely the inflammatory and antigenic responses in the host to be discussed later. That this fusion protein was delivered to the recipient was shown by the significant levels of IL-2 delivered to the serum (Fig. 3) and the intraperitoneal organs such as kidney, spleen, and liver (Fig. 5). Most important, the targeting of the fusion protein to the tumor was achieved as shown with immunohistochemical analysis (Fig. 2C).

Once the fusion protein binds to tumor cells through the antibody portion, the local concentration of IL-2 is increased, which in turn should attract and bind to host T cells in the vicinity through the IL-2 receptor. This local activation of T cells presented within the proper major histocompatibility complex (MHC) I context, should activate the downstream signals required in T-cell activation for cell-mediated killing. IL-2 occupies a central role in the augmentation of cell-mediated immune responses by stimulating and supporting a broad range of immune cells in vitro and in vivo, including T and B cells, macrophages, and natural killer cells (NK), as well as stimulating the production of lymphokine-activated killer (LAK) cells derived from NK cells that can effectively mediate antibody-dependent cellular toxicity. These multiple immune responses caused by the delivery of IL-2 should account for the significantly increased levels of tumor cell apoptosis (Fig. 7-I) as well as necrosis (Fig. 7-II). The twofold increase in apoptotic and necrotic indices in the tumors from mice receiving encapsulated sFvIL-2 cells is consistent with localized IL-2 mediated therapy (Gillies et al., 1992). Other cytokines such as IL-4 or IL-13 have been delivered via xenogeneic encapsulated cells for the treatment of collagen-induced arthritis, and efficacy was also demonstrated by a significant reduction in the severity of the articular disease (Bessis et al., 1999). Hence, the delivery of a bioactive cytokine to a localized site is an achievable goal.

The end-point analysis of tumor development is perhaps of greater clinical importance. It is now shown that the delivery of the fusion protein via microencapsulated cells can lead to a significant retardation of tumor progression, concomitant with an improved life span in the treated animals (Fig. 6). However, the improvements in clinical outcomes are short-lived, lasting for the first 7–10 days after implantation before tumor progression resumed at the untreated rate. This coincided with a dramatic decrease in circulating level of IL-2 (Fig. 3, day 21), as well as an emerging high level of antibodies against the fusion protein (Fig. 4). Furthermore, the encapsulated cells retrieved from the implanted animals at this later time point (day 21) were no longer secreting the fusion protein (Table 1, in vivo studies).

The loss of long-term efficacy observed above may be attributed in part to the xenogeneic human origin of the fusion protein. When a xenogeneic (human factor IX into mice) or a novel therapeutic gene product (mouse β-glucuronidase to a null-mutant mouse) was delivered, an antigenic response is triggered, associated with rapid clearance of the delivered transgene product (Hortelano et al., 1996), and loss of long-term efficacy (Ross et al., 2000a). However, such antigenic response can be suppressed (Potter et al., 1998), leading to restoration of effective delivery of the trangene product (Ross et al., 2000a). Because no host immune responses were triggered by alginate encapsulated monkey CV1 cells secreting murine IL-4, IL-5 or interferon-γ (IFN-γ) (all type II cytokines) in

![Fig. 7](image-url) Pathologic indices of the tumor. From the hematoxylin and eosin (H&E)-stained slides, mitotic, apoptotic, and necrotic indices were compared among the tumors from three groups of mice: untreated controls, mock-treated controls implanted with encapsulated parental cells, and treated mice implanted with encapsulated cells secreting the fusion protein (n = 3 per group). Significant difference from untreated and sham controls (**p < 0.01, ***p < 0.001).
mice, or encapsulated Chinese hamster ovary (CHO) cells secreting the murine IFN-α (a type-1 cytokine) (Savelkoul et al., 1994), it is likely that the lack of long-term IL-2 delivery observed (Table 1, Fig. 6) is a problem that may be resolved by using a murine-derived fusion protein instead. This is supported when syngeneic proteins were delivered in other studies using this technology. These studies confirmed the delivery of transgene products for up to 178 days in mice receiving mouse growth hormone (Al-Hendy et al., 1995).

However, concurrent with the observed decrease in the delivery of IL-2 into serum (Fig. 3) and increase in neutralizing antibodies against the fusion protein (Fig. 4), we also observed fibroblastic tissue reaction surrounding the microcapsules containing C2C12sFvIL-2 cells (Fig. 2A-I). Such inflammatory response was not observed in mice implanted with encapsulated parental C2C12 cells (data not shown). The microcapsules were encapsulated by an acute and chronic inflammatory response with neutrophils and fibrous tissue. The surrounding native tissue did show some local necrosis, with only a few viable cells remaining within the capsules. This inflammatory response may have caused the loss of continued fusion protein production from the microcapsules after 21 days of implantation (Table 1, in vivo studies). However, no indicators of chronic inflammation were observed. Although the antibody response (Fig. 4) is likely because a humanized protein was being delivered, the tissue responses, including the acute inflammation and the high local necrosis are consistent with high levels of IL-2 bioactivity in the peritoneal cavity, a reaction also observed in rats (Siegel and Puri, 1991). Hence, although the delivery of cytokines via this approach has been shown to be feasible, such immune responses to the delivered cytokines should certainly be assessed and anti-inflammatory medication may be indicated. As of yet, no general rule can be applied toward the effects of delivering potent immune-stimulating compounds from such implantable device, since no host immune responses were triggered by alginate-encapsulated monkey CV1 cells secreting murine IL-4 or IL-5 in mice, or encapsulated CHO cells secreting the murine IFN-α (a type-1 cytokine) (Savelkoul et al., 1994).

In theory, recombinant molecules with ability to disrupt cancer progression via a variety of metabolic pathways may be considered for this delivery platform (Cirone et al., 2001). Efficacy in using encapsulation for the treatment of pancreatic cancer by enhanced prodrug metabolism (Kröger et al., 1999), and treatment of glioma by endostatin delivery (Joki et al., 2001; Read et al., 2001) has already been demonstrated. The advantages of this approach are efficiency, safety, and potential cost effectiveness. Once a recombinant cell line has been created, it can be used for all recipients requiring the same recombinant product replacement. It can be stored indefinitely until needed, thoroughly characterized for quality-control purposes before implantation, and requires no patient-specific genetic manipulation. The approach is surgically benign via injection with syringe or catheter. Standard cell lines for implantation are genetically modified by chemical transfection without recourse to viral vectors, thus removing concerns about viral recombination, uncontrolled dissemination, insertional mutagenesis or host-cell damage from irreversible genetic alteration of the recipient’s own cells. Furthermore, the microcapsules are potentially retrievable. In principle, the “treatment” can be terminated when desired by removing the microcapsules in case of unanticipated adverse reaction, thus offering additional control and safety. In addition, the recombinant product is delivered in situ continuously and in low doses, thus obviating the need for product purification and repeated bolus injection. The costs of product purification and repeat administrations, and the toxicity of high-dose delivery by bolus injection are considerably reduced.

In conclusion, an alternative approach to treating cancer through systemic delivery of a tumor-targeted cytokine with microencapsulation of recombinant cells is a feasible goal. The many advantages of this delivery system are further enhanced by the platform nature of this technology, amenable to the delivery of molecules important in a variety of pathways that can intervene cancer progression (Cirone et al., 2001). For these reasons, delivery of recombinant gene products for cancer therapy via microencapsulation is a scientifically and clinically viable concept (Chang et al., 1999).

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