The Antibody-Mediated Targeted Delivery of Interleukin-15 and GM-CSF to the Tumor Neovasculature Inhibits Tumor Growth and Metastasis

Manuela Kaspar, Eveline Trachsel, and Dario Neri

Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, Zurich, Switzerland

Abstract

Tumor-targeting immunocytokines represent a new class of anticancer pharmaceutical agents, which often display a superior therapeutic index compared with the corresponding unconjugated cytokines. In this article, we have studied the anticancer properties of interleukin-15 (IL-15) and granulocyte macrophage colony-stimulating factor (GM-CSF), fused to the human antibody fragment scFv(L19), specific to the EDB domain of fibronectin, a marker of angiogenesis. The immunocytokines L19-IL-15 and L19-GM-CSF were expressed in mammalian cells and purified to homogeneity, revealing no loss of cytokine activity in in vitro assays. Furthermore, the ability of the two immunocytokines to selectively localize to tumors in vivo was confirmed by biodistribution analysis with radioiodinated protein preparations. L19-IL-15 and L19-GM-CSF displayed a potent antitumor activity both in s.c. and in metastatic F9 and C51 murine models of cancer in immunocompetent mice. This therapeutic action was superior compared with IL-15–based and GM-CSF–based fusion proteins, containing antibodies of irrelevant specificity in the mouse, which were used as non–tumor-targeting controls. For both L19-IL-15 and L19-GM-CSF immunocytokines, CD8+ T cells seemed to mostly contribute to the therapeutic action as shown by in vivo cell depletion experiments. The results presented in this article are of clinical significance, considering the fact that the sequence of EDB is identical in mouse and man and that the tumor-targeting ability of the L19 antibody has been extensively shown in clinical trials in patients with cancer.

Introduction

Many proinflammatory recombinant cytokines display potent anticancer activities, but their preclinical and clinical use is often limited by the fact that unacceptable toxicities can be encountered already at very low cytokine doses, preventing escalation to therapeutically active concentrations. In most cases, cytokines do not preferentially accumulate at the tumor site following i.v. administration. For this reason, the antibody-based targeted delivery of cytokines to the tumor environment seems to be a promising strategy for enhancing the therapeutic index of these potent anticancer agents (1, 2). The targeted delivery of bioactive agents to the tumoral neovasculature seems to be a particularly promising anticancer therapeutic modality, considering the fact that a vigorous neovasculature development is a characteristic feature of aggressive solid tumors and that tumor blood vessels are readily accessible for i.v. administered therapeutic agents (2, 3).

The EDB domain of fibronectin is one of the best-characterized markers of angiogenesis described so far (4, 5). This 91-amino acid domain is typically absent in human plasma and in normal adult tissues (exception made for the endometrium in the proliferative phase and some vessels in the ovaries) but is strongly expressed in most aggressive solid tumors, with a prominent vascular and/or stromal pattern of staining (6, 7). EDB is identical in sequence between mouse and man, thus facilitating preclinical studies in the syngeneic immunocompetent setting. Furthermore, the tumor-targeting ability of the high-affinity human antibody L19 (8), specific to EDB, has been well established both in animal models of cancer (9–15) and in patients with solid tumors (16).

A recombinant fusion protein, consisting of the L19 antibody in scFv format fused to human interleukin (IL)-2 (17, 18), is currently being investigated in multicenter phase II clinical studies in Italy and Germany. Furthermore, the L19 antibody in SIP format radiolabeled with iodine-131 (12, 13, 15) is being investigated in phase II radioimmunotherapy clinical trials, whereas a recombinant fusion protein between L19 and human tumor necrosis factor (TNF) is entering clinical studies for applications in oncology.

The L19 antibody is one of the best-characterized antibodies in terms of number of therapeutic derivatives, which have been tested in preclinical models of cancer for their therapeutic activity and in biodistribution studies. L19 derivatives include fusions to procoagulant factors (19), enzymes (20), charged proteins (21, 22), and cytokines, such as IL-2 (17, 18), TNF (23–25), vascular endothelial growth factors (26), IL-12 (24, 27, 28), IFN-γ (29), and IL-10 (30). Furthermore, the L19 antibody has been chemically coupled to radionuclides (9–15), photosensitizers (31, 32), and drugs with cleavable linkers. Among these derivatives, L19-cytokine fusion proteins possibly represent the most promising class of anticancer therapeutic agents.

To explore the anticancer potential of immunocytokines using antibodies of proven tumor-targeting ability, such as L19, it is imperative to continue expanding our investigations to a larger set of cytokines. In fact, L19-cytokine fusions have displayed therapeutic activities in incurable models of murine cancer, thus justifying the ongoing clinical development programs. Furthermore, different

Requests for reprints: Dario Neri, Institute of Pharmaceutical Sciences, Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, Wolfgang-Pauli-Str. 10, ETH Hönggerberg, HCI G396, CH-8093 Zurich, Switzerland. Phone: 41-44-633-74-01; Fax: 41-44-633-13-58; E-mail: neri@pharma.ethz.ch.

1 D. Neri, unpublished results.
L19-cytokine fusions display different tumor-targeting performance in vivo when assessed by quantitative biodistribution studies. These investigations shed light on the molecular determinants, which result in efficient in vivo targeting. Finally, different L19-cytokine fusions often display potent antitumor activity using different mechanisms of action and may display synergistic therapeutic effects when used in combination.

In this study, we have investigated the anticancer therapeutic properties of IL-15 and granulocyte macrophage colony-stimulating factor (GM-CSF), fused to the L19 antibody in scFv format. IL-15 is a potent proinflammatory cytokine, whose mechanism of action partially overlaps with the one of IL-2. Both cytokines stimulate the proliferation of T cells, induce the generation of CTLs, facilitate the proliferation of and the synthesis of immunoglobulin by B cells, and induce the generation and persistence of natural killer (NK) cells (33). However, in many adaptive immune responses, IL-2 and IL-15 have distinct and often competing roles. IL-2, through its unique role in activation-induced cell death and its participation in maintenance of peripheral Treg cells, is involved in the elimination of self-reactive T cells. By contrast, IL-15 is important in the maintenance of long-lasting, high-avidity T-cell responses and it achieves this by supporting the survival of CD8+ memory T cells. Therefore, IL-15 might be a better choice than IL-2 for fusion with the vascular tumor-targeting antibody L19 and for the treatment of cancer.

GM-CSF is a cytokine associated with the growth and differentiation of hematopoietic cells. Furthermore, it is a potent immunostimulator with pleiotropic effects, including the augmentation of antigen presentation in a variety of cells, increased expression of MHC class II on monocytes and adhesion molecules on granulocytes and monocytes, and amplification of T-cell proliferation (34–36). Previous work by the Penichet and Morrison groups (36) had shown that a recombinant fusion between an IgG specific to HER-2/neu and GM-CSF can induce a substantial growth retardation for mouse tumor cells stably transfected with the human HER-2/neu gene (37). Furthermore, recombinant human GM-CSF (Leukine) is a pharmaceutical product, which is indicated for use following induction of chemotherapy in patients with acute myelogenous leukemia to shorten time to neutrophil recovery and to reduce the incidence of severe and life-threatening infections. Additionally, it is used in multiple stem cell transplantation settings for acceleration of myeloid recovery.

Both L19-IL-15 and L19-GM-CSF were expressed in mammalian cells and purified to homogeneity. The two immunocytokines were shown to be active in vitro and to target tumors in vivo. Furthermore, both fusion proteins displayed a substantial antitumor activity in both s.c. and metastatic tumors in syngeneic mice. For acceleration of myeloid recovery, both fusion proteins displayed a substantial antican-...
cloned into the mammalian cell expression vector pcDNA3.1(+) using the HindIII and NotI sites of the vector.


HEK-293 cells were stably transfected with the previously described plasmids and selection was carried out in the presence of G418 (0.5 μg/mL). Clones of G418-resistant cells were screened for expression of the fusion protein by ELISA using recombinant EDB domain of human fibronectin or lysozyme as antigens and an anti-His, tag antibody (Sigma) for detection as described. The fusion proteins were purified from cell culture medium by affinity chromatography over antigen columns as described previously (9, 41). The size of the fusion proteins was analyzed in reducing and nonreducing conditions on SDS-PAGE and in native conditions by fast protein liquid chromatography gel filtration on a Superdex S-200 size exclusion column (Amersham Pharmacia Biotech).

Deglycosylation

To deglycosylate purified L19-IL-15 and IL-15-L19, 40 μg protein was incubated with 2,500 units PNGase F for 20 h at 37°C.

Bioactivity Assay

Cytokine activity of L19-IL-15, IL-15-L19, and HyHEL10-IL-15 was determined by doing a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) dye reduction assay on CTLL-2 (42). CTLL-2 cells were seeded into 96-well plates at the concentration of 5 × 10^4 per well in 200 μL of complete medium containing varying amounts of hIL-15 standard or the fusion proteins at a maximum of 10 ng/mL IL-15 equivalents and serial dilutions. After 72 h, 20 μL of 5 mg/mL MTT (Sigma) in PBS were added to each well. After 2 to 4 h, the plate was centrifuged and cells were lysed with DMSO (Fluka) and read for absorbance at 570 nm. The experiment was done in quadruplicates.

The cytokine activity of monoclonal and homodimeric L19-GM-CSF as well as HyHEL10-GM-CSF was determined by doing a proliferation assay on murine mast cells MC/9 (43). Cells were seeded into 96-well plates at the concentration of 5 × 10^5 per well in 200 μL of complete medium containing varying amounts of recombinant murine GM-CSF standard or the fusion proteins at a maximum of 5 ng/mL GM-CSF equivalents and serial dilutions. After 72 h, 20 μL of 5 mg/mL MTT (Sigma) in PBS were added to each well. After 2 to 4 h, the plate was centrifuged and cells were lysed with DMSO (Fluka) and read for absorbance at 570 nm. The experiment was done in triplicates.

Biodistribution Experiments

The in vivo targeting performance was evaluated by biodistribution analysis as described before (14, 17). Brieﬂy, purified L19-IL-15, IL-15-L19, and L19-GM-CSF were radioiodinated and injected into the tail vein of immunocompetent 129SvEv mice bearing s.c. implanted F9 murine teratocarcinoma. Mice were sacriﬁced 24 h after the injection (2 μg, 6 μCi for L19-IL-15; 2.5 μg, 5 μCi for IL-15-L19; and 2 μg, 2 μCi for L19-GM-CSF/mouse). Radioiodinated immunocytokines were also studied alone or mixed with a molar excess of unlabeled (“cold”) protein to evaluate the dose dependency of the tumor-targeting properties of L19-GM-CSF and L19-IL-15. Organs were weighed and radioactivity was counted with a Packard Cobra gamma counter. Radioactivity content of representative organs was expressed as the percentage of the injected dose per gram of tissue (%ID/g).

Tumor Mouse Models

Tumor-bearing mice were obtained by injecting s.c. 10^6 F9 murine teratocarcinoma cells in 10- to 12-week-old female 129SvEv mice or 10^6 C51 murine colon adenocarcinoma cells in 10- to 12-week-old female BALB/c mice, respectively. All mice were purchased from Charles Rivers Laboratories.

Formal i.v. injections of immunocytokines were followed to fuse IL-15 at the COOH terminus or at the NH2 avidity due to its bivalent nature.

The sequential fusion of the GM-CSF gene yielded an immunocytokine, which coexisted in a monomeric and noncovalent homodimeric form (“diabody”) of the immunocytokines was chosen. This format increases the size of the immunocytokines over the renal threshold and shows increased avidity due to its bivalent nature.

The sequential fusion of the scFv(L19) gene with the GM-CSF gene yielded an immunocytokine, which coexisted in a monomeric and noncovalent homodimeric form (Fig. 1A). Shortening the scFv(L19) linker from a 14-amino acid peptide to a 5-amino acid GSSGG sequence resulted, as expected (44, 45), in the formation of a stable homodimeric immunocytokine (Fig. 1B). A similar strategy was followed to fuse IL-15 at the COOH terminus or at the NH2 terminus of the scFv(L19) in diabody format with the GSSGG linker, yielding the immunocytokines L19-IL-15 (Fig. 1C) and IL-15-L19 (Fig. 1D), respectively. Only the homodimeric immunocytokines were later used for in vivo experiments.

Following similar experimental approaches, we used scFv-(HyHEL10), an antibody fragment specific to hen egg lysozyme as an antibody of irrelevant specificity in the mouse, for the construction of immunocytokines, which were used as negative controls in tumor therapy experiments.
The fusion proteins were cloned and expressed in stably transfected HEK-293 cells and purified to homogeneity by affinity chromatography on antigen columns (Fig. 1).

**Activity assays of huIL-15 and muGM-CSF.** The biological activity of huIL-15 and muGM-CSF was determined by their ability to induce proliferation of the cytokine-dependent cell lines CTLL-2 and MC/9, respectively, using the colorimetric MTT dye reduction assay.

Figure 2 shows that only the COOH-terminal fusions of scFv and IL-15 displayed EC\textsubscript{50} values comparable with the one of recombinant huIL-15 when tested for their ability to proliferate the cytokine-dependent CTLL-2 cells, whereas the immunocytokine IL-15-L19 showed no biological activity at all.

L19-IL-15 and IL-15-L19 were deglycosylated with PNGase F (an amidase that cleaves between the innermost GLcNAc and asparagine residues from N-linked glycoproteins) to assess if their different glycosylation state could influence their different in vitro behavior. Both immunocytokines IL-15-IL-19 and IL-15-L19 had the same size after deglycosylation as confirmed by SDS-PAGE analysis (Fig. 2B), and the deglycosylated IL-15-IL-19 was active in the CTLL-2 proliferation assay (Fig. 2C).

The biological activity of all GM-CSF immunocytokines was comparable with the one of the recombinant cytokine in the MC/9 cell proliferation assay (Fig. 2D).

**Biodistribution studies.** The tumor-targeting ability of L19-IL-15, IL-15-L19, and L19-GM-CSF was tested by quantitative biodistribution analysis in 129SvEv mice bearing s.c. F9 tumors using i.v. injections of radioiodinated protein preparations. L19-IL-15 displayed a preferential accumulation at the tumor site 24 h after injection. The pharmacokinetic profile did not substantially change at doses ranging between 2 and 62 \( \mu \)g (Fig. 3A). Similarly, IL-15-L19 showed high tumor uptake values after 24 h (4.1% ID/g) but somewhat higher values in normal organs, ranging between 0.7% and 2.8% ID/g (Fig. 3A). Tumor to blood ratios were comparable with 5.3:1 for L19-IL-15 and 4:1 for IL-15-L19. For the sake of comparison, the fusion protein L19-IL-2 in the same tumor model exhibited 4% ID/g in the tumor at 24 h, with a tumor to blood ratio of 28 (17).

L19-GM-CSF exhibited only modest tumor to organ ratios at 24 h (Fig. 3B) when administered at low dose (1 \( \mu \)g). However, the tumor-targeting performance increased when the immunocytokine dose was increased (at a 2 \( \mu \)g dose, tumor to organ ratios ranging...
between 2 and 12) or when the radiiodinated protein was coadministered with a 20- or 30-fold molar excess on unlabeled fusion protein to saturate GM-CSF receptors on leukocytes.

**Therapy experiments with s.c. F9 and C51 murine tumors in immunocompetent mice.** 129SvEv mice bearing F9 tumors, or BALB/c mice bearing C51 colon adenocarcinomas, were chosen as s.c. murine models of cancer for the assessment of the therapeutic action of L19-IL-15, IL-15-L19, and L19-GM-CSF. Because L19-GM-CSF had exhibited a dose-dependent tumor-targeting performance, this fusion protein was tested at different doses (15, 30, and 60 μg per injection) in therapy experiments in the F9 s.c. tumor model. Only a dose of 60 μg per injection (daily injections over 4 days) lead to significant tumor growth inhibition (data not shown). In the following therapy experiments, the fusion proteins were administered at a dose of 75 μg (thrice every second day) corresponding to an i.v. injected volume of 300 μL. At this dose, no side effects could be detected for both fusion proteins. The immunocytokines started to form higher-order noncovalent oligomers at concentrations >0.25 mg/mL, thus preventing escalation above the 75 μg dose.

Figure 4 shows that both L19-IL-15 and L19-GM-CSF substantially inhibited tumor growth compared with control mice treated with saline \( P = 0.008 \) at day 15 for L19-IL-15; \( P = 0.005 \) for L19-GM-CSF at day 14 for F9 tumors (Fig. 4A and B); \( P = 0.001 \) at day 14 for L19-IL-15; \( P = 0.026 \) for L19-GM-CSF at day 14 for C51 tumors (Fig. 4C and D). IL-15-L19 could also substantially inhibited tumor growth compared with control mice treated with saline (\( P = 0.038 \) at day 17; data not shown). However, L19-IL-15 showed a stronger therapeutic benefit.

By contrast, HyHEL10-IL-15 and HyHEL10-GM-CSF did not significantly inhibit tumor growth compared with saline (\( P = 0.1 \) and 0.07 at days 15 and 14, respectively; Fig. 4A and B), showing the contribution of the L19-mediated cytokine targeting to the therapeutic effect.

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Figure 2. Activity assays of huIL-15 and muGM-CSF. The effect of huIL-15 on the proliferation of CTLL-2 cells was tested in vitro. A, L19-IL-15, HyHEL10-IL-15, IL-15-L19 (fully glycosylated), and recombinant huIL-15 as positive control were added at different concentrations to CTLL-2 cells, and after 72 h, viable cells were visualized by MTT incorporation. B, SDS-PAGE gel of L19-IL-15 and IL-15-L19 before and after treatment with PNGase F to deglycosylated fusion proteins. 1, deglycosylated IL-15-L19; 2, glycosylated IL-15-L19; 3, deglycosylated L19-IL-15; 4, glycosylated L19-IL-15. C, activity assay of IL-15-L19 after complete deglycosylation with PNGase F. D, GM-CSF activity assay. The effect of muGM-CSF on the proliferation of MC/9 mast cells was tested in vitro. L19-GM-CSF, HyHEL10-GM-CSF, and recombinant muGM-CSF as positive control were added at different concentrations to MC/9 cells, and after 48 h, viable cells were visualized by MTT incorporation. The experiment was done in triplicates. Points, mean; bars, SE.
Therapy experiments in metastatic models of F9 and C51 tumors. When injected i.v., F9 tumor cells may give rise to liver metastases (29, 40), whereas C51 cells preferentially yield lung metastases (27). We used these animal models to assess the antimetastatic potential of L19-IL-15 and L19-GM-CSF. The immunocytokines were administered i.v. thrice (days 3, 5, and 7 following tumor cell injection). Twenty-one days after tumor cell inoculation, mice were sacrificed and metastatic load was assessed by counting foci in relevant organs (Fig. 5). Both immunocytokines displayed a clear antimetastatic activity compared with control mice treated with saline (L19-IL-15: \( P = 0.0008 \) for F9 tumors and \( P = 0.001 \) for C51 tumors; L19-GM-CSF: \( P = 0.014 \) for F9 tumors and \( P = 0.0008 \) for C51 tumors). L19-IL-15 was strikingly active against F9 tumors (three of five free of metastases), whereas L19-GM-CSF did better in the C51 lung metastasis setting.

**In vivo T-cell depletion and response to therapy.** To elucidate the T-cell contribution to the antitumoral activity of L19-IL-15 and L19-GM-CSF, we did therapy studies in mice bearing s.c. F9 tumors, depleting CD4+ or CD8+ T lymphocytes. Depleting antibodies were administered 6 days before tumor implantation to ensure >95% cell depletion at the time of tumor cell inoculation. Mice received a therapeutic dose of immunocytokines at days 5, 7, and 9 after tumor cell implantation. Figure 5 clearly shows how CD4+ T-cell depletion has no significant effect on the immunocytokine-mediated anticancer activity of IL-15 and GM-CSF immunocytokines.
antitumor effect of both fusion proteins (\(P = 0.93\) for L19-IL-15 and 
\(P = 0.28\) for L19-GM-CSF), whereas CD8\(^+\) T-cell depletion 
completely abrogates the therapeutic effect (\(P = 0.03\) for L19-IL-15 
and \(P = 0.018\) for L19-GM-CSF). These data indicate a crucial 
contribution of CD8\(^+\) T lymphocytes as mediators for the 
therapeutic activity of L19-IL-15 and L19-GM-CSF.

**Discussion**

In this article, we have described the cloning, expression, and 
anticancer properties of two novel immunocytokines (L19-IL-15 
and L19-GM-CSF), which recognize the EDB domain of fibronectin, 
a marker of angiogenesis. Both immunocytokines were shown to 
preferentially localize in tumors following i.v. injection and to 
potentiate inhibit the growth of s.c. and metastatic F9 and C51 
tumors in immunocompetent murine models.

The L19 antibody is uniquely suited for the systematic 
investigation of the therapeutic potential of antibody-based 
anticancer pharmaceuticals because it recognizes a tumor-
associated antigen, which is virtually absent in all normal tissues 
and is strongly expressed in the majority of aggressive solid tumors, 
with a prominent perivascular pattern of staining (12, 17, 22, 
30–32). Furthermore, EDB is identical in sequence among different 
species (mouse, rat, rabbit, dog, monkey, and man), which allows 
the use of immunocompetent animal models with the same antibody, 
which will be used for clinical studies. Finally, the L19 
antibody has been extensively characterized in biodistribution and 
imaging studies both in animal models of pathology and in patients 
with cancer. Two phase II clinical trials are currently in progress 
with L19-IL-2 and the L19 antibody in SIP format, labeled with 
iodine-131, whereas L19-TNF has completed monkey toxicology 
studies and is about to enter clinical testing. In this respect, the 
comparative analysis of the therapeutic potential of different L19 
derivatives allows a clinically relevant evaluation of various 
antibody functionalization strategies. More than 30 derivatives of 
the L19 antibody have been characterized until now by biodis-
tribution studies and by in vivo therapy experiments.

The partially overlapping immunologic activities of IL-15 and 
IL-2, together with the consideration that L19-IL-2 is in advanced 
clinical studies, raise questions about relative advantages and 
disadvantages for L19-IL-2 and L19-IL-15. In mouse models of 
cancer, both biopharmaceuticals display comparable tumor-
targeting efficacy. The clinical translation of L19-IL-2 has been 
greatly facilitated by the fact that IL-2 is an approved biopharma-
ceutical. On the other hand, both IL-15 and L19-IL-15 display an 
excellent safety profile. In murine models of cancer and vascular 
leak syndrome, IL-15 is approximately six times less toxic than IL-2, 
although the therapeutic index for IL-15 is 3-fold higher than the 
one calculated for IL-2 (46). In our study, no weight loss could be 
detected for 225 \(\mu\)g cumulative dose of L19-IL-15. The use of higher 
doses in mice will require improved formulations to prevent 
antibody oligomerization. In humans, the possibility to administer 
larger infusion volumes facilitates clinical development.

The gain of biological activity of IL-15-L19 following deglycosy-
lation is consistent with previous reports on a mutant of huIL-15 
that is not active on CTLL-2 cells (47). This protein contains the
mutations Asp8Ser and Gln108Ser and can still bind to the cytokine receptor but shows no biological activity at all. IL-15-L19, in contrast to L19-IL-15, is fully glycosylated (Fig. 2) and one of the glycosylated asparagines (Asn112) is structurally close to the essential Gln108, and this additional modification completely abolishes the in vitro activity of IL-15-L19.

In addition to L19-IL-15, this article reports for the first time a fusion protein between a tumor-targeting scFv fragment and GM-CSF. In a previous study, the groups of Penichet and Morrison had described the tumor inhibition properties of a recombinant fusion between an IgG specific to HER-2/neu and muGM-CSF (37). We normally prefer to engineer immunocytokines based on antibody fragments (e.g., scFv fragments) because the use of full immunoglobulin leads to the construction of multifunctional therapeutic proteins, which may engage Fc receptors and activate complement, in addition to antigen binding and cytokine receptor activation. Furthermore, recombinant immunocytokines devoid of Fc portion typically lead to lower uptake values in liver and spleen as well as to better tumor to organ ratios at earlier time points.

Biodistribution studies done at different antibody doses of L19-GM-CSF clearly revealed an improved tumor-targeting performance at higher doses (Fig. 3). These findings are in line with previous observations made by our group for the study of other immunocytokines. For example, the fusion protein L19-IFN-γ displays a superior tumor-targeting performance in tumor-bearing mice, which are knocked out for the IFN-γ receptor, compared with immunocompetent mice. These data suggest a possible competition between the antibody-mediated tumor targeting and the capture of immunocytokines by cells, which express the cytokine receptor and soluble receptor, respectively. In the mouse, this competition is clearly detectable for cytokines, which feature abundant and high-affinity receptors (e.g., IFN-γ and GM-CSF), but is not obviously detectable for potent immunostimulatory cytokines, which are used at ultralow doses (e.g., IL-12, IL-2, and TNF; refs. 17, 23, 28).

Tumor therapy experiments with depletion of CD4+ and CD8+ T cells (Fig. 6) clearly revealed a functional role for CD8+ T lymphocytes in the anticancer activity of both L19-IL-15 and L19-GM-CSF. This observation is different compared with the situation encountered with L19-IL-2, where the activation and tumor infiltration of NK cells was shown to be a main determinant of the immunocytokine therapeutic activity. Indeed, the therapy of F9 tumors in immunocompetent mice or nude mice gave comparable results in the case of L19-IL-2 (17). The promising anticancer activities observed for L19-IL-15 and L19-GM-CSF, together with their easy expression and excellent stability, strongly motivate clinical development programs based on these two biopharmaceuticals. The excellent tumor-targeting ability of the L19 antibody and the conservation of EDB between mouse and man suggest that the therapeutic activities observed in immunocompetent murine models may be at least in part be translated to patients in the oncology setting. However, it is worth

Figure 6. T-cell depletion of 129SvEv mice bearing s.c. F9 tumor and response to L19-IL-15 and L19-GM-CSF therapy. 129SvEv mice were depleted from CD4+ or CD8+ T cells before F9 tumor cell implantation. When tumors were clearly palpable, mice were grouped (n = 5) and therapy was started as described in Materials and Methods. Arrows, mice were injected with either L19-IL-15 (A) or L19-GM-CSF (B) at different time points.

remembering that tumor therapy studies done in mice with human antibody-based pharmaceuticals can only be done in the acute administration setting (i.e., three or four injections in up to 8 days) due to potential immunogenicity of the human protein in rodents. Only clinical studies can reveal the true pharmacological potential of L19-IL-15 and of L19-GM-CSF for the therapy of cancer.

References

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