Protective and Curative Potential of Vaccination with Interleukin-2-Gene-transfected Cells from a Spontaneous Mouse Mammary Adenocarcinoma

Federica Cavallo, Francesco Di Pierro, Mirella Giovarelli, Alberto Gulino, Alessandra Vacca, Antonella Stoppacciaro, Marco Forni, Andrea Modesti, and Guido Forni

CNR-Immunogenetics and Histocompatibility Center at the Institute of Microbiology, University of Turin, Via Sanlena 9, Turin [F. C., F. D-P., M. G., G. F.]; Department of Experimental Medicine, University of L’Aquila, 67100, Collemaggio [A. G.]; Department of Biomedicine, University La Sapienza, Viale R. Elena 324, 00161, Rome [A. S., A. V.]; Regina Margherita Children’s Hospital, Piazza Polonia 40, 10126 Turin [M. F.]; and Institute of Experimental and Social Medicine, University of Chieti, Via Vestini, 66013, Chieti, Italy [A. M.]

Abstract

The potential of interleukin 2-gene-transfected tumor cells to prevent tumor growth and cure established tumors was evaluated using cells from a spontaneous, invasive, and metastasizing mouse mammary adenocarcinoma. Tumor cells engineered to secrete interleukin 2 initially trigger a local inflammatory reaction that leads to inhibition of established parental adenocarcinomas, as well as an antigenically unrelated fibrosarcoma. The ensuing systemic immunity selectively inhibits subsequent parental cell challenges and cures established parental adenocarcinomas and their lung metastases, although less effectively as the neoplastic mass increases. Multiple injections of interleukin 2-gene-transfected tumor cells may thus be considered a new form of vaccination in the management of minimal residual disease and incipient metastases.

Introduction

Several data have shown that the local presence of cytokines in a tumor growth area triggers a potent antitumor reaction in both experimental models (1-3) and clinical trials (4). IL-2 appears of particular interest due to its central regulatory role in the immune response. It is a progression factor for T-helper and T-cytotoxic lymphocytes, which participates in B-lymphocyte activation, boosts natural killer cells, generates lymphokine activated killer activity (for a review see Ref. 5), and triggers nonspecific cytotoxicity in macrophages (6) and neutrophils (7). Studies with IL-2 that is repeatedly injected at the tumor site or around tumor-draining lymph nodes (1, 2), as well as with IL-2 directly released by IL-2 gene transduced tumor cells (for reviews see Refs. 2 and 3), have shown that it provides the signals for the efficient induction of both a nonspecific and a specific antitumor immune response. The nonspecific, delayed type of hypersensitivity reaction first elicited involves a knotty cross-talk between granulocytes, macrophages, fibroblasts, and CD4+ T-lymphocytes that often results in the induction of a tumor-specific, CD8+ T-lymphocyte mediated immune memory (1-3).

These data suggest that the local availability of IL-2 increases tumor immunogenicity and stress its use as a component of new tumor vaccines. Phase I clinical trials based on the injection of IL-2 gene transduced tumor cells are underway (8, 9), while the ability of these cells to cure established tumors has not been extensively explored in mouse models. Moreover, the biological meaning of the experimental data is difficult to determine, since proper staging of the established tumors is not reported (10-12).

To obtain a realistic assessment of the potential of IL-2 gene transduced tumor cells to vaccinate mice before tumor challenge and cure those with established tumors, we used an expression vector to introduce the complementary DNA coding for mouse IL-2 into the cells of a spontaneous, invasive, and metastasizing mouse mammary adenocarcinoma (TS/A) that arose in a female of a mouse strain of low tumor incidence (1).

Materials and Methods

In Vitro Cultures. Cell cultures were performed with sterile, disposable flasks and plates from Nunc, Roskilde, Denmark, at 37°C in a humidified 5% CO2 atmosphere, using RPMI 1640 supplemented with 10% fetal calf serum, 100 units/ml penicillin, 100 units/ml streptomycin, 50 μg/ml gentamycin, and 2.5 × 10⁻⁵ M 2-β-mercapto-ethanol (Flow Laboratories, Opera, Italy).

Tumors. TS/A-pc derive from the eighth in vitro passage of a tumor cell line established from the first in vivo transplant of a moderately differentiated mammary adenocarcinoma which arose spontaneously in a BALB/cnAnCr female mouse. TS/A-pc express major histocompatibility complex class I, but not class II, molecules and do not stimulate a syngeneic antitumor response in vivo nor in vivo (1, 13). Four × 10⁵ are about the minimal 100% TS/A-pc tumor-inducing doses in mice of the BALB/cAnCr (BALB/c mouse from Charles River Laboratories, Calco, Italy) of its origin used in these experiments. When necessary, TS/A-pc were treated with 60 μg/ml of Mit-C (Sigma, St. Louis, MO) × 10⁵ cells/ml for 30 min at 37°C (13). CE-2 is a methylcholanthrene induced sarcoma of BALB/c mice that does not cross-react antigenically with TS/A-pc (13). Two × 10⁵ cells are the minimal 100% CE-2 tumor inducing dose.

In Vivo Evaluation of Tumor Growth. Seven-week-old female BALB/c were challenged (day 0) s.c. in the left flank or i.v. with the lethal dose of TS/A-pc or CE-2 cells. For 120 days, mice were inspected twice weekly and s.c. neoplastic masses were measured with calipers in the two perpendicular diameters in a fashion blind to the group in which they had been treated. Mice with a tumor >4 mm mean diameter were classed as tumor bearers. Mice were killed for humane reasons when the tumor exceeded 10 mm mean diameter. Following i.v. challenge, mice were killed when moribund. Care of mice was in accordance with the European community and Italian guidelines.

IL-2 Gene Transfection and IL-2 Titration. TS/A-pc were transfected by electroporation with the linearized BCMGneo (neomycin resistance gene) (TS/A-neo cells) or BCMGneo-IL-2 plasmids (14), cloned, and cultured in selective medium as previously described (13). The IL-2 produced by 1 × 10⁵ transfected cells cultured for 48 h in 1 ml of medium was evaluated as the ability to support the growth of the murine T-cell line CTLL. The IL-2 titer was calibrated against the Biological Response Modifiers Program Reference reagent human IL-2 (lot ISDP-841) (13).

Morphological Analysis. For histological evaluation, groups of 4 mice were killed 1, 7, and 14 days after challenge. Tissues were fixed, embedded in paraffin, sectioned at 4 mm, and stained with hematoxylin-eosin. Metastases were counted in lungs injected with 2 ml of 15% black India ink.
Statistical Analysis. Each experiment was performed independently four times using groups consisting of 5-6 mice. Because they gave homogeneous results the data were cumulated. The significance of differences in tumor takes was determined by Fisher's exact method; those in latency and survival time were determined by the two-tailed Wilcoxon's signed rank test.

Results

In Vivo Behavior of Parental and IL-2 Gene Transfected TS/A Adenocarcinoma Cells. In BALB/c mice, a s.c. challenge with TS/A-neo or TS/A-pc in the middle of the left flank gives rise to a rapid growing tumor (Fig. 1), while an i.v. challenge with TS/A-pc kills all mice in 30 days through the overgrowth of multiple lung metastases (Fig. 2).

A previous study has shown that a challenge of several TS/A clones releasing more than 30 units of IL-2 are promptly rejected by BALB/c mice. Comparison of the protection provided by these clones against a subsequent TS/A-pc challenge showed that clone B6.3600, which releases 3600 units of IL-2, was more effective than those releasing lower or higher amounts (13). The growth and rejection patterns of B6.3600 cells injected s.c. and i.v. are illustrated in Figs. 1 and 2.

Immunization Potential. To compare the immunizing potential of IL-2 producing and nonproducing TS/A cells against a subsequent TS/A-pc challenge, mice received a single or multiple injections of 1 × 10^5 B6.3600 cells, Mit-C TS/A-neo, or Mit-C TS/A-pc in the left or right flank, starting 30 days before TS/A-pc challenge (day 0) s.c. in the left flank or i.v. While a single injection of Mit-C TS/A-neo and Mit-C TS/A-pc did not provide any protection, significant protection was provided by B6.3600 cells. Multiple Mit-C TS/A-neo or Mit-C TS/A-pc injections were required to secure a significant protection, while full protection was provided by similar multiple injections of B6.3600 cells. No differences in immunization efficacy were found when immunizations were performed in the area of subsequent s.c. tumor challenge (data not shown) or contralaterally. Takes of the antigenically unrelated CE-2 fibrosarcoma were never impaired, showing that the protection elicited by preimmunization is restricted to TS/A-pc (Fig. 3).

Local Curative Potential. To evaluate whether these vaccinations can also affect the growth of 1, 7 or 14 day-old tumors, mice received a single or multiple s.c. injections of Mit-C TS/A-neo or Mit-C TS/A-pc or B6.3600 cells in the same tumor challenge area or contralaterally. Since these vaccinations began at the TS/A-pc staging days, their curative potential was assessed against s.c. neoplastic masses of known size (Fig. 1).

The local reaction activated by the injection of B6.3600 cells in the right flank near the TS/A-pc growing tumors very effectively impaired tumor growth (Fig. 4). A single or multiple injections completely
suppressed 1-day TS/A-pc tumors. Seven injections cured 60% of mice with 7-day TS/A-pc tumors, whereas the 4 weekly injections were less effective. Multiple injections also led to the rejection of large 14-day TS/A-pc tumors in about 30% of mice. To evaluate the specificity of this local reaction, other mice were challenged with the CE-2 fibrosarcoma in the right flank. Full protection was obtained with a single or multiple B6.3600 cell injections in the CE-2 tumor growth area 1 day after challenge, showing that the reaction elicited is not restricted to TS/A-pc. Single or multiple local injections of Mit-C TS/A-neo or Mit-C TS/A-pc never impaired the growth of both TS/A-pc and CE-2 tumors (data not shown).

Systemic Curative Potential. The systemic immunity elicited by multiple injections of B6.3600 cells in the right flank cured a significant number of mice bearing established contralateral TS/A-pc tumors, although its efficacy decreased from 25–30% for 1-day tumors to 15–20% and 8–18% for 7- and 14-day tumors, respectively. In these tumor-bearing mice, single or multiple contralateral injections of Mit-C TS/A-neo or Mit-C TS/A-pc never impaired the progression of established TS/A-pc tumors (data not shown). The growth of 1-, 7-, and 14-day CE-2 fibrosarcomas was never impaired by B6.3600 vaccination, indicating that the systemic immunity elicited is restricted to TS/A-pc tumors.

Significant protection against 1- and 7-day old lung metastases was also provided by multiple s.c. injections of B6.3600 cells in mice challenged i.v. with TS/A-pc (Fig. 4).

Discussion

The data presented here show that two distinct reaction patterns are elicited by the local presence of IL-2. The first powerful reaction leads to the rejection of both IL-2 gene transfected TS/A cells and intermingled TS/A-pc or antigen unrelated CE-2 cells. It directly depends

---

Fig. 2. Survival and lung metastases of BALB/c mice challenged i.v. with $4 \times 10^4$ TS/A-pc and B6.3600 cells. To better outline metastases, lungs were treated with black India ink as described in "Materials and Methods" and examined under a dissecting microscope. No differences in their number and size were evident in moribund mice challenged with either TS/A-pc or B6.3600 cells. Right, small and large metastases (arrowheads; H, heart) in a lung of a mouse challenged with TS/A-pc.

Fig. 3. Protective immunity elicited by vaccinations with Mit-C TS/A-pc (□), Mit-C TS/A-neo (●), and B6.3600 (◆) cells before tumor challenge. Groups of at least 24 mice challenged s.c. in the right flank or i.v. with either TS/A-pc or CE-2 cells at day 0 received a single or multiple contralateral s.c. injections of $1 \times 10^5$ Mit-C TS/A-pc, Mit-C TS/A-neo, and B6.3600 cells. Identical protection was observed in mice immunized ipsilaterally to the tumor challenge or when tumor challenges were performed 30 days after last vaccination (data not shown). In all experiments vaccination with B6.3600 cells provided a protection significantly higher ($P > 0.05-0.001$) than vaccination with Mit-C TS/A-pc and Mit-C TS/A-neo.
on the IL-2 released by IL-2 gene transfected tumor cells acting as biological pumps and is analogous to that activated by repeated injections of exogenous IL-2 around initial mouse (1, 6) and human (4) tumors. IL-2 releasing cells appears to trigger this reaction more effectively than injections of exogenous IL-2 (1, 2), probably since their IL-2 secretion is continuous. The quantity of IL-2 released by B6.3600 cells is governed by a feed back mechanism, increasing at first as they expand and then decreasing in function of the intensity of the IL-2 activated reaction. The features of this local and nonspecific reaction resemble those of delayed type hypersensitivity. Killing is mostly the work of neutrophils activated by IL-2 (13) or by secondary proinflammatory chemokines elicited by IL-2 (2).

A tumor-specific immunity follows this nonspecific antitumor reaction. The combined effect of IL-2 and a massive load of dead tumor cell debris after the destruction of IL-2 releasing tumor cells appears to secure both efficient tumor antigen presentation by IL-2 activated dendritic cells and macrophages displaying an enhanced expression of major histocompatibility complex class I and II glycoproteins and the activation of CD8+ long-lasting and tumor specific effecter cells (1, 2, 13). This passage from a nonspecific to a specific reaction allows IL-2 gene transfected tumor cells to trigger a systemic immunity and be used as live antitumor vaccine. These vaccinations never enhanced the take of the parental tumor but constantly and specifically protected against its subsequent challenges and cured a significant number of mice bearing both parental tumors and established metastases, although their efficacy decreased as the tumor mass increased. Mice vaccinated through repeated injections of B6.3600 are protected both against s.c. and i.v. challenges, even if the overall vaccination efficacy is lower against lung metastases than s.c. tumors.

These findings provide a straightforward evaluation of the vaccination potential of IL-2 gene transfected tumor cells in different settings and fit in well and complete several observations on the preimmunizing and curative potential of IL-2 releasing tumor cells (1-3, 10-12). They are strengthened by the consideration that they were obtained with a spontaneous and rapidly invasive adenocarcinoma against which it is difficult to induce a significant cell reactivity (1, 13).

Although direct comparison of these mice data with the human scenario is not possible, they nevertheless hold out the hope that this new kind of vaccine could be used in the management of minimal residual disease and small incipient metastases.

Acknowledgments

We wish to thank Dr. Robin Foa for helpful suggestions and Dr. John Iliffe for critical review of the manuscript.

References


Protective and Curative Potential of Vaccination with Interleukin-2-Gene-transfected Cells from a Spontaneous Mouse Mammary Adenocarcinoma

Federica Cavallo, Francesco Di Pierro, Mirella Giovarelli, et al.


Updated version  Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/53/21/5067

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, use this link http://cancerres.aacrjournals.org/content/53/21/5067. Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.