Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 7430–7434, August 1994
Immunology

Persistence of dormant tumor cells in the bone marrow of tumor cell-vaccinated mice correlates with long-term immunological protection

KHASHAYARSHA KHAZAIE*, SOPHIA PRIFTI*, PHILLIP BECKHOVE*, ANDREAS GRIESBACH*, STEPHEN RUSSELL†, MARRY COLLINS†, AND VOLKER SCHIRRMACHER*

*German Cancer Research Center, Department of Tumor Immunology, Im Neuenheimer Feld 280, D-69120 Heidelberg, Federal Republic of Germany; and †Institute of Cancer Research, Chester Beatty Laboratories, Fulham Road, London SW3 6JB, United Kingdom

Communicated by Max L. Birnstiel, March 16, 1994 (received for review September 23, 1993)

ABSTRACT **Live proliferation-competent and irradiated proliferation-incompetent L5178 murine lymphoma cells (Eb cell line) were compared for their potency to induce systemic anti-tumor immunity in syngeneic DBA/2 mice. The tumorigenic potential *in vivo* of live Eb cells was suppressed through local secretion of interleukin 4 (IL4) or alternatively by injection of parental cells at a site refractory to tumor growth. Inoculation of nontumorigenic doses of live Eb or Eb-IL4 cells led to long-lasting specific and systemic T-cell-mediated anti-tumor response requiring both CD4+ and CD8+ T lymphocytes. Irradiated cells offered only limited short-term protection, which could be marginally improved by IL4. The more effective protection offered by vaccination with live tumor cells correlated with rapid migration and persistence of tumor cells in the bone marrow of host animals after tumor cell inoculation. In contrast, irradiated Eb-lacZ cells had a short persistence. Tumor cells recovered from the bone marrow of host animals injected with live Eb-IL4 cells still expressed IL4. These observations indicate that in the course of vaccination with live Eb or Eb-IL4 cells, a fraction of these cells escaped destruction by host mechanisms and persisted in a dormant state in the bone marrow for long periods of time. Persistence of dormant tumor in the bone marrow correlated with the duration of anti-tumor immunity.**

Failure to develop effective tumor-rejection immune responses has been suggested to be due to a deficiency of help generated within the immune system (1, 2). In accordance with this postulate, the potential immunotherapeutic effects of a number of lymphokines have been tested through immunization of syngeneic or immunodeficient animals with tumor cell lines that have been engineered to secrete particular lymphokines for help. Protocols using multiple vaccinations at optimal doses have been successful in identifying combinations of tumor systems and lymphokines where local lymphokine secretion is effective for systemic anti-tumor protection (3) or even in the therapy of established micrometastases (4). Other recent investigations (using single-vaccination protocols) have found that, with the exception of granulocyte/macrophage-colony-stimulating factor, local secretion of lymphokines achieve little if any improvement on the immunogenicity of the irradiated parental tumor cells (5, 6).

The present investigation addresses the influence of tumor cell viability (when used as vaccine) on the induction and maintenance of long-lasting anti-tumor immunity. To this aim we have employed the well-characterized L5178 lymphoma line Eb, which expresses characteristic K^d-associated tumor antigens and elicits specific cytotoxic T-cell responses in immunized or tumor-bearing syngeneic hosts (7–9). Vaccination of syngeneic DBA/2 mice with live tumor cells was achieved either through local secretion of interleukin 4 (IL4), which is known to render tumor cells nontumorigenic (10, 11), or through the inoculation of viable parental Eb cells into a site refractory to the growth of these cells [intra-ear pinna (i.e.)]. The fate of tumor cells in the host was followed by using Eb cells that were genetically marked with the bacterial *lacZ* gene. Our observations highlight the significance of tumor cell viability, migration, and persistence in a dormant state for the establishment of effective long-term T-cell-mediated anti-tumor immunity.

MATERIALS AND METHODS

Recombinant Retroviruses, Transduction, and Characterization of Modified Tumor Cells. The pZIPneo retroviral vector (12) expressing a synthetic cDNA encoding murine IL4 was packaged in the GPE+86 packaging cell line (13). The retroviral vector harboring the bacterial *lacZ* gene fused to a nuclear transport signal sequence (14) and the CRIP packaging cell line (15) transfected with this vector were kindly provided by O. Danos. Infected Eb cells were selected for growth in the presence of G418 or stained to reveal nuclear β-galactosidase activity microscopically (16) or by flow cytofluorometry (17). Poly(A)-tailed RNA was prepared and analyzed as described (18). To generate Eb-IL4-lacZ cells, Eb-lacZ cells were superinfected with the IL4-encoding virus described above. Individual clones were obtained by limiting dilution. Murine IL4 produced by modified Eb cells or by 3T3 BMG cells (19) was titrated on the IL4-dependent T-cell line F4/4 (20) by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) colorimetric assay (21). Rat monoclonal IgG antibody to mouse IL4, 11b11 (22), completely blocked the growth response of F4/4 cells. One unit of activity was defined as the amount of IL4 that induced 50% maximal proliferation. So, for example, if 100 μl of culture supernatant from BMG cells induced 50% maximal proliferation of F4/4 cells at a dilution of 1:320, the supernatant contained 3200 units/ml.

Vaccinations, Histological Staining, and Cell Depletions. Tumor cells were washed, suspended in phosphate-buffered saline, and injected in a volume of 50 μl i.e., 100 μl s.c. or i.m., or 200 μl i.p. The cells were irradiated at 50 Gy (5000 rads) with a ^{137}Cs source (Gammacell 1000; Atomic Energy, Ottawa). Irradiation did not abrogate secretion of cytokine *in vitro* over the course of 7 days. GK 1.5 [rat monoclonal IgG antibody to mouse CD4 (23)] and 53672 [rat monoclonal IgG antibody to mouse CD8 (24)] were used for histological staining. For T-cell depletion, a single pretested effective dose (25) of depleting anti-CD8 (YTS-169.4.2, purified IgG;

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: IL4, interleukin 4; i.e., intra-ear pinna.

2.6 mg) or anti-CD4 (GK 1.5, purified IgG; 0.5 mg) monoclonal antibodies was used i.p. per mouse.

RESULTS

We introduced and expressed cDNAs to the murine IL4 gene and/or bacterial *lacZ* gene into the Eb tumor line by retroviral mediated gene transfer. Northern blot analysis (Fig. 1*A*) showed the presence of IL4-encoding viral transcripts in a clone (Eb-IL4) selected for this study, and a bioassay (Fig. 1*B*) revealed biologically active IL4 (70 units/ml) in its culture supernatant. Eb-IL4-lacZ cells produced about 10-fold less IL4 than the parental Eb-IL4 cells. Eb-IL4 cells formed no tumors in DBA/2 mice over 240 days of observation. The level of IL4 secretion determined the extent of the host response. Thus, Eb-IL4-lacZ cells developed regressive tumors in some DBA/2 mice, whereas parental Eb cells produced progressive tumors. Histological analysis showed secretion of IL4 *in situ* by inoculated Eb-IL4 cells but not by Eb cells (Fig. 2 *A* and *B*). The local secretion of IL4 induced a local nonspecific inflammatory response characterized by eosinophilic granulocytes surrounding the IL4-stained tumor cells (Fig. 2*A*), whereas $CD4^+$ or $CD8^+$ lymphocytes were clearly absent (Fig. 2 *D* and *F*). No clear inflammatory response was seen with parental Eb cells (Fig. 2*B*). These findings corroborate previous observations (10, 11, 26, 27) and suggest that the *in vivo* growth of Eb-IL4 cells is hindered, possibly through a non-T-cell-mediated host defense mechanism.

Efficacy of Tumor Cell Vaccine Cannot Be Entirely Explained by the Local Secretion of IL4. Subcutaneous inoculation of a relatively high dose of irradiated cells (10^7 cells) was required for achieving effective short-term immunity in DBA/2 mice against challenge with parental Eb tumor cells. Local secretion of IL4 improved the efficacy of the vaccine with irradiated parental Eb cells by 20–30% (compare Fig. 3 *C* and *F* with *B* and *E*). Vaccination with 10^5 irradiated Eb-IL4 or irradiated Eb cells did not confer immunity against challenge by parental tumor cells (data not shown), whereas the same dose of live Eb-IL4 cells provided 90% survival (compare Fig. 3 *A* and *D*). Resistance to tumor challenge was characterized by a lower percentage of tumor takes as well as by delayed tumor outgrowth and tumor regression.

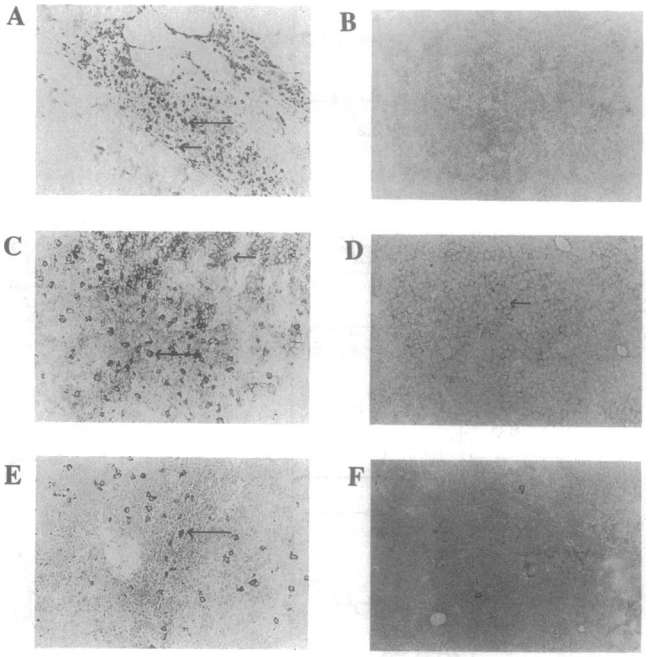

FIG. 2. Effect of Eb-IL4 vaccination on inflammatory host responses to challenge with parental Eb tumor cells. *A*, *B*, *D*, and *F* represent controls of nonvaccinated mice injected i.m. with 10^5 live Eb-IL4 (*A*, *D*, and *F*) or Eb (*B*) tumor cells. *C* and *E* show inflammatory secondary responses of Eb-IL4-vaccinated mice (10^5 Eb-IL4 cells s.c.) which were challenged after 2 weeks with 10^5 live Eb cells just like the control (*B*). All histological slides were prepared 7 days after tumor cell injection. Immunohistochemical staining was performed with anti-IL4 (*A* and *B*), anti-CD8 (*C* and *D*), or anti-CD4 (*E* and *F*) monoclonal antibody followed by peroxidase-labeled second antibody. Large arrows in *C* and *E* point, respectively, toward $CD8^+$ or $CD4^+$ inflammatory T cells; small arrows in *C* and *D* show the Eb or Eb-IL4 tumor cells, which are weakly $CD8^+$. *C* shows the edge of the tumor challenge site. Tumor cells from the Eb-IL4 injection site (*A*) but not from an Eb injection site (*B*) are stained for IL4 (large arrows). The Eb-IL4 injection site is characterized by infiltrating peroxidase-positive granulocytes (*A*, small arrow), mostly eosinophiles, but extremely poor in $CD8^+$ (*D*) and $CD4^+$ (*F*) T lymphocytes. (×200.)

The protection was specific, as (*i*) the vaccinated mice were not protected against challenge by ESb cells, a closely

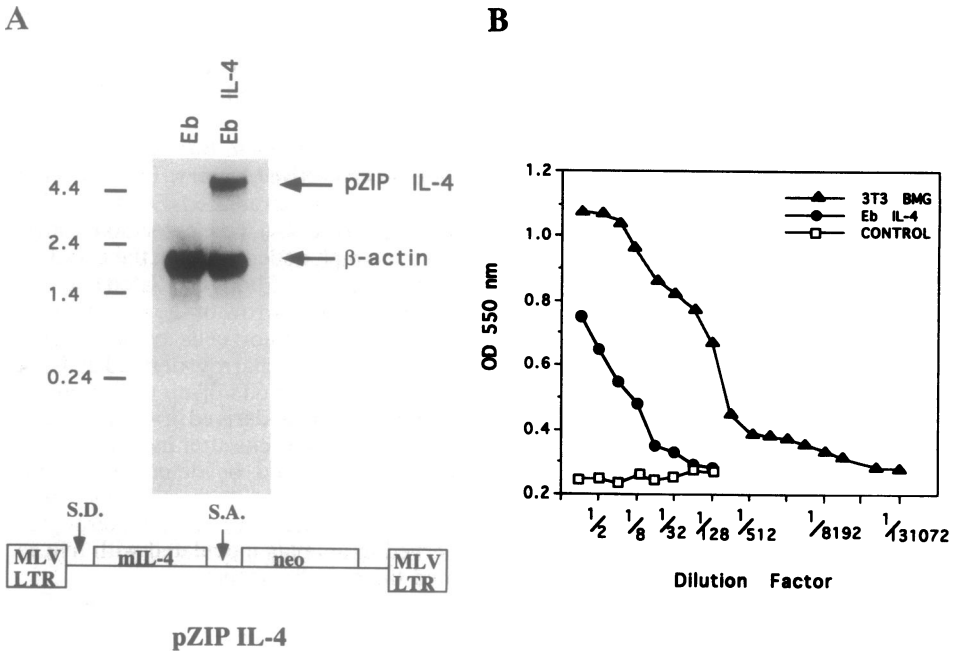

FIG. 1. IL4 expression and secretion in Eb-IL4 transfectants. (*A*) Northern blot analysis. Poly(A)-tailed RNA (5 μg per lane) from Eb and Eb-IL4 cells was electrophoresed through 1% agarose gel and blotted onto a nitrocellulose filter. IL-4 and β-actin-specific transcripts were visualized by hybridization to the corresponding [α-^{32}P]dATP-labeled cDNAs. A schematic diagram of the retroviral vector used is shown. S.D., splice donor site; S.A., splice acceptor site; MLV LTR, murine leukemia virus long terminal repeat; mIL-4, mouse IL4 cDNA; neo, neomycin (G418)-resistance marker. RNA size markers in kilobases are indicated at left. (*B*) Titration of IL4 activity in supernatant medium from the Eb and Eb-IL4 cell lines or the previously characterized IL4-producing cell line 3T3 BMG. The Eb and Eb-IL4 lines produced 0 and 70 units of IL4 per ml per 10^6 cells in 24 hr, respectively. A confluent culture of 3T3 BMG cells produced 3200 units of IL4 per ml, which was saturating for growth of the indicator cells.

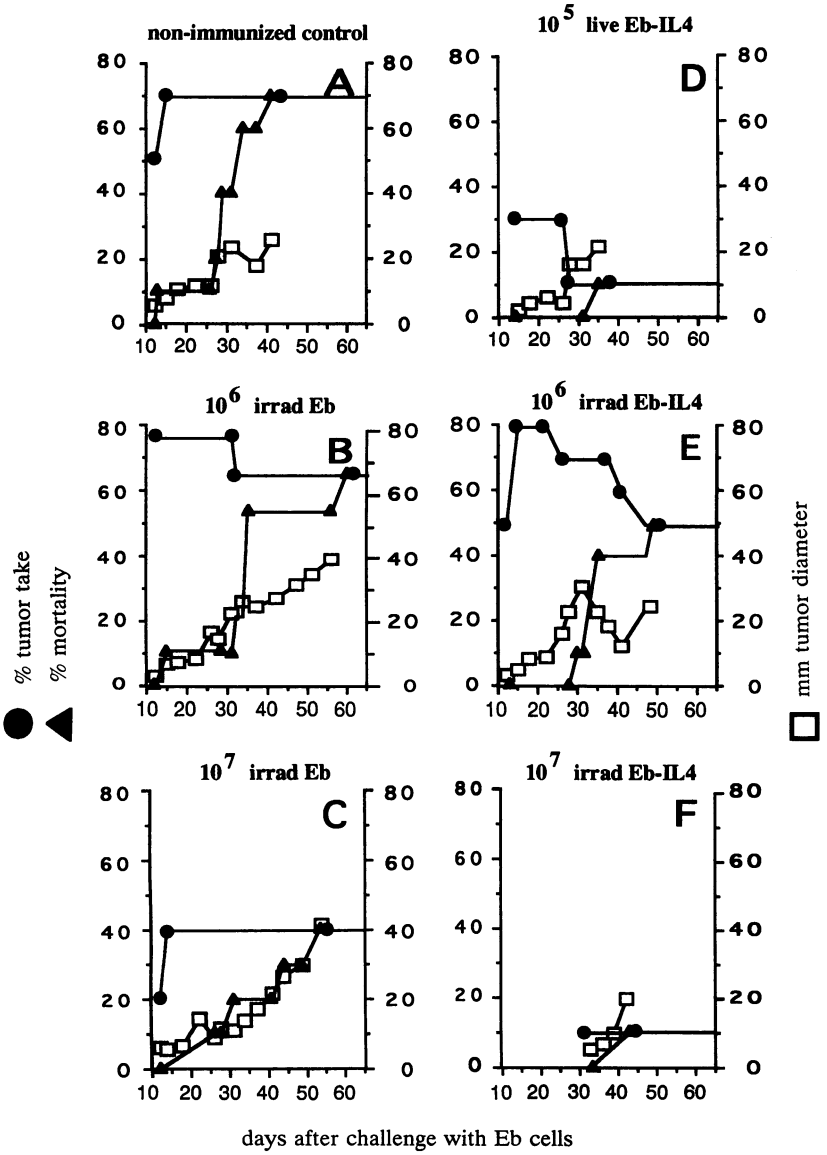

FIG. 3. Efficacy of live vs. irradiated tumor-cell vaccine and influence of local IL4 secretion. Immunity against live Eb cells was induced by immunization with either a small number (10^5) of live Eb-IL4 cells (*D*) or by higher numbers (10^6 and 10^7) of irradiated Eb (*B* and *C*) or Eb-IL4 (*E* and *F*) cells. DBA/2 mice (10 per group) were immunized by s.c. injection of irradiated Eb or Eb-IL4 cells and then challenged 2 weeks later with 10^5 live parental Eb cells. Groups were immunized as follows: 10^6 irradiated Eb cells (*B*); 10^7 irradiated Eb cells (*C*); 10^6 irradiated Eb-IL4 cells (*E*); 10^7 irradiated Eb-IL4 cells (*F*). A separate group of 10 mice were vaccinated s.c. with 10^5 live Eb-IL4 cells (*D*) and challenged as above. As control (*A*), nonimmunized mice were inoculated with 10^5 parental Eb cells. Tumor growth and survival was assessed twice per week. ●, % tumor take: number of animals bearing tumors, expressed as percentage of total number of inoculated animals; ▲, % mortality: number of animals dead due to tumor load, expressed as percentage of total number of inoculated animals; □, tumor diameter in millimeters (mean of tumor-positive animals)

related but antigenically distinct cell line (28) (data not shown), (*ii*) the site of Eb tumor challenge inoculation was strongly infiltrated with mature $CD8^+$ and $CD4^+$ T lymphocytes (Fig. 2 *C* and *E*), and (*iii*) depletion of host T cells with anti-CD8 or anti-CD4 monoclonal antibodies 2 weeks after vaccination led to a complete loss of anti-tumor immunity (data not shown).

Tumor Cell Viability Determines Vaccine Potency and Duration of Protective Anti-Tumor Memory. The anti-tumor immunity induced by irradiated cells was effective only at high doses and declined with time after vaccination (Table 1, Exp. A, groups II–IV). In contrast, s.c. or i.e. vaccination with live Eb-IL4 (Table 1, Exp. A, group V, and Exp. B, groups VI–IX) or i.e. vaccination with live Eb cells (Exp. B, groups I–V) was potent and long lasting, at relatively low cell doses. Injection of live Eb cells i.e. did not lead to tumor growth. In these experiments the dose of parental Eb cells used for challenge was increased to 2×10^6 to produce 100% mortality in the control group. The state of protective immunity induced by live-cell vaccination was extremely long lasting as demonstrated by the survival of all immunized animals challenged after 6 months with 2×10^6 parental Eb cells (Table 1, Exp. C). These results suggested a higher efficacy of live-tumor-cell vaccination, which was reflected not only in the short-term potency but also the duration of protective anti-tumor memory.

Live Eb cells were as good as live Eb-IL4 cells in providing short-term (2 weeks; Table 1, compare Exp. A, group V, and Exp. B, group III) or long-term (4–8 weeks; compare Exp. B, groups III–V and VI–IX) anti-tumor protection. It is therefore likely that the major effect of IL4 on protective memory, at least in this model system, was through enabling the use of live-cell vaccination.

Persistence of Dormant Tumor Cells in Bone Marrow Correlates with Immunological Protective Memory. Following the i.e. injection of 3×10^6 live or irradiated Eb-lacZ cells, β-galactosidase-stained cells (Fig. 4*A*) were detectable within 4 hr of i.e. injection in the lymph nodes draining the ear (data not shown). Comparable numbers of both irradiated and live cells were detectable in the bone marrow of the host animals 1 week after inoculation. Live tumor cells persisted at a similar level (>30 cells per 10^6 bone marrow derived cells) in the bone marrow for follow-up periods of up to 2 months, while the number of bone marrow-derived irradiated cells declined with time (Fig. 4*B*). By 8 weeks after inoculation, no more irradiated Eb-lacZ cells could be detected. As expected, no primary tumors developed in the i.e. inoculated mice. These observations correlated with the long-term protective memory observed in animals inoculated with live as compared to irradiated tumor-cell vaccines.

To establish the fate of IL4 gene-modified Eb cells, Eb-IL4-lacZ or Eb-IL4 cells were injected s.c. (10^6 cells per

Table 1. Long-term protective immunity after vaccination with live or irradiated (irrad.) tumor cells

Group	Vaccination (day 0)	% survival after challenge with Eb cells			
		Day 14	Day 28	Day 56	Day 180
	Exp. A (s.c. vaccination)				
I	No vaccination	30	20	20	—
II	1×10^7 Eb irrad.	60	30	40	—
III	1×10^7 Eb-IL4 irrad.	90	60	70	—
IV	1×10^5 Eb-IL4 irrad.	30	20	20	—
V	1×10^5 Eb-IL4 live	90	90	100	—
	Exp. B (i.e. vaccination)				
I	No vaccination	0	0	0	—
II	2×10^6 Eb live	80	80	—	—
III	2 (or 5) $\times 10^5$ Eb live	90	90	(100)*	—
IV	5×10^4 Eb live	—	—	60	—
V	5×10^3 Eb live	—	—	10	—
VI	2×10^6 Eb-IL4 live	—	100	—	—
VII	2 (or 5) $\times 10^5$ Eb-IL4 live	—	60	(95)*	—
VIII	5×10^4 Eb-IL4 live	—	—	10	—
IX	5×10^3 Eb-IL4 live	—	—	0	—
	Exp. C (i.e. vaccination)				
I	No vaccination	—	—	—	10
II	1×10^6 Eb-IL4 live	—	—	—	100

DBA/2 mice (at least 10 per group) were inoculated s.c. or (B&C) i.e., with the indicated numbers of irradiated or live Eb or Eb-IL4 cells, suspended in 50 μl of phosphate-buffered saline, and were challenged once after 14, 28, 56, or 180 days by s.c. injection of 1×10^5 (Exp. A), 2×10^6 (Exp. B), or 2×10^6 (Exp. C) Eb cells suspended in 100 μl of phosphate-buffered saline. Tumor growth was assessed twice weekly for up to 2 months after inoculation. Percent survival was assessed 2 months after challenge.

*Values in parentheses indicate mice vaccinated with 5×10^5 tumor cells.

animal). The Eb-IL4-lacZ cells produced small tumors which became visible from 2 to 4 weeks and regressed completely within 4 to 6 weeks after inoculation. Eb-IL4-lacZ cells were detected in bone marrow cell preparations 10, 14, and 28 days after inoculation (1–8 cells per 10^6 bone marrow cells). In mice which had regressed tumors, 3 months after inoculation the level of Eb-IL4-lacZ in the bone marrow had reached 80–120 per 10^6 cells (pool of cells from two mice). It was possible to select for the outgrowth of tumor cells from bone marrow of tumor-free mice vaccinated with Eb-IL4 or Eb-IL4-lacZ cells by culturing the cells in RPMI 1640 containing 5% fetal bovine serum for >3 weeks. The bone marrow-derived and *in vitro* expanded Eb-IL4 and Eb-IL4-lacZ cells were, respectively, strongly and weakly positive for the expression of IL4, whereas bone marrow-derived Eb-lacZ cells were negative, as revealed by cytoplasmic staining for IL4 (data not shown). These observations suggest that a fraction of Eb-IL4 or Eb-IL4-lacZ cells escape destruction by host mechanisms and persist in a dormant state in the bone marrow for long periods of time.

DISCUSSION

Optimal presentation and persistence of antigen are likely to be the most important determinants of T-cell-mediated immunity (29, 30), the major effector mechanism in the vast majority of experimental models of anti-cancer immunotherapy. The present investigation has demonstrated that the exposure of viable Eb T-lymphoma tumor cells to the immune system and the persistence of such cells in the host animal are the most important factors in the establishment and long-term maintenance of anti-tumor immunity and mem-

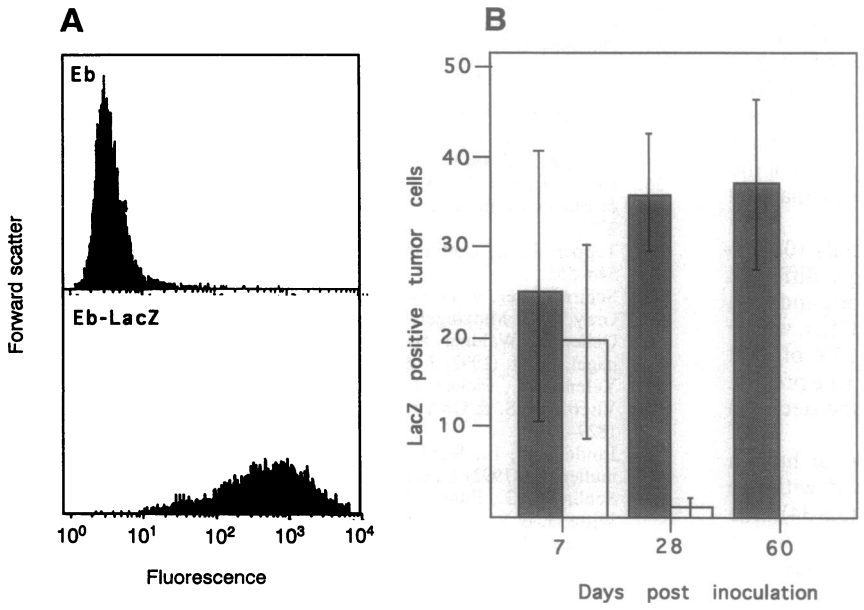

FIG. 4. Migration and persistence of tumor cells in inoculated animals. (A) Flow cytofluorometric analysis (10,000 events) showing uniform β-galactosidase (LacZ) staining (fluorescein digalactoside) of Eb-lacZ cells used for injection into host animals. (*B*) Quantitation of bone marrow-derived tumor cells. DBA/2 mice were injected i.e. with 3×10^6 live (filled bars) or irradiated (open bars) Eb-lacZ cells. At every time point, bone marrow cells were isolated from three animals per group and pooled, and duplicate samples of 10^6 cells were collected by centrifugation and stained with 5-bromo-4-chloro-3-indolyl β-D-galactoside. Average results from two independent experiments are presented as number of tumor cells per 10^6 bone marrow cells. In mice receiving irradiated Eb-lacZ cells no tumor cells were detected 60 days after inoculation.

ory. To address questions related to anti-tumor immunity and cell viability, two different experimental approaches were used: (*i*) s.c. injection of nontumorigenic IL4-secreting tumor cells as vaccine and (*ii*) injection of viable parental tumor cells at a site refractory to tumor growth. Migration and persistence of *lacZ*-expressing tumor cells in the host was followed by enzymatic staining.

While live tumor cells persisted in the bone marrow of vaccinated animals for months, irradiated tumor cells persisted only for a few weeks. The differential persistence of live vs. irradiated tumor cells in the bone marrow correlated with their contrasting potential to maintain long-lasting anti-tumor immunity. Thus, when the challenge was made later than 4 weeks after vaccination, only live tumor vaccines could effectively protect the mice against tumors: the mice were fully protected even when challenged with parental cells 6 months after vaccination. The T-cell-mediated nature of the protective response was demonstrated by (*i*) the infiltration of $CD4^+$ and $CD8^+$ cells at the site of challenge, (*ii*) the abolishment of immunological protection through antibody-mediated depletion of T cells and, (*iii*) the demonstration of an *in vitro* tumor-specific cytotoxic activity by T cells isolated from immune mice (data not shown).

It is difficult to imagine such long lasting T-cell-mediated protection without the persistence of tumor antigen. Indeed, it was possible to demonstrate the presence of Eb-lacZ or Eb-IL4-lacZ or Eb-IL4 cells in the bone marrow of tumor free mice inoculated up to 3 months earlier and to expand these cells *in vitro*. It is therefore very likely that in these instances the host response was sufficient to prevent tumor growth but not sufficient to eradicate all tumor cells. A small number of tumor cells were able to evade local immune reactions and to migrate to the bone marrow, where they persisted for long periods of time. The use of irradiated cells in vaccination was clearly suboptimal with respect to the required vaccine dose and the duration of protection, correlating with the short persistence of tumor cells in the host. Under such conditions local secretion of IL4 improved the vaccine efficacy by 20–30% (expressed in terms of percent survivors). These observations indicate that interaction of tumor and immune cells is not limited to the site of vaccination and that the persistence of viable tumor cells in the host (bone marrow) may be a critical factor in the efficacy and duration of the anti-tumor immune response.

In other studies, vaccination with live IL4-secreting plasmacytoma J558L cells in syngeneic BALB/c mice (26) or B16 melanoma cells in syngeneic C57BL/6 mice (5) had only moderate benefits in comparison to vaccination with irradiated tumor cells. In the study with J558L cells a significantly better anti-tumor protection was seen with mice which had survived inoculation with viable tumor cells plus *Corynebacterium parvum* as compared to those which had been vaccinated with irradiated tumor cells plus *C. parvum* (26). Neither of these studies had involved a systematic investigation of the duration of the immune protection, nor was the bone marrow checked for content of tumor cells.

The relatively low number of dormant Eb cells (0.001–0.01% of bone marrow-derived cells) is strikingly different from what was recently reported for an antibody-induced dormancy model of a murine B-cell lymphoma (BCL_1), where suspected dormant tumor cells constituted about 1% of total spleen cells (31). However in contrast to Eb cells (the present report), dormancy of BCL_1 cells was unstable and declined with a constant rate.

Presence of tumor cells in the bone marrow of human cancer patients has been reported and correlated with the likelihood of tumor recurrence and metastasis (32–34). We propose that the bone marrow may act as a site of tumor dormancy, providing persistent antigenic stimulation and immune control. Such immune control, however, may not last forever, and breakage of tumor dormancy could potentially lead to tumor recurrence. Better understanding of the nature of tumor dormancy in the bone marrow is likely to have a strong impact on future strategies for the control of neoplastic disease.

We thank Dr. Michael Stöck for his help in the initiation of this work and Dr. Thomas Blankenstein for his interest and support. We are grateful to Dr. Peter Ternesz and Dr. Reinhard Zawatsky for critical reading of the manuscript. This work was supported by the Dr. Mildred Scheel Stiftung fuür Krebsforschung (W20/88/Schi 1). S.P. has been supported by the State Scholarship Foundation of Greece (Second Division).

1. Fearon, E. R., Pardoll, D. M., Itaya, T., Golumbek, P., Levitsky, H. I., Simons, J. W., Karasuyama, H., Vogelstein, B. & Frost, P. (1990) *Cell* **60**, 397–403.
2. Russel, S. J. (1990) *Immunol. Today* **11**, 196–200.
3. Porgador, A., Gansbacher, B., Bannerji, R., Tzehoval, E., Gilboa, E., Feldman, M. & Eisenbach, L. (1993) *Int. J. Cancer* **53**, 471–477.
4. Porgador, A., Bannerji, R., Watanabe, Y., Feldman, M., Gilboa, E. & Eisenbach, L. (1993) *J. Immunol* **150**, 1458–1470.
5. Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Pardoll, D. & Mulligan, R. C. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 3539–3543.
6. Hock, H., Dorsch, M., Kunzendorf, U., Uberla, K., Qin, Z., Diamantstein, T. & Blankenstein, T. (1993) *Cancer Res.* **53**, 714–716.
7. Schirrmacher, V. (1979) *Int. J. Cancer* **43**, 367–377.
8. Schirrmacher, V., Shantz, G., Clauer, K., Komitowski, D. & Zimmermann, H. P. (1979) *Int. J. Cancer* **23**, 233–244.
9. Schirrmacher, V., Leidig, S. & Griesbach, A. (1991) *Cancer Immunol. Immunother.* **33**, 299–306.
10. Golumbek, P. T., Lazenby, A. J., Levitsky, H. I., Jaffee, L. M., Karasuyama, H., Baker, M. & Pardoll, D. M. (1991) *Science* **254**, 713–716.
11. Tepper, R. L., Pattengale, P. K. & Leder, P. (1989) *Cell* **57**, 503–512.
12. Cepko, C., Roberts, B. & Mulligan, R. (1984) *Cell* **37**, 1053–1062.
13. Markowitz, D., Goff, S. & Bank, A. (1988) *J. Virol.* **62**, 1120–1124.
14. Danos, O. & Heard, J. M. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8377–8381.
15. Danos, O. & Mulligan, R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 6460–6464.
16. Sane, J. R., Rubenstein, J. L. R. & Nicolas, J. F. (1986) *EMBO J.* **5**, 3133–3142.
17. Fiering, S. N., Roeder, M., Nolan, G. P., Micklem, D. R., Parks, D. R., & Herzenberg, L. A. (1991) *Cytometry* **12**, 291–301.
18. Vennström, B. & Bishop, M. (1982) *Cell* **28**, 135–143.
19. Karasuyama, H. & Fritz, M. (1988) *Eur. J. Immunol.* **18**, 97–104.
20. Hültner, L. & Dörmer, P. (1989) *J. Immunol.* **142**, 3440–3446.
21. Mosmann, T. (1983) *J. Immunol. Methods* **65**, 55–63.
22. Ohara, J. & Paul, W. E. (1985) *Nature (London)* **315**, 333–336.
23. Dialynas, D. P., Quan, Z. S., Wall, K. A., Pierres, A., Quintáns, J., Loken, M. R., Pierres, M. & Fitch, F. W. (1983) *J. Immunol.* **131**, 2445–2451.
24. Ledbetter, J. A. (1979) *Immunol. Rev.* **47**, 63–90.
25. Schild, H. -J., Kyewski, B., Von Hoegen, P. & Schirrmacher, V. (1987) *Eur. J. Immunol.* **17**, 1863–1866.
26. Hock, H., Dorsch, M., Kunzendorf, U., Qin, Z., Diamantstein, T. & Blankenstein, T. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2774–2778.
27. Tepper, R. I., Coffman, R. L. & Leder, P. (1992) *Science* **257**, 548–551.
28. Schirrmacher, V. (1989) *Interdiscip. Sci. Rev.* **14**, 291–303.
29. Gray, D. & Matzinger, P. (1991) *J. Immunol.* **174**, 969–974.
30. Oehen, S., Waldner, H., Kündig, T. M., Hengartner, H. & Zinkernagel, R. M. (1992) *J. Exp. Med.* **176**, 1273–1281.
31. Yefenof, E., Picker, L. J., Scheuermann, R. H., Tucker, T. F., Vitetta, E. S. & Uhr, J. W. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 1829–1833.
32. Lindemann, F., Schlimok, G., Dirschedl, P., Witte, J. & Riethmuller, G. (1992) *Lancet* **340**, 685–689.
33. Schlimok, G., Funke, I., Pantel, K., Strobel, F., Lindemann, F., Witte, J. & Riethmuller, G. (1991) *Eur. J. Cancer* **27**, 1461–1465.
34. Brugger, W., Bross, K. J., Glatt, M., Weber, F., Mertelsmann, R., Kanz, L. (1994) *Blood* **83**, 636–640.