IN VIVO THERAPY OF A MURINE B CELL TUMOR (BCL₁) USING ANTIBODY-RICIN A CHAIN IMMUNOTOXINS*

By KEITH A. KROLICK, JONATHAN W. UHR, SHIMON SLAVIN,
AND ELLEN S. VITETTA

From the Department of Microbiology, The University of Texas Health Science Center at Dallas, Dallas, Texas 75235; and the Department of Medicine A, Hadassah-Hebrew University, Jerusalem, Israel

The coupling of tumor-reactive antibodies to toxic agents as a way of directing such agents to tumor cells in vivo offers the promise of highly selective therapy. During the past several years, there have been numerous reports describing the use of antibody coupled to drugs (reviewed in 1) and toxic peptides (immunotoxins) (2–18) to kill tumor cells in vitro. The tumoricidal effect of these immunotoxins is highly specific and requires only minute doses.

To explore further the therapeutic potential of immunotoxins, we chose a well-defined mouse tumor model (BCL₁) (19). The tumor is a B cell leukemia of monoclonal origin, bearing surface IgM and IgD molecules (20, 21) that express a unique idiotype (22). The tumor can be transferred with 1–10 cells (17, 23), and its growth pattern can be accurately measured by indirect immunofluorescence using an anti-idiotypic (anti-Id)[1] antibody (24). Animals bearing the tumor survive for several months, despite massive tumor burdens (5×10^9–1×10^{10} cells/mouse).

We have demonstrated that immunotoxins directed against the immunoglobulin (Ig) on the surface of BCL₁ cells can kill virtually all tumor cells in a suspension of bone marrow or spleen (7, 17). In the present study, we treated mice bearing advanced BCL₁ tumors by intravenous administration of tumor reactive immunotoxin. The approach was to eliminate the vast majority of the tumor burden by nonspecific cytoreductive therapy. The residual tumor cells were then killed by intravenous administration of an immunotoxin reactive with the idiotype or isotype of the surface Ig of the tumor cells. The results of these studies indicate that such immunotoxins are highly effective at inducing prolonged remissions in tumor-bearing mice provided that sufficient prior cytoreduction has been achieved.

Materials and Methods

The BCL₁ Tumor. BALB/c mice, 8–10 wk of age, were obtained from Cumberland Farms, Clinton, TN. Mice were injected intravenously with 10^6 BCL₁ cells, and therapy was begun 6–8 wk later. At this point in time, the mice contained 1–2×10^9 tumor cells in the spleens and 2–4×10^8 tumor cells per ml of blood. There were also large numbers of tumor cells in the liver (1×10^9) and small numbers (~10–20%) of tumor cells in the bone marrow. Such mice would

[1] *Abbreviations used in this paper:* FACS, fluorescence-activated cell sorter; GARIg, goat anti-rabbit immunoglobulin; Id, BCL₁ immunoglobulin idiotype; PBL, peripheral blood leukocyte; RAMIg, rabbit anti-mouse immunoglobulin; rabbit anti-δ, rabbit anti-mouse δ chain; rabbit anti-OVA, rabbit anti-ovalbumin; TLI, total lymphoid irradiation.

survive another 4-6 wk in the absence of therapy. Before the initiation of therapy, the mice were staged by determining the number of Id$^+$ cells in the spleens and blood. This was accomplished by indirect immunofluorescent staining with rabbit anti-Id and fluorescein isothiocyanate (FITC) goat anti-rabbit Ig (GARIg) (22, 24). Stained cells were then quantified on the fluorescence-activated cell sorter (FACS) (FACS III; B-D FACS Systems, Becton, Dickinson & Co., Sunnyvale, CA).

Antibodies

ANTI-ID. Using a technique similar to that described by Levy and Dilly (25), nonsecreting BCL$_1$ tumor cells were fused to nonproducing hypoxanthine-aminopterin-thymidine sensitive myeloma cells (Sp2/0). A large portion of the hybridomas that survived the selection medium secreted Id-positive IgM$_\lambda$ into the medium. One BCL$_1$ × Sp2/0 hybridoma was recloned and then injected intraperitoneally into mineral oil-primed BALB/c mice. Large quantities of the IgM$_\lambda$ were purified from the ascites by conventional chromatographic techniques. Anti-Id antibody was then generated by immunizing rabbits with the BCL$_1$ IgM$_\lambda$ and removing antibodies directed against isotypic heavy and light chain determinants by adsorption on Sepharose-mouse Ig and Sepharose-MOPC-104E (μ, λ). As demonstrated by radioimmunoassay and immunofluorescence staining, the anti-Id bound to the BCL$_1$ IgM$_\lambda$ and the BCL$_1$ tumor cells but not to normal serum Ig, a panel of unrelated paraproteins, or to normal spleen cells from BALB/c mice (22, 24). The anti-Id antibody was affinity purified on Sepharose bound to the BCL$_1$ IgM$_\lambda$.

RABBIT ANTI-MOUSE δ CHAIN (RABBIT ANTI-δ). Affinity-purified rabbit anti-δ was prepared by immunizing rabbits with the TEPC-1017 ($\delta\kappa$) paraprotein. The anti-δ antibodies were affinity purified on Sepharose-TEPC-1033 ($\delta\kappa$) (26) and absorbed with Sepharose-mouse Ig to remove all anti-light chain specificities. This anti-δ reacts with the heavy chain of cell surface IgD on both normal mouse B cells and on BCL$_1$ cells (26).

RABBIT ANTI-OVALBUMIN (ANTI-OVA). The serum from rabbits immunized with commercial ovalbumin was affinity purified on Sepharose-OVA. These antibodies were used as controls in all experiments and, as determined by FACS analysis, did not bind to normal spleen cells or to BCL$_1$ cells.

RABBIT ANTI-MOUSE IG (RAMIG). Rabbit serum was prepared by immunizing rabbits with purified mouse Ig, and the specific antibody was eluted from Sepharose-mouse Ig as described previously (26). This antibody bound to both normal B cells and BCL$_1$ cells.

GOAT ANTI-RABBIT IG (GARIG). Goats were immunized with purified rabbit IgG, and the antibody was affinity purified on Sepharose-rabbit IgG. The affinity-purified antibody was conjugated to FITC as described previously (27).

ANTI-THY-1. The 30-H-12 rat hybridoma cells (28) were obtained from Dr. Noel Warner, Becton, Dickinson & Co., Palo Alto, CA. The IgG$_\kappa$ antibodies secreted by these cells bind to both Thy-1.1 and Thy-1.2 determinants. The medium from these hybridoma cells was treated with an equal volume of saturated ammonium sulfate, and the precipitate was centrifuged and resuspended at 1 mg/ml in phosphate-buffered saline, pH 7.3 (PBS).

RABBIT ANTI-MOUSE γ CHAIN. F(ab')$_2$ fragments of rabbit anti-mouse γ chain [F(ab')$_2$ rabbit anti-mouse γ] were prepared as described previously (29). Fragments were conjugated to FITC (27).

Preparation of Immunotoxins. Antibodies were conjugated to the A chain of ricin as described previously (7). Briefly, the A chain from *Ricinus communis* agglutinin II (Vector Laboratories, Burlingame, CA) was isolated by ion exchange chromatography after reduction of the disulfide bond that links the A chain to the B chain. The concentrated ricin A chain was stored in 0.05% 2-mercaptoethanol. Thiol groups were introduced into affinity-purified antibodies by using a 30-fold excess of *N*-succinimidyl-3-(3-pyridyldithio)-propionate (SPDP) (Pharmacia Fine Chemicals, Uppsala, Sweden). A fivefold molar excess of ricin A chain was mixed with the thiolated antibodies, and the mixture was dialyzed extensively against PBS, pH 7.8, to initiate disulfide exchange-mediated coupling of ricin A chain to the antibody. The antibody-A chain conjugates were chromatographed on Sephacryl-200 to remove free A chain and the majority of the uncoupled antibody. The conjugates were evaluated and tested by radioimmunoassay (7) and by analysis on sodium dodecyl sulfate-polyacrylamide gels. The antibody-A chain conjugates were used within 48 h.

Total Lymphoid Irradiation (TLI) (30, 31). X rays were delivered by a General Electric Maxitron 300 (General Electric Co., Wilmington, MA) at a rate of 60–70 rad/minute. Mice received a total of 200 rad per treatment and a total of 8–10 treatments over a period of 8–10 d. The source-to-skin distance was 72 cm, and 2.0 mm Cu filters were used. Dosimetry was verified using a calibrating ionizing chamber and by lithium fluoride thermoluminescence dosimeter. BALB/c mice were anesthetized with nembutal at a dose of 70 μg/g body weight and were positioned in the lead apparatus described by Slavin, et al. (30). The major lymph nodes, thymus, and spleen were exposed to the x rays. The majority of the skull, ribs, lungs, hind legs, and tails were shielded with lead.

Splenectomy. TLI-conditioned mice were anesthetized with nembutal at 70 μg/g body weight. The spleens were exposed through a midline abdominal incision, the pedicle was tied off with 00 silk, and the spleens were excised. The incision was closed with metal clips. The clips were allowed to remain in place for the duration of the experiments.

Indirect Immunofluorescence and Analysis on the FACS. Indirect immunofluorescence analysis was performed as described previously (22, 24). Briefly, cells suspended in PBS containing 10 mM sodium azide were treated with optimum concentrations of the primary antibodies (rabbit anti-δ, rabbit anti-Id, RAMIg, rabbit anti-OVA, normal rat IgG, or monoclonal rat anti-Thy-1) for 15 min at 4°C. Cells were then washed and resuspended in PBS-azide containing either FITC-GARIg or FITC-F(ab')$_2$ R anti-mouse γ. After a 10-min incubation on ice, the cells were washed twice in PBS-azide and fixed at 4°C in 4% buffered paraformaldehyde. For analysis, the cells were centrifuged and resuspended in PBS. The labeled cells were analyzed on the FACS. A laser light of 488 nm was used at an intensity of 300 MV. Living and dead cells were distinguished by near-forward angle light scatter; dead cells were excluded from the analysis. The fluorescent signal was determined with a photomultiplier potential of 500 V and a gain setting of 4. Fluorescent and scatter signals were standardized daily by using gluteraldehyde-fixed chicken erythrocytes.

Administration of Immunotoxin and Staging of Treated Animals. After 8–10 doses of 200 rad TLI ($\pm$ splenectomy), animals were injected intraveneously with two doses of 20 μg of immunotoxin 5–7 d apart. At weekly or biweekly intervals thereafter, mice were bled by retro-orbital punctures, and the white cell count was determined. After leukemic relapse, cells from the peripheral blood were stained with rabbit anti-Id and FITC-GARIg to confirm their tumor origin.

Results

Effect of TLI on Regression of the Tumor. As shown in Fig. 1, after 8–10 fractionated doses of 200 rad TLI, the number of tumor cells in both the spleen and blood of tumor-bearing mice was reduced by 90–95%. Without further treatment, the levels of Id$^+$ cells in the spleen began to increase within a week and reached 50–80% of pretreatment levels 1–2 wk after the termination of TLI. Id$^+$ cells were detectable in the blood 1.5–2 wk after the completion of TLI and reached pretreatment levels 2 wk later. The rapid relapses indicated the persistence of large numbers of tumor cells in the spleen, liver, and bone marrow.

Effect of Immunotoxins after TLI. Because of the rapid reappearance of tumor cells in the spleen and blood, immunotoxins were injected within several days after the completion of TLI. Although cytoreduction was optimum at this time, we estimate that 10^8–10^9 tumor cells remained in the mice. One of two types of immunotoxin were then injected into the TLI-conditioned mice. The first was a rabbit anti-Id immunotoxin that was specific for the BCL$_1$ tumor cells, as demonstrated previously by in vitro studies (7). The second immunotoxin was a rabbit anti-δ that is not a tumor-specific antibody. Nevertheless, the levels of serum IgD in mice are very low (32, 33), there are few B cells remaining in TLI-conditioned mice (31), and the
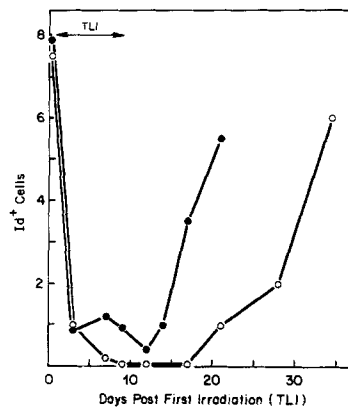
Fig. 1.   Effect of TLI on elimination of BCL₁ tumor cells from the spleens and blood of tumor-bearing mice. Mice were treated with nine daily doses of 200 rad TLI, and the spleens and blood of the animals were monitored for the presence of Id$^+$ tumor cells. ●, × 10^{-8} cells/spleen; ○, × 10^{-7} cells/ml blood.
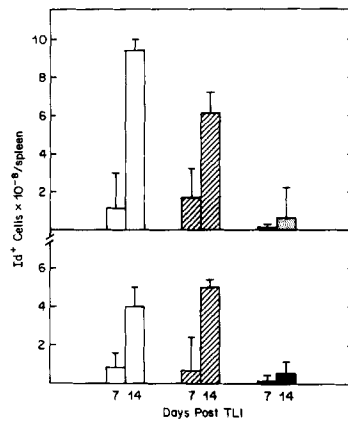

Fig. 2.   The effect of immunotoxin on the reappearance of tumor cells in the spleens of BCL₁ mice after nine doses of 200 rad TLI. After TLI, mice were injected with a single 20-μg dose of either rabbit anti-OVA-A chain, rabbit anti-Id-A chain, or rabbit anti-mouse δ-A chain. An additional group was not injected. At 7 and 14 d after TLI, the mice were killed and the spleens were examined for Id$^+$ cells. There were four mice per group. □, no treatment; ▨, anti-OVA-A; ▩, anti-Id-A; ■, anti-δ-A.

residual B cells lack surface IgD (R. May and E. Vitetta, manuscript in preparation). Therefore, the rabbit anti-δ immunotoxin was highly selective for the BCL₁ tumor cells. Moreover, any normal B cells that would be killed during administration of this immunotoxin would be reconstituted from stem cells in the shielded portions of the bone marrow. As shown in Fig. 2, the administration of either of these immunotoxins caused a measurable delay in the reappearance of tumor cells in the spleens of the treated mice. However, by 4 wk after treatment (data not shown), these mice had relapsed with progressive BCL₁ disease. These experiments indicated that the administration of immunotoxin delayed relapse but that significant numbers of tumor cells escaped the effect of the immunotoxin and eventually repopulated the spleens of the treated mice.

Effect of Immunotoxins after TLI and Splenectomy.   Rather than increase the amount of

immunotoxin administered, we chose to further reduce the tumor burden before treatment with immunotoxin. Therefore, TLI-conditioned mice were splenectomized 2 d after the completion of TLI. Postsurgical survival of these mice was >90%. 2 d after splenectomy, mice were injected with 20 μg of specific or control immunotoxin or were left untreated. The injections were repeated 1 wk later. As shown in Fig. 3, control mice (untreated or injected with rabbit anti-OVA-A chain) showed elevated white counts in the blood 4 wk after the completion of TLI (and splenectomy) and died 2-3 wk later. At the peak of leukemia, the blood cells from these mice were 70–80% Id$^+$. In contrast, the mice treated with rabbit anti-δ-A chain showed no reemergence of leukemic cells in the blood for a period of 14 wk after TLI, at which point the experiment was terminated. Furthermore, peripheral blood cells were obtained from nine mice treated with rabbit anti-mouse δ-A chain at 10 wk and again at 14 wk after completion of TLI. The blood samples were pooled, and 0.5 ml (containing 3×10^6 cells) was injected into each of three normal recipients. Recipients have remained tumor free for the 6 mo of observation after adoptive transfer (data not shown). In comparing the data in Figs. 2 and 3, it is evident that one of the critical features in successful immunotoxin therapy is sufficient nonspecific cytoreduction of the tumor mass before immunotoxin administration. As shown in Table I, similar experiments were performed with a total of 87 mice, and the average remission time after TLI, splenectomy, and anti-δ immunotoxin therapy (38 mice) was significantly greater than after nonspecific cytoreduction alone (29 mice) or nonspecific cytoreduction followed by control immunotoxin (20 mice). There were, however, instances in which late leukemic relapses were observed in mice treated with rabbit anti-mouse δ-A chain (57% of the mice relapsed). Nevertheless, in every mouse treated with the specific immunotoxin, there was a significant prolongation of remission. In three of four experiments, the mice appeared tumor free at 12–16 wk. It is possible, therefore, that these mice were cured. We do not know the reasons for the relapses observed in one of the experiments. The most likely causes are differences in the extent of nonspecific
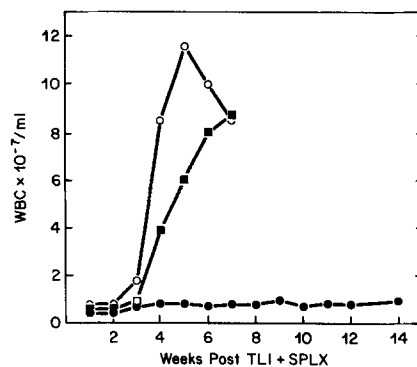

Fig. 3. Effect of TLI, splenectomy, and administration of immunotoxin on leukemic relapse of BCL$_1$-bearing mice. After nine doses of TLI and splenectomy, mice were injected with two doses of 20 μg of anti-δ or control immunotoxin or were not injected. There were nine mice per group. Leukemic relapse was monitored by determining the number of white cells in the blood of the treated mice. The control mice were all dead at 7 wk after TLI. The rabbit anti-mouse δ-A chain-treated group was monitored for a period of 14 wk post-TLI, at which point the experiment was terminated. O, no treatment; ■, anti-OVA-A; ●, anti-δ-A.

TABLE I

Effect of Immunotoxin on the Prevention of Leukemic Relapse in Mice Treated with TLI and Splenectomy

A *Individual Experiments*

Experiment	Number of mice/group	Average remission time in weeks*		
		No treatment	Anti-OVA-A	Anti-δ-A
1	9	4	4	14‡
2	4	4	4	12‡
3	18	4	ND§	8
4	7	6	6	16‖

B *Summary*

Treatment	Number of Mice	Average remission time in weeks
None	29	4.5
Anti-OVA-A	20	3.8
Anti-δ-A	38	11.2¶

* End of remission defined as >2.5 × 10^7 leukocytes/ml.
‡ No relapses observed, at which point experiments were terminated.
§ Not done.
‖ Experiment still in progress.
¶ Estimates of average remission time are minimum estimates because experiments 1, 2, and 4 were terminated or are in progress.

cytoreduction and/or differences among the batches of rabbit anti-mouse δ-A chain (affinity differences ?) that were used during the course of these experiments.

Effect of TLI, Splenectomy, and Treatment with Unconjugated Antibody. There are reports indicating that administration of antibody alone may be effective in the therapy of various types of tumors (18, 34–41). It was important, therefore, to compare the ability of conjugated vs. unconjugated rabbit anti-mouse δ to induce remission in BCL$_1$-bearing mice after TLI and splenectomy. As seen in Fig. 4, mice injected with rabbit anti-mouse δ-A chain showed no evidence of tumor cells in the blood, whereas animals injected with identical doses of antibody alone showed early leukemic relapses. There was no significant difference between the animals receiving no injection or administration of anti-δ alone (data not shown). These results emphasize the advantage of the immunotoxin compared with antibody alone. It is possible, of course, that administration of very large amounts of unconjugated anti-δ might have had an inhibitory effect on tumor growth.

Immunofluorescence Analysis of Cells Obtained from Mice in Remission. Because animals had been treated with an antibody-A chain conjugate directed against cell surface IgD, it was of interest to determine whether mice in remission had normal numbers of IgD-bearing B cells in their blood. Therefore, 14 wk after the completion of therapy (TLI, splenectomy, and treatment with anti-δ immunotoxin), peripheral blood cells from mice in remission were stained with either rabbit anti-mouse δ, rabbit anti-Id or monoclonal rat anti-Thy-1, and the appropriate secondary fluoresceinated antibody. Cells from normal BALB/c mice were stained in parallel. As shown in Table II, mice in remission contained higher percentages and absolute numbers of IgD$^+$ B cells in their blood than normal mice. Id$^+$ cells were not detectable. The number of T cells
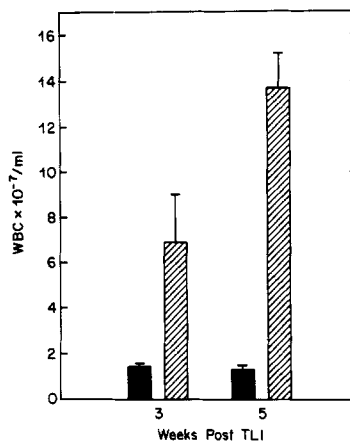
Fig. 4.   The effect of anti-δ-A chain or anti-δ alone on the reappearance of leukemia in BCL₁-bearing mice. After TLI and splenectomy, mice were injected with two doses, 1 wk apart, of 20 μg of either conjugated or unconjugated antibody, and the number of peripheral blood cells was determined at 3 and 5 wk after TLI. There were four mice per group. ▬, anti-δ-A; ▨, anti-δ.

TABLE II

Immunofluorescence Analysis of Peripheral Blood Lymphocytes from Mice in Remission

Staining antibody	Percent of PBL that stain	
	Mice in remission*	Normal mice
Anti-δ	42	29
Anti-Thy-1	55	66
Anti-Id	3	4
Normal Ig	3	3

* Blood counts ranged from 1-2 × 10⁷ cells/ml; in normal mice such counts are 0.5-1 × 10⁷ cells/ml.

was normal. An increase in B lymphocytes in the blood has previously been observed in mice that have undergone TLI (31). These results indicate that despite the extensive immunosuppression resulting from TLI, splenectomy, and treatment with an anti-δ immunotoxin (potentially reactive with most normal B cells), there was reconstitution of the IgD⁺ B cell compartment presumably from stem cells in the bone marrow.

Discussion

The present studies have shown that in vivo administration of an immunotoxin that was reactive with, but not specific for, a tumor cell (antibody to the δ chain of IgD) was instrumental in inducing prolonged remissions and possible eradication of a murine B cell leukemia. The strategy was to use mice with enormous tumor burdens (>3 × 10⁹ cells), to reduce the massive tumor burden by nonspecific cytoreduction (fractionated total lymphoid irradiation and splenectomy), and to then administer a tumor-reactive immunotoxin to kill the remaining tumor cells. The success of this approach is illustrated by the fact that each of the 38 animals so treated had a significant remission and that in three of four experiments (representing about one-half the mice) the duration of the remission was 12–16 wk, at which time the

experiments were terminated or are currently in progress. BCL_1-bearing mice subjected to nonspecific cytoreduction and injection with anti-OVA immunotoxin or no immunotoxin were all dead 7 wk after splenectomy. In addition, transfer of peripheral blood cells from animals in prolonged remission into normal syngeneic mice did not cause tumor in the recipients at a time after transfer when 1–10 BCL_1 cells would have caused tumor in the recipients. It could be argued that an anti-tumor response was transferred with the peripheral blood cells and that this putative immunity (although highly diluted in the normal recipients) might still be effective in suppressing growth of a small number of tumor cells. In addition, although mice in remission might not have tumor cells in the blood, they may still harbor such cells in other organs, e.g. lymph nodes, brain, testes, etc. This may indeed be the case after splenectomy. Thus, we are unable to conclude at this time whether there was a small number of tumor cells held in check by a host anti-tumor immune response or whether the mice were tumor free.

Our initial in vitro studies were performed with an anti-Id immunotoxin to specifically kill BCL_1 cells, and our preliminary in vivo results with anti-Id immunotoxin were encouraging. However, antibody against the BCL_1 Id is difficult to generate in large quantities, and its use is restricted to the BCL_1 tumor. In contrast, large amounts of heterologous (29) or monoclonal (42) anti-δ antibody can be easily raised. Anti-δ immunotoxins should be effective in treating mice bearing BCL_1 tumors because (a) BCL_1 tumor cells bear IgD (20, 21); (b) serum IgD levels in the mouse are extremely low (32, 33); (c) IgD-bearing normal B cells are virtually eliminated after TLI (31); and (d) IgD^- pre-B cells, immature B cells (IgM^+IgD^-), or stem cells should repopulate the B cell compartment after the elimination of mature IgD-bearing B cells by TLI and immunotoxin therapy. The success of this approach in the present experiment has important implications for the potential treatment of human B cell neoplasms such as chronic lymphocytic leukemia or non-Hodgkins lymphomas. Thus, the majority of such tumors bear IgD (43). Serum IgD levels are also low in the human (44) and probably even lower in patients with B cell tumors who are immunosuppressed because of the tumor (45) and/or because of chemotherapy. Thus, an anti-IgD immunotoxin, in contrast to anti-Id immunotoxin, might be viewed as a potentially "universal" immunotoxin for the management of a large portion of human B cell tumors.

We attribute the relative effectiveness of in vivo therapy with immunotoxins in the BCL_1 tumor model to a number of factors: (a) BCL_1 cells are highly susceptible to killing by the immunotoxins in vitro (7, 17); (b) the immunotoxins used remained biologically active after admixture with fresh mouse serum, i.e., significant disulfide exchange or inactivation by serum enzymes did not occur; (c) earlier in vitro results suggest that all BCL_1 tumor cells express surface Ig (17); (d) extensive nonspecific cytoreduction could be achieved and its degree of effectiveness could be accurately measured by indirect immunofluorescence using the anti-Id reagent in conjunction with FACS analysis; (e) the amount of immunotoxin needed to kill a particular number of BCL_1 cells could also be estimated based on in vitro experiments; and (f) BCL_1-bearing mice are characteristically in an immunosuppressed state (46), so that after TLI, which is in itself immunosuppressive (31), tumor-bearing mice cannot mount an antibody response to the heterologous immunotoxin. Thus, the characterization of the BCL_1 tumor model and the results of the in vitro studies using

immunotoxins formed a firm foundation for the development of a rational in vivo approach.

There have been several reports of in vivo use of immunotoxins in which some anti-tumor effects were obtained. One of the most provocative of the studies is that of Moolton et al. (4), who conjugated whole diphtheria toxin to antibodies raised to SV40-transformed cells and affinity purified the antibody on such cells. Repeated injections with these conjugates caused long-lasting remissions in a proportion of hamsters bearing SV40-induced lymphomas but did not effect established SV40-induced sarcomas. In more recent experiments performed by Blythman et al. (16), T lymphoma cells and anti-Thy-1.2-ricin A chain conjugates were injected into the peritoneal cavity of mice at virtually the same time. A significant number of the treated animals failed to develop tumors. In the studies of Trowbridge and Domingo (18), nude mice carrying a human melanoma tumor were treated with an anti-transferrin receptor antibody alone or with the antibody coupled to ricin A chain. It was found that in either case the tumor was eliminated. These results did not, however, indicate a functional role for the toxic portion of the immunotoxin.

The present studies also emphasize the advantage of an immunotoxin compared to antibody itself for in vivo therapy. Unconjugated anti-δ antibody did not induce remission in mice bearing the BCL$_1$ tumor. Nevertheless, results of therapy with antibody alone or in combination with added complement have been encouraging in some other tumor systems (18, 34–41). The most successful of these experiments have involved protocols in which antibody is given simultaneously with or shortly after the injection of tumor cells so that tumor burdens are extremely small. Such results have been described by Lanier et al. (36), who demonstrated that administration of an anti-Id prolonged the survival of mice bearing a B cell tumor. Kirsch and Hammerling (40) showed that high doses of monoclonal antibodies would inhibit the growth of the T cell tumor ASL.1, but only when antibody was given within 24 h of tumor inoculation. Trowbridge and Domingo (18), using a monoclonal antibody reactive with a human melanoma growing in nude mice, and Bernstein et al. (35), using monoclonal anti-Thy-1 antibodies in AKR mice bearing thymomas, induced curtailment of tumor growth in a high proportion of animals. In humans with leukemia, monoclonal or conventional antibodies reactive with tumor cells caused substantial but transient decreases in tumor growth (34, 37–39, 41). It is probable, therefore, that a proportion of tumors are susceptible to this type of therapy because of complement-mediated killing, removal of tumor cells by the reticuloendothelial system, or through a cell-mediated defense mechanism. However, large amounts of Ig must be used, and it is likely that the host will eventually respond with an antibody response to the heterologous Ig. In the case of monoclonal antibodies, the response could include an anti-idiotypic component. The advantages of using immunotoxins are that (a) much smaller amounts of antibodies can be used; (b) under optimum conditions every tumor cell might be susceptible to killing by an immunotoxin that binds to it; and (c) normal B cells that are specifically reactive with determinants on the immunotoxin (via the B cell's antigen-specific receptors) might be killed by this contact, thereby abrogating a potential antibody response to the immunotoxin.

There are a number of potential problems in considering immunotoxin therapy for human tumors beyond the problems of toxicity, precise quantification of tumor burden, and amount of immunotoxin to be administered. Thus, immunotoxins in the

systemic circulation might not be able to reach tumor cells in solid tumors, particularly in those with a large connective tissue component. All the cells from a particular tumor might not bear the relevant determinant, and, even if they do, the emergence of immunotoxin-resistant clones might occur at a significant incidence. These resistant clones could lack the surface antigen in question, be unable to transport the immunotoxin into the cell, or be resistant to the lethal effects of the A chain. Large amounts of circulating tumor antigen might compete successfully with the immunotoxins for surface antigen on the tumor cells. Another potential problem is the cross-reactivity of monoclonal anti-tumor antibodies with normal tissues. Considering the fact that a few immunotoxin molecules can kill a cell, modest cross-reactions could cause tissue damage. In addition, Fc binding to certain types of normal cells might also cause nonspecific toxicity in vivo. We therefore believe that it is important to obtain further information from experiments with animal models before using immunotoxins in humans.

Summary

Prolonged remissions were induced in mice bearing advanced BCL$_1$ tumors by the combined approach of nonspecific cytoreductive therapy and administration of a tumor-reactive immunotoxin. Thus, the vast majority of the tumor cells (~95%) were first killed by nonspecific cytoreductive therapy using total lymphoid irradiation (TLI) and splenectomy. The residual tumor cells were then eliminated by intravenous administration of an anti-δ immunotoxin. In three of four experiments, all animals treated in the above fashion appeared tumor free 12–16 wk later. In one experiment, blood cells from the mice in remission were transferred to normal BALB/c recipients, and the latter animals have not developed detectable tumor for the 6 mo of observation. Because 1–10 adoptively transferred BCL$_1$ cells will cause tumor in normal BALB/c mice by 12 wk, the inability to transfer tumor to recipients might indicate that the donor animals were tumor free. In the remainder of the animals treated with the tumor-reactive immunotoxin there was a substantial remission in all animals, but the disease eventually reappeared. In contrast, all mice treated with the control immunotoxin or antibody alone relapsed significantly earlier (3–4 wk after splenectomy).

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