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Eradication of large solid tumors in mice with an immunotoxin directed against tumor vasculature

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ABSTRACT **Antibody-based therapy of solid tumors has met with limited success, chiefly because solid tumors are relatively impermeable to macromolecules. This problem could be circumvented by attacking the readily accessible endothelial cells of the tumor vascular bed. We have developed a model to test this "vascular targeting" approach in which cytokine gene transfection of the tumor cells causes them to induce an experimental marker selectively on tumor vascular endothelium. An anti-tumor endothelial cell immunotoxin caused complete occlusion of the tumor vasculature and dramatic regressions of large solid tumors. By contrast, a conventional anti-tumor cell immunotoxin of equivalent *in vitro* potency produced only minor, transient antitumor effects but, when combined, the two immunotoxins induced permanent complete remissions in over half of the animals. These experiments indicate that immunotoxins directed against recently described markers on vascular endothelial cells in human tumors could provide a general treatment for solid tumors in humans.**

Immunotoxins constructed by using chemical (1) or molecular (2) approaches have proved effective against lymphomas and leukemias (3) but thus far clinical results in carcinoma and melanoma patients have been disappointing (2, 4). The principal reason for this is that immunotoxins and other antibody conjugates permeate poorly and unevenly into solid tumors. Typically, only 0.001–0.01% of the injected dose of an antibody localizes to each gram of tumor in humans (5). The poor penetration is due to several interrelated factors. First, dense packing of tumor cells and the fibrous tumor stroma present a formidable physical barrier to macromolecular transport. Second, elevated interstitial pressure in the tumor core hinders extravasation and fluid convection (6, 7). Third, the antibody entering the tumor tends to become specifically adsorbed in perivascular regions by the first tumor cells encountered, leaving none to reach tumor cells at more distant sites (8).

A solution to the problem of poor penetration of antibodies into solid tumors would be to attack the endothelial cells lining the blood vessels of the tumor rather than the tumor cells themselves. The vascular endothelial cells are directly accessible to circulating therapeutic agents, and, since thousands of tumor cells are reliant on each capillary for oxygen and nutrients, even limited damage to the tumor vasculature should produce an avalanche of tumor cell death (9). In addition, the approach should be applicable to numerous types of solid tumors because all rely on their vasculature for growth (10). Despite its appeal, the "vascular targeting" approach has not hitherto been testable because antibodies with adequate specificity for tumor vascular endothelial cells have not been available (9). The recent discovery of markers of tumor endothelial cells in human cancers (11, 12) accentuates the need for animal models of vascular targeting with which to study the efficacy and pharmacology of this therapeutic strategy.

We have exploited the ability of cytokines to activate specific genes in vascular endothelial cells in order to develop a murine model to test the concept of antibody-directed targeting of tumor vasculature (13). A neuroblastoma cell line was transfected with the murine interferon γ (IFN-γ) gene by using a retroviral vector (14). When the transfectant C1300(Muγ) cells are grown subcutaneously in BALB/c *nu/nu* mice, they secrete IFN-γ, which activates capillary and venular endothelial cells within the tumor mass to express class II antigens of the major histocompatibility complex (MHC). Vascular endothelial cells in normal mouse tissues do not express MHC class II antigens unless activated by IFN-γ (15, 16), although MHC class II antigens are constitutively expressed by B cells, macrophages, and some epithelial cells (13). Similarly, a class II-negative murine endothelial cell line was induced to express class II antigens by activation with recombinant murine IFN-γ or C1300(Muγ) tumor-conditioned medium *in vitro* (13). When an anti-class II antibody was injected intravenously into C1300(Muγ) tumor-bearing mice, it localized within 1 hr to all tumor vascular endothelial cells. By contrast, a tumor-specific antibody directed against the unique MHC class I antigen of the tumor allograft, which strongly stained all C1300(Muγ) cells by fluorescence-activated cell sorting and in tissue sections of subcutaneous tumors, accumulated slowly and was restricted to perivascular regions of the tumor even 48 hr after injection (13). In this report, we describe the antitumor effects of ricin A-chain immunotoxins (17), prepared with anti-class II and anti-class I antibodies, in mice bearing large solid C1300(Muγ) tumors.

MATERIALS AND METHODS

Animals. BALB/c *nu/nu* mice were purchased from Simonsen Laboratories (Gilroy, CA). All animals were maintained in microisolation units on sterilized food and water. Tetracycline HCl (Vedeo, St. Joseph, MO) was added to drinking water at a final concentration of 1.1 mg/ml (18).

Cells and Culture Conditions. The C1300 neuroblastoma cell line was established from a spontaneous tumor, which arose in an A/Jax mouse in 1940 (19). The C1300(Muγ) 12 line, here referred to as C1300(Muγ), was derived by transfection of C1300 cells with the murine IFN-γ gene using the IFN-γ expression retrovirus pSVX(MuγΔAs) (14). Both lines carry the MHC haplotype *H-2K*^{k}, *I-A*^{k}, *I-E*^{k}, *D*^{d}. The SVEC 4-10 murine endothelial cell line, here referred to as SVEC, was kindly provided by M. Edidin (Department of Biology, The Johns Hopkins University, Baltimore) and was derived by immortalization of lymph node endothelial cells from a C3H (*H-2*^{k}) mouse with simian virus 40 (15). C1300 and SVEC cells were grown in modified Eagle's medium (MEM) sup-

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Abbreviations: dgA, deglycosylated ricin A chain; IFN-γ, interferon γ; MHC, major histocompatibility complex.

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plemented with 10% (vol/vol) fetal calf serum (FCS), 2.4 mM L-glutamine, 200 units of penicillin and 10 μg of streptomycin per ml, 100 μM nonessential amino acids, 1 μM sodium pyruvate, and 18 μM Hepes. C1300(Muγ) cells were maintained in the same medium supplemented with G418 (1 mg/ml) (Geneticin; Sigma). Cultures were maintained at 37°C in a humidified atmosphere of 90% air/10% CO_2 in regular tissue culture flasks or, when large quantities were required for *in vivo* experiments, in cell factories (Baxter, Grand Prairie, TX). For some experiments, SVEC cells were cultured with recombinant IFN-γ, tumor conditioned medium, or neutralizing anti-IFN-γ antibody as described (13).

Monoclonal Antibodies. The M5/114.15.2 (here referred to as M5/114) and 11-4.1 hybridomas were purchased from the American Type Culture Collection and were grown in MEM/10% FCS. The antibodies were purified from culture supernatant by precipitation in 50% ammonium sulfate and affinity chromatography on protein G (M5/114) or protein A (11-4.1). The rat IgG2b antibody, M5/114, detects an epitope on I-A^d and I-E^d molecules, hereafter referred to collectively as Ia^d, on cells from BALB/c mice (20) and also cross-reacts with an epitope on I-E^k molecules on induced SVEC cells (13). The mouse IgG2a antibody 11-4.1 recognizes H-$2K^k$ but not H-$2K^d$ molecules (21) and so binds to H-$2K^k$ on C1300 and C1300(Muγ) cells but is unreactive with MHC antigens from BALB/c mice. A rat IgG2b anti-human CD7 antibody, Campath-2, was generously provided by G. Hale (Department of Pathology, University of Cambridge, Cambridge, England) and was used as the isotype-matched control antibody for M5/114. A mouse IgG2a anti-human CD7 antibody, WT-1, was the kind gift of W. Tax (Sint Radbondzeikenhuis, Nijmegen, The Netherlands) and was used as a control for 11-4.1.

Preparation of Deglycosylated Ricin A (dgA) Chain. The ricin A chain was purified by the method of Fulton *et al.* (22). dgA was prepared as described (17). For conjugation with antibodies, the A chain was reduced with 5 mM dithiothreitol (DTT) and subsequently separated from DTT by gel filtration on a column of Sephadex G-25 in phosphate-buffered saline (pH 7.5) containing 2 mg of Na_2EDTA per ml.

Preparation of Immunotoxins. IgG immunotoxins were prepared by using the 4-succinimidyloxycarbonyl α-methyl(2-pyridyldithio)toluene linking agent described by Thorpe *et al.* (17). Briefly, 4-succinimidyloxycarbonyl α-methyl(2-pyridyldithio)toluene dissolved in dimethylformamide was added to the antibody solution [7.5 mg/ml in borate buffer (pH 9.0)] to give a final concentration of 0.11 mM. After 1 hr, the derivatized protein was separated from unreacted material by gel chromatography on a Sephadex G-25 column and mixed with freshly reduced ricin A chain. The solution was concentrated to ≈3 mg/ml and allowed to react for 3 days. Residual thiol groups were inactivated by treating the immunotoxin with 0.2 mM cysteine for 6 hr. The solution was then filtered through a Sephacryl S-200 HR column in 0.1 M phosphate buffer (pH 7.5) to remove unreacted ricin A, cysteine, and aggregates. Finally, the immunotoxin was separated from free antibody by chromatography on a blue Sepharose CL-6B column equilibrated in 0.1 M sodium phosphate buffer (pH 7.5) according to the method of Knowles and Thorpe (23). All immunotoxin preparations contained >90% 180-kDa product consisting of one molecule of IgG and one molecule of ricin A chain, as assessed by analytical SDS/PAGE.

Cytotoxicity Assays. C1300, C1300(Muγ), and SVEC cells suspended at 10^5 cells per ml in MEM/10% FCS were distributed in 100-μl vol into the wells of flat-bottomed microtiter plates. For some assays, SVEC cells were suspended in C1300- or C1300(Muγ)-conditioned medium or MEM supplemented with recombinant IFN-γ as indicated. Immunotoxins in the same medium were added (100 μl per well) and the plates were incubated for 24 hr at 37°C in an atmosphere of humidified 10% CO_2/90% air. After 24 hr, the cells were pulsed with [^{3}H]leucine (2.5 μCi per well; 1 Ci = 37 GBq) for another 24 hr. The cells were then harvested onto glass fiber filters with a Titertek harvester and the radioactivity on the filters was measured with a liquid scintillation spectrometer (LKB; Rackbeta). The percentage of reduction in [^{3}H]leucine incorporation, as compared with untreated control cultures, was used as the assessment of killing.

Antitumor Experiments. For establishment of solid tumors, a mixture of 1.4×10^7 C1300 cells and 6×10^6 C1300(Muγ) cells in 200 μl of MEM/30% FCS was injected subcutaneously into the right anterior flank of BALB/c *nu/nu* mice. Fourteen days later, when the tumors had grown to 0.8–1.2 cm in diameter, the mice were separated into groups of 5–10 animals and injected intravenously with 200 μl of immunotoxins, antibodies, or diluent. Perpendicular tumor diameters were measured at regular intervals and tumor volumes were estimated according to the following equation (24):

$$\text{volume} = \frac{\text{smaller diameter}^2 \times \text{larger diameter} \times \pi}{6}.$$

For histopathological analyses, animals were killed at various times after treatment and the tumors were excised immediately into 4% (vol/vol) formalin. Paraffin sections were cut and stained with hematoxylin and eosin.

RESULTS

Cytotoxicity of Immunotoxins to Activated Endothelial Cells and Tumor Cells *in Vitro*. The two immunotoxins M5/114-dgA and 11-4.1-dgA were approximately equally potent at inhibiting protein synthesis by their appropriate target cells *in vitro*, as shown in Fig. 1. M5/114-dgA inhibited protein synthesis of SVEC cells activated with recombinant IFN-γ or C1300(Muγ)-conditioned medium by 50% at 0.1–0.3 nM (Fig. 1 *Upper*). Similarly, 11-4.1-dgA inhibited protein synthesis by cells recovered from subcutaneous C1300/C1300(Muγ) tumors by 50% at 0.2 nM (Fig. 1 *Lower*). The cytotoxic effects of both immunotoxins were antigen specific, since isotype-matched immunotoxins prepared from antibodies of irrelevant specificity (human CD7) were not toxic to either cell type at 100 nM. Also, neither immunotoxin was toxic to nontarget cells, including unstimulated endothelial cells, at 100 nM. Importantly, M5/114-dgA, which reacts with I-E^k antigens expressed weakly by C1300(Muγ) cells (13), was not toxic to tumor cells at concentrations below 10 nM (Fig. 1 *Lower*), which strongly suggested that any *in vivo* antitumor effects mediated by this immunotoxin must be via an indirect mechanism, probably disruption of blood supply. Campath-2 and WT-1 immunotoxins were specifically cytotoxic to $CD7^+$ human T-cell lines (unpublished data).

Antitumor Effects in Mice Bearing Large Solid Tumors. A single intravenous injection of the anti-tumor endothelial cell immunotoxin M5/114-dgA into mice bearing large (≥1 cm in diameter) solid tumors induced potent, dose-dependent antitumor effects (Fig. 2). Responses were minor and transient in animals treated with 20 μg of M5/114-dgA but, at a dose of 40 μg, marked regressions were achieved in all animals. Tumors collapsed to an average of a quarter of their initial volume, sometimes reaching almost unmeasurable dimensions, before regrowing 7–10 days later. Higher doses (≥100 μg) of M5/114-dgA were highly effective but were toxic in some cases. By contrast, a high dose (100 μg) of the tumor-specific immunotoxin 11-4.1-dgA induced only minor transient effects on tumor growth, but when the two immunotoxins were used in combination, synergistic antitumor effects were achieved and five of eight of the treated animals

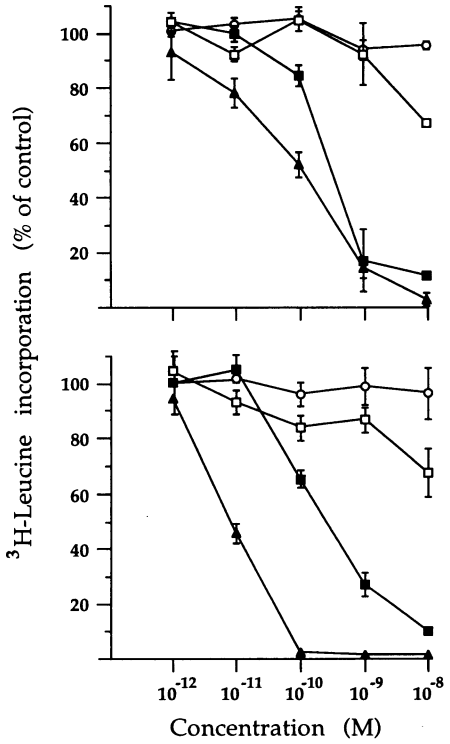

FIG. 1. Cytotoxicity of anti-class II and anti-H-2K^k immunotoxins to target cells *in vitro*. (*Upper*) SVEC cells were incubated for 48 hr with M5/114-dgA after having incubated them for 72 hr in regular medium (○), medium supplemented with recombinant IFN-γ (200 international units/ml) (▲), C1300(Muγ)-conditioned medium (■), or C1300(Muγ)-conditioned medium supplemented with neutralizing anti-IFN-γ antibody (200 neutralizing units/ml) (□). (*Lower*) Cells recovered from subcutaneous C1300/C1300(Muγ) tumors were incubated for 48 hr with ricin (▲), 11-4.1-dgA (■), M5/114-dgA (□), or WT-1-dgA (○). Similar results were obtained in two other experiments. Data points are geometric means of triplicate measurements of [^{3}H]leucine incorporated by the cells during the final 24-hr period of culture expressed as a percentage of the incorporation in untreated cultures. Bars are 1 SEM.

cleared their tumors and remained disease-free. These effects were specific since equivalent doses of the unconjugated antibodies or of control immunotoxins of irrelevant specificity had no antitumor effects.

The gross appearance of tumors treated with M5/114-dgA changed dramatically within 2 days (Fig. 3). Massive hemorrhaging of the tumor vascular bed caused the tumors to assume a blackened, bruised appearance reminiscent of the effects when Meth-A fibrosarcomas are treated with bacterial endotoxin (25) or tumor necrosis factor type α (26). Over the next 5–7 days, the tumors collapsed to form a flat scabrous plug that subsequently detached, leaving a small avascular area of scar tissue in animals treated with M5/114-dgA and 11-4.1-dgA. Some tumors became fully coagulated before significant hemorrhaging could occur and so progressed to the final stage illustrated without blackening or scabbing of the tumor mass. In mice given M5/114-dgA alone, the tumors regrew from a ring around the original site and reinvaded the core of dead tumor tissue.

Histological Observations. A study of the time course of the events in tumor-bearing mice treated with M5/114-dgA revealed vascular endothelial cell destruction as the first visible event, occurring as early as 2 hr after administration of the immunotoxin (Fig. 4*a*). Degeneration of the endothelial cell layer induced a wave of platelet adhesion and activation followed by fibrin deposition. By 6 hr, many blood vessels in the tumor were occluded with thrombi and had been stripped

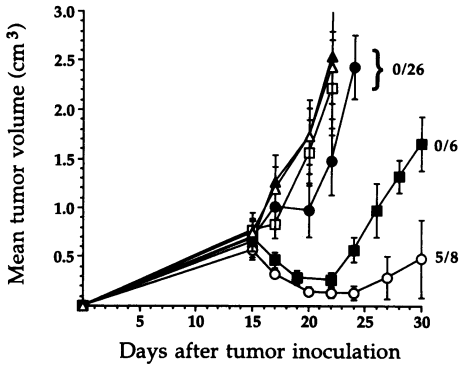

FIG. 2. Induction of tumor regression by anti-class II immunotoxins. C1300(Muγ) tumor-bearing BALB/c *nu/nu* mice were injected intravenously with 20 μg (□) or 40 μg (■) of M5/114-dgA, 100 μg of 11-4.1-dgA (●), or 100 μg of 11-4.1-dgA plus 40 μg of M5/114 dgA (○). Mice in control groups received 200 μl of diluent (△) or 100 μg of an isotype-matched immunotoxin of irrelevant specificity [Campath-2-dgA, anti-human CD7 (▲)]. Mice treated with equivalent doses of unconjugated antibodies (M5/114, 11-4.1) displayed no retardation in tumor growth rate. Immunotoxin doses refer to total protein content. Error bars indicate SEM. Also indicated is number of complete tumor remissions per total number of mice in each experimental group. Similar results were obtained in two other experiments.

of their endothelial cell lining (Fig. 4*b*). At this time, the tumor cells themselves were morphologically unchanged. By 24 hr, all vessels contained mature thrombi and the surrounding tumor cells had pyknotic nuclei (Fig. 4*c*). By 48 hr, massive tumor cell degeneration and autolysis had occurred (Fig. 4*d*).

The cause of tumor regrowth in mice treated with M5/114-dgA alone was investigated by histopathological examination of treated tumors (Fig. 5). Three days after injection of M5/114-dgA, a median section showed hemorrhagic necrosis throughout the tumor, most advanced in the core. However, a thin cuff of tumor, 5–10 cells thick, survived on the extreme periphery (Fig. 5 *Inset*) of the tumor and it was these cells that subsequently proliferated to cause the regressions in animals treated with M5/114-dgA alone.

Lack of Damage to Normal Tissues. Previous studies had shown that class II antigens are expressed by several normal cell populations in BALB/c *nu/nu* mice, including some

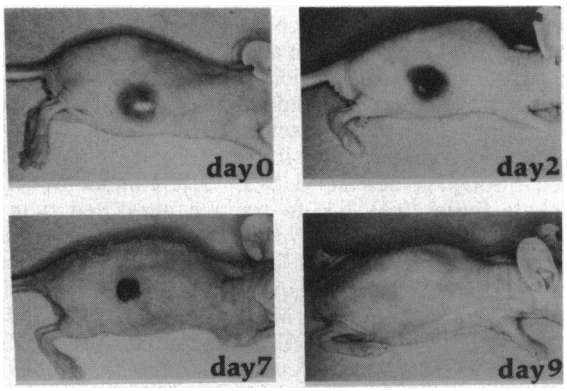

FIG. 3. Gross appearance of subcutaneous tumors treated with anti-class II immunotoxins. Mice were inoculated with tumor cells and treated with 40 μg of M5/114-dgA as described. Before treatment (day 0), tumors were pink/purple, indicating florid vascularization. Two days after treatment, massive intratumoral hemorrhage caused a blackened discoloration, and by day 7 the tumor mass had largely collapsed into a scabrous tissue plug, which later dropped off to reveal a white, avascular nodule of dead tumor tissue (day 9). Representative mice at different stages of therapy are shown.

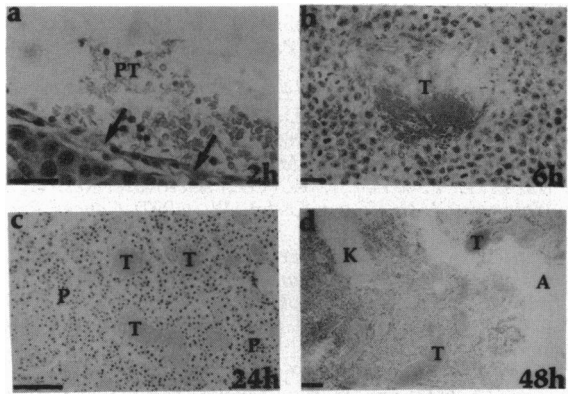

FIG. 4. Time course of vascular thrombosis and tumor necrosis after administration of M5/114-dgA. (*a*) Two hours: a few endothelial cells had become denuded with exposure of the underlying subendothelial extracellular matrix (arrows). Platelet (PT) adhesion and aggregation on the damaged areas was visible. (*b*) Six hours: many vessels had become completely occluded by mature fibrin thrombi (T). (*c*) Twenty-four hours: all vessels were thrombotic and surrounding tumor cells had pyknotic nuclei (P). (*d*) Forty-eight hours: tumor necrosis had advanced and areas of pyknosis, karyolysis (K), and autolysis (A) were apparent. Hematoxylin and eosin stain. (Bars: *a* and *b*, 15 μm; *c* and *d*, 60 μm.)

intestinal and renal tubular epithelial cells, B cells, a minority of bone marrow cells, Kupffer cells, Langerhans cells, and macrophages in most organs (13). However, therapeutic doses (≥40 μg) of M5/114-dgA did not cause detectable damage to class II-positive epithelial cells or to Kupffer or Langerhans cells, as assessed by histopathological analyses at various times after treatment (unpublished data). It would be expected that readily accessible class II-positive cells in primary and secondary lymphoid organs would be killed by the immunotoxin, but these effects were apparently temporary because, 20 days after treatment, all mature bone marrow cell populations and splenic B-cell compartments were normal (unpublished data).

DISCUSSION

The findings from this experimental model validate the concept of tumor vascular targeting and, in addition, demonstrate that this strategy is complementary to that of direct tumor targeting. The theoretical superiority of vascular targeting over the conventional approach was established by comparing the *in vivo* antitumor effects of two immunotoxins—one directed against tumor vascular endothelium and the other directed against the tumor cells themselves—in the same model. The immunotoxins were equally potent against their respective target cells *in vitro* but, while 100 μg of the tumor-specific immunotoxin had practically no effect against large solid C1300(Muγ) tumors, as reported previously in other systems (27, 28), as little as 40 μg of the anti-tumor endothelial cell immunotoxin caused complete occlusion of the tumor vasculature and dramatic tumor regressions.

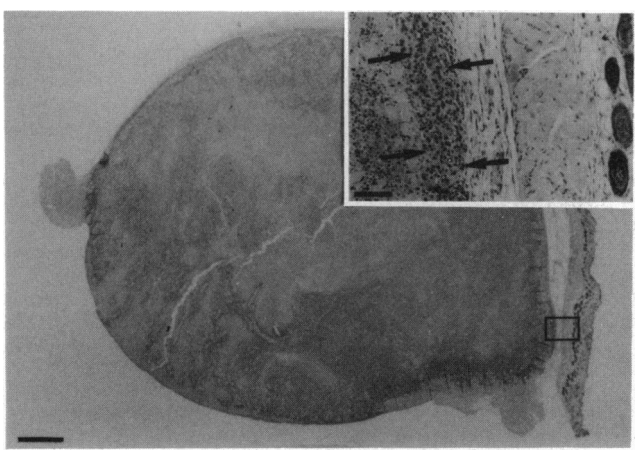

FIG. 5. Microscopic appearance of tumors from mice treated with anti-class II immunotoxin 72 hr earlier. (Bar = 1.0 mm.) (*Inset*) Almost the entire tumor mass is necrotic; only a thin cuff of tumor cells at the extreme tumor periphery adjacent to normal tissue survives (arrows). Hematoxylin and eosin stain. (Bar = 40 μm.)

Despite causing thrombosis of all blood vessels within the tumor mass, the anti-tumor endothelial cell immunotoxin was not curative because a small population of malignant cells at the tumor–host interface survived and proliferated to cause the observed relapses 7–10 days after treatment. The proximity of these cells to intact capillaries in adjacent skin and muscle suggests that they survived because they could derive their nutrition from the extratumoral blood supply, which was not induced to express class II antigens (13). However, these same tumor cells could be killed by the anti-tumor immunotoxin, probably because they were readily accessible to the immunotoxin because of the florid vasculature and lower interstitial pressure in those regions of the tumor (29). Importantly, the complementary nature of the killing achieved with the antitumor vasculature and anti-tumor immunotoxins resulted in improved antitumor effects when both reagents were administered. Thus, over half of the mice receiving both immunotoxins achieved lasting complete remissions.

The time course study demonstrated that the anti-class II immunotoxin exerted its antitumor activity via the tumor vasculature since endothelial cell detachment and diffuse intravascular thrombosis clearly preceded any changes in tumor cell morphology. In contrast with the anti-tumor immunotoxin, the onset of tumor regression in animals treated with the anti-tumor endothelial cell immunotoxin was rapid. Massive necrosis and tumor shrinkage were apparent 48–72 hr after injection. Focal denudation of the endothelial lining was evident within 2–3 hr, in keeping with the finding that protein synthesis by IFN-induced endothelial cells is reduced by 10% within 2 hr (unpublished data). Because only limited endothelial damage is required to upset the hemostatic balance and initiate irreversible coagulation, many intratumoral vessels became thrombosed quickly thereafter, with the result that tumor necrosis began within 6–8 hr of administration of the immunotoxin. This illustrates several of the strengths of vascular targeting in that an avalanche of tumor cell death swiftly follows destruction of a minority of tumor vascular endothelial cells (9). Thus, in contrast to conventional tumor cell targeting, anti-endothelial immunotoxins could be effective even if they have short serum half-lives and only bind to a subset of tumor endothelial cells. The anti-tumor immunotoxin 11-4.1-dgA killed large numbers of tumor cells, giving rise to discrete islands of necrosis surrounding intact blood vessels (unpublished data). However, many cells in poorly vascularized regions of the tumor were inaccessible to the immunotoxin (13), and this surviving fraction proliferated rapidly so that the overall effect of the anti-tumor immunotoxin on tumor growth was minimal.

MHC class II antigens are not unique to vascular endothelial cells. They are expressed constitutively on B cells, bone marrow myelocytes, cells of monocyte–macrophage lineage, and on some renal and gut epithelial cells in BALB/c *nu/nu* mice (13). It would therefore be anticipated that damage to these normal tissues would result if anti-class II immunotoxin were to be administered. However, anti-class II immunotoxins administered intravenously to antibiotic-treated BALB/c *nu/nu* mice were no more toxic to the mice than are immunotoxins having no reactivity with mouse tissues. There are a number of possible explanations for this

surprising result. First, anti-class II antibodies injected intravenously did not appear to reach the epithelial cells or the monocytes–macrophages in organs other than the liver and spleen (13). Presumably, this is because the vascular endothelium in most organs is tight, not fenestrated as it is in the liver and spleen, and so the antibodies must diffuse across basement membranes to reach the class II-positive cells. Second, hepatic Kupffer cells and probably other cells of monocyte–macrophage lineage were not killed by the anti-class II immunotoxin even though it binds to them (13). No morphological changes in the Kupffer cells were visible even several days after administration of the immunotoxin. Cells of monocyte–macrophage lineage are generally resistant to ricin A-chain immunotoxins (30), probably because internalized immunotoxins are routed directly to lysosomes and metabolized as part of the cells' degradative physiologic function. Third, although B cells and bone marrow myelocytes were probably killed by the immunotoxin, they were efficiently replaced from the stem cell pool because early bone marrow progenitor cells do not express class II antigens (31).

It was important to maintain mice on oral antibiotics to prevent toxicity of anti-class II immunotoxins to the small intestine. In animals not given tetracycline HCl, some ileal villous endothelial cells were induced to express class II antigens by IFN-γ secreted by thymus-independent intraepithelial lymphocytes responding to gut flora. Antibiotic treatment diminished local T-cell accumulation and abolished endothelial cell class II antigen expression (13). Tetracycline itself had no effects on tumor growth because mice injected with diluent or control immunotoxins showed the same kinetics of tumor growth whether or not they were given the antibiotic (unpublished data).

Although this model system cannot be directly transferred to the clinic, the findings described in this report demonstrate the therapeutic potential of the vascular targeting strategy against large solid tumors and highlight the need for antibodies recognizing endothelial cells in human tumors. Numerous differences between tumor blood vessels and normal ones have been documented (9, 29, 32), which suggest that the approach could be applicable in humans. Tumor endothelial markers could potentially be induced directly by tumor-derived angiogenic factors (33) or cytokines (34, 35) or could relate perhaps to the rapid proliferation (36) and migration (33) of endothelial cells during neovascularization. Indeed, several candidate anti-tumor endothelial cell antibodies have recently been described. The antibodies FB-5, against endosialin (11), and E9 (12) have been reported to be highly selective for tumor vascular endothelial cells. Two related antibodies, TEC-4 and TEC-11, raised in this laboratory against carcinoma-stimulated human endothelial cells, show strong reactivity against vascular endothelial cells in a wide range of malignant tumors but little or no staining of vessels in benign tumors or normal tissues (F.J.B., P. Tazzari, P. Amlot, A. F. Gazdar, E. S. Vitetta, and P.E.T., unpublished data). Vascular targeting could therefore become a valuable approach to the therapy of disseminated solid cancers for which there are currently no effective treatments.

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