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Prevention of immunotoxin-mediated vascular leak syndrome in rats with retention of antitumor activity

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ABSTRACT **Immunotoxins are hybrid molecules composed of a cell-surface binding domain and a protein toxin moiety that together target specific cell populations for elimination. These agents represent a promising approach for the treatment of many human diseases, most notably cancer. However, it has recently become clear that many immunotoxins when used in human clinical trials induce vascular leak syndrome (VLS), restricting the administration of doses necessary to achieve good therapeutic responses. The lack of an appropriate animal model has hindered efforts to understand and prevent immunotoxin-induced VLS. We have found that in rats, intravenous administration of the single-chain immunotoxin BR96 sFv-PE40 results in symptoms that closely resemble VLS seen in human immunotoxin trials. A large fluid accumulation in the thoracic cavity was observed, along with an increase in hematocrit and body weight and a decrease in serum albumin. The VLS was apparent within 24 hr after administration of immunotoxin and was seen in both immunocompetent and athymic rats. Similar symptoms were not found in mice even at lethal doses. Prophylactic administration of the corticosteroid dexamethasone resulted in prevention of VLS and survival of rats injected with what would otherwise be lethal doses of BR96 sFv-PE40. Prophylactic treatment with dexamethasone in rats xenografted with human tumors either did not inhibit or minimally inhibited the antitumor activity of BR96 sFv-PE40. The use of prophylactic corticosteroids should be considered for immunotoxin clinical trials, since it may improve therapeutic efficacy by decreasing the dose-limiting toxicity of VLS.**

Vascular leak syndrome (VLS) is the dose-limiting toxicity found in many clinical trials utilizing immunotoxins, including those prepared with blocked ricin, ricin A chain (in native and deglycosylated forms), and saporin (1–10). VLS is characterized in humans by hypoalbuminemia, weight gain, and edema, resulting from the extravasation of fluids and proteins from the vascular system into the periphery. VLS restricts the use of immunotoxins in humans and in many cases necessitates either a significant reduction in dose or a complete cessation of therapy. While peripheral edema is clinically manageable, pulmonary edema can be life threatening. Recently, VLS was found to have contributed to the death of two B-cell lymphoma patients who were treated with anti-CD22-deglycosylated ricin A chain (9). Other toxic effects in patients treated with immunotoxins may be a result of VLS, including tachycardia, nausea, aphasias as a result of cerebral edema, and myocardial damage (4).

Other proteins also have been found to induce VLS in humans. Systemic administration of the cytokine interleukin 2 (IL-2) results in the development of VLS when approaching doses that may provide antitumor efficacy in patients with metastatic cancer (11, 12). VLS has also been observed in patients following treatment with granulocyte/macrophage colony-stimulating factor or the anti-GD3 antibody R24 (13, 14).

BR96 sFv-PE40 is a single-chain immunotoxin fusion protein that has been shown to cure established human tumor xenografts in both athymic rats and mice (15, 16). In this study, a rat model for VLS was established following administration of BR96 sFv-PE40 at doses beyond those required for cures of tumor xenografts in rodent models. The identification of an animal model for VLS that closely approximates the human VLS response to immunotoxins provides an opportunity to evaluate specific drugs for their ability to block immunotoxin-induced VLS and to determine whether they affect the antitumor activity of BR96 sFv-PE40.

MATERIALS AND METHODS

Reagents. The single-chain immunotoxin fusion protein BR96 sFv-PE40 was expressed in *Escherichia coli* and purified as described (16). Diphenhydramine hydrochloride was purchased from Elkins-Sinn (Cherry Hill, NJ). Cyclosporine A (CsA) was purchased from Sandoz Pharmaceutical. 15-Deoxyspergualin (DSG) was purchased from Nippon Kayaku (Tokyo). Dexamethasone (Dex) was purchased from Anpro Pharmaceuticals (Arcadia, CA).

Toxicity Studies. Six- to 8-week-old female Wistar Furth and Rowett, *nu/nu* (athymic) rats (Harlan–Sprague–Dawley) were i.v. injected with various amounts of BR96 sFv-PE40 (0.25–4 mg/kg). After 24 hr, they were euthanized by exposure to CO_2, and the tissues were analyzed using gross and microscopic techniques. Cardiac blood was collected from comatose animals and placed either in serum collection tubes for blood chemistry analysis or in EDTA tubes for complete blood count (CBC). Hydrothorax fluid was collected from separate animals by placing the carcass in dorsal recumbancy, carefully removing the ventral chest wall, and aspirating fluid using a 5-ml syringe and 21-gauge needle.

Histopathology of Rat Lungs Following Immunotoxin Therapy. Trachea, lungs, and heart were removed as a unit and the lungs were gently inflated with ≈2 ml of fixative solution delivered via the trachea. These organs, as well as the chest wall, diaphragm, liver, kidney, and spleen, were fixed for at least 48 hr in 10% neutral buffered formalin. Fixed tissues were processed for paraffin embedding, sectioned at 6 μm, and stained with hematoxylin/eosin for histologic evaluation.

Treatment of Rats Carrying H3396 Tumor Xenografts. H3396 tumor xenografts were established in athymic rats as described (16). Groups ($n = 6$) were either left untreated, i.v. injected with a single dose of BR96 sFv-PE40 (0.5 mg/kg), pretreated with three i.p. administrations of Dex (1 mg/kg) at 50, 26, and 2 hr prior to immunotoxin administration (0.5 mg

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Abbreviations: VLS, vascular leak syndrome; Dex, dexamethasone; DSG, 15-deoxyspergualin; CsA, cyclosporine A; CBC, complete blood count; SGOT, serum glutamic–oxaloacetic transaminase; SGPT, serum glutamic–pyruvic transaminase; IL-2, interleukin 2.

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Table 1. Immunotoxin-mediated, dose-dependent hydrothorax in rats

IT dose, mg/kg (mg/m^2)	Hydrothorax, ml	Hematocrit, %	Albumin, g/dl
0	0	38.9	3.6
0.25 (1.475)	0	39.9	3.8
0.5 (2.95)	0	39.7	3.5
1.0 (5.9)	0–3*	39.5	3.2
2.0 (11.8)	>5†	42.9	2.6
3.0 (17.7)	>5†	56.1	2.5
4.0 (23.6)	>5†	55.5	2.6

BR96 sFv-PE40 was (i.v.) administered in a volume of 0.2 ml in phosphate-buffered saline (PBS). Fluid accumulation into the thoracic cavity was collected 24 hr after administration of immunotoxin (IT). (Two to four animals were treated at each dose.)

*Two of three rats had no fluid and one of three had 3 ml of hydrothorax fluid.

†Hydrothorax fluid >5 ml indicates a range of 5–8 ml.

of BR96 sFv-PE40 per kg), or pretreated with three i.p. injections of DSG (10 mg/kg) and immunotoxin as above.

RESULTS

Identification of Immunotoxin-Mediated Hydrothorax in Rats. Wistar Furth immunocompetent rats were i.v. administered with a single dose of BR96 sFv-PE40 (0.25–4.0 mg/kg). Necropsy was performed 24 hr following immunotoxin administration. The appearance of large amounts of clear fluid in the thoracic cavity (hydrothorax) was found to be dose dependent with doses of 2.0 mg/kg or greater resulting in fluid accumulations of >5 ml (Table 1). At 1 mg/kg, there was either small or no fluid accumulation and below 1 mg/kg, no fluid was detected. Acute toxicity was clinically apparent in rats treated at 2–4 mg/kg. Rats treated with 4 mg of BR96 sFv-PE40 per kg (and not euthanized earlier) died within 72 hr. The cause of death in these rats was determined to be asphyxiation and other complications as a result of fluid accumulation in the lungs and thoracic cavity occurring within 48–72 hr after administration of BR96 sFv-PE40. Comparatively, doses of 0.25 and 0.5 mg/kg were previously used to promote tumor regressions without toxicity in xenografted rats (16).

Associated with an increase in thoracic fluid accumulation, there was a slight increase in hematocrit at 2 mg/kg and a dramatic increase at 3 and 4 mg/kg. There was also an opposing effect on serum albumin as decreased levels were found at BR96 sFv-PE40 doses of 2 mg/kg or higher (Table 1). Hematocrit and serum albumin levels in the normal range were observed with BR96 sFv-PE40 doses of <2 mg/kg. Additionally, an increase in body weight (up to 10%) was associated with the onset of the hydrothorax in rats administered with BR96 sFv-PE40 at 1.5 mg/kg (maximum tolerated dose) within 7 days of administration (data not shown).

Histologic Evaluation. Histopathology was performed on rats sacrificed 24 hr after treatment with 2 mg of BR96 sFv-PE40 per kg to assess tissue damage to major organs. Diffuse hepatocellular degeneration or, less frequently, mild liver necrosis was occasionally observed. However, major histologic lesions were confined to the lungs of rats treated with immunotoxin. The principal histologic lesion was characterized by the accumulation of light pink fluid in the peribronchovascular space consistent with edema fluid (Fig. 1 *A* and *B*). The edema fluid filled lymphatics and dissected and expanded adjacent connective tissues. Alveolar walls were normal to very slightly thickened and there was scant to moderate pleural mesothelial cell hypertrophy. It was common for fluid accumulated in perivascular spaces to contain small numbers of mixed, but primarily mononuclear, inflammatory cells (Fig. 1*C*). In <25% of cases, lesions were slightly to moderately more severe with alveolar walls being prominently thickened and adjacent air spaces containing increased numbers of macrophages along with fewer polymorphonuclear leukocytes, lymphocytes, and erythrocytes (Fig. 1*D*).

Lack of VLS Symptoms in Mice. The hydrothorax found in rats following administration of 2–4 mg of BR96 sFv-PE40 per kg was not seen in immunocompetent mice (Table 2). Serum albumin was constant and only a slight increase in hematocrit was observed at 2–4 mg of immunotoxin per kg, doses that are lethal to mice. Severe hepatic lesions were found to be the dose-limiting toxicity in mice. Thus rats, but not mice, can serve as a model for BR96 sFv-PE40-induced VLS.

Assessment of Prophylactic Drug Treatment on Rats Prior to Immunotoxin Therapy. Utilizing this immunotoxin-induced rat VLS model, experiments were initiated to evaluate the effect of several antiinflammatory and immunosuppressant drugs for ability to block hydrothorax. The drugs that were chosen were diphenhydramine (antihistamine), CsA (immunosuppressant), DSG (immunosuppressant), and Dex (antiinflammatory/immunosuppressant). Rats were treated with

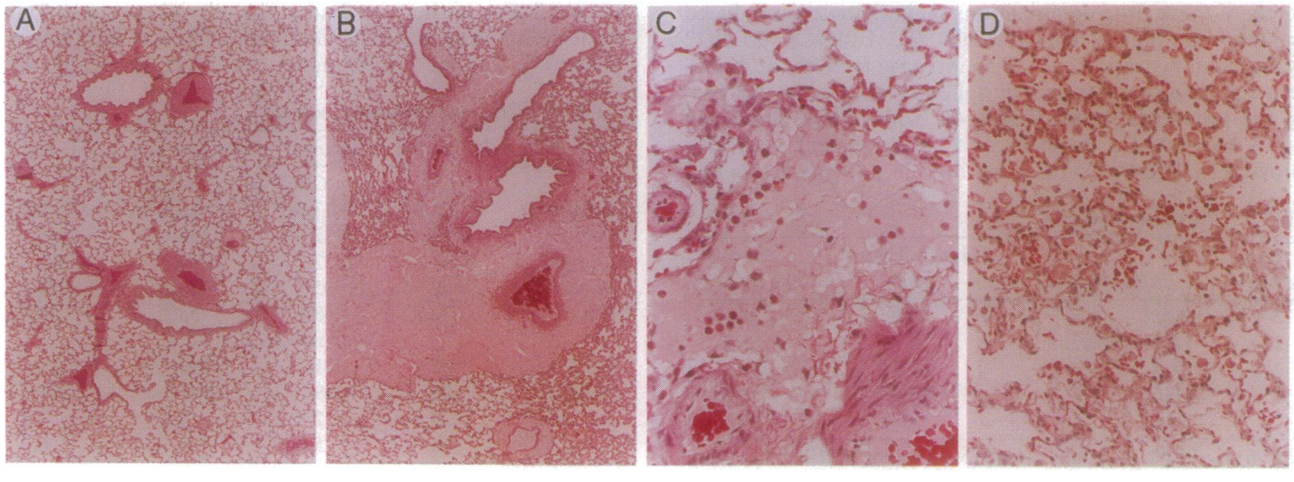

FIG. 1. Histologic evaluation of BR96 sFv-PE40-treated rat lungs. (*A*) Lung section taken from an untreated rat. (*B*) Histologic section of lung taken from a rat treated 24 hr earlier with a single dose of BR96 sFv-PE40 at 2 mg/kg. The peribronchovascular spaces are markedly widened and contain pink edema fluid. (*C*) Scattered within the edema fluid are scant to moderate numbers of mixed inflammatory cells. (*D*) Representative alveolar section from the most severe expression of alveolitis, characterized by accumulation of alveolar macrophages mixed with fewer polymorphonuclear leukocytes, lymphocytes, and widely dispersed clumps of fibrin. (*A* and *B*, ×20; *C*, ×110; *D*, ×70.)

Table 2. Lack of hydrothorax in mice

IT dose, mg/kg (mg/m^2)	Hydrothorax, ml	Hematocrit, %	Albumin, g/dl
0	0	36.4	3.15
2.0 (6.0)	0	42.8	3.3
4.0 (12.0)	0	46.0	3.3

BR96 sFv-PE40 was administered and fluid accumulation was measured as in Table 1.

either diphenhydramine, 26 and 2 hr prior to immunotoxin administration, or CsA, DSG, or Dex, 50, 26, and 2 hr prior to administration of BR96 sFv-PE40 (2 mg/kg). Dex pretreatment effectively blocked BR96 sFv-PE40-induced VLS in rats, with no detectable fluid accumulation in the thoracic cavity (Table 3). A variable though reproducible reduction in BR96 sFv-PE40-induced hydrothorax (0.5–5 ml) was observed in DSG-pretreated rats, while neither CsA nor diphenhydramine was effective at blocking VLS (Table 3). These results show that Dex is a potent inhibitor of immunotoxin-induced VLS and DSG has a variable inhibitory effect on VLS.

Survival of Rats Administered with Lethal Doses of BR96 sFv-PE40. Since VLS is the dose-limiting toxicity in BR96 sFv-PE40-treated rats, we evaluated the ability of these drugs to prevent immunotoxin-induced death. Rats were pretreated as described above, followed by administration of a lethal dose of BR96 sFv-PE40, 2 mg/kg, and were observed for survival until death or through day 10, postimmunotoxin treatment. All rats treated with 2 mg of BR96 sFv-PE40 per kg and no prophylaxis succumbed within 72 hr. All Dex-pretreated rats survived the otherwise lethal dose of immunotoxin (Table 3). No evidence of hydrothorax or other gross lesions was detected at necropsy. Neither CsA nor diphenhydramine prophylaxis resulted in prolongation of survival. Surprisingly, DSG, which reduced hydrothorax variably in BR96 sFv-PE40-treated rats, was ineffective in prolonging survival. Rats injected with Dex either at the same time or 24 hr following BR96 sFv-PE40 administration was unable to prevent hydrothorax or prolong survival. Thus, exposure to Dex, prior to immunotoxin administration, is necessary for the prevention of hydrothorax.

Effects of BR96 sFv-PE40 and VLS-Blocking Drugs on Blood Chemistry and CBC in Rats. VLS induced by immunotoxins has been shown to be associated with hematologic changes. Since Dex and, to a lesser extent, DSG, were found to block VLS in BR96 sFv-PE40-treated rats, we investigated the effects of these drug combinations on immunotoxin-induced changes in rat blood chemistry and CBC. BR96 sFv-PE40 (2 mg/kg) administration caused an increase in polymorphonuclear cells and hematocrit in peripheral blood, while decreasing the monocyte count (Table 4). Total white blood cell counts were within normal limits. Pretreatment with Dex followed by BR96 sFv-PE40 resulted in a leukocytosis and neutrophilia along with a rise in hematocrit, both predictable side effects of Dex (17, 18). As expected, the lymphocyte count was depressed in these rats. DSG pretreatment had a minor suppressive effect on all immunotoxin-induced hematologic changes and ameliorated the elevated hematocrit induced by BR96 sFv-PE40 (Table 4). However, DSG caused a pronounced decrease in monocytes consistent with the general myelosuppression effect of DSG that had been previously reported (19).

Table 3. Prevention of VLS

IT dose, mg/kg	Pretreatment	Hydrothorax,* ml	Survival at 10 days†
2	None	>5	0/10
2	Diphenhydramine	>5	0/3
2	CsA	>5	0/3
2	DSG	0.5–5	0/5
2	Dex	0	10/10

BR96 sFv-PE40 was administered i.v. in 0.2 ml of PBS either alone or 2 hr after the last pretreatment dose of diphenhydramine (1 mg/kg, i.p., daily for 2 days), CsA (100 mg/kg, s.c., daily for 3 days), DSG (10 mg/kg, i.p., daily for 3 days), Dex (1 mg/kg, i.p., daily for 3 days). IT, immunotoxin (BR96 sFv-PE40).

*Fluid collected 24 hr after administration of IT.

†Number alive/total number.

The effects of various treatments on blood chemistry were also compared at 2 mg of BR96 sFv-PE40 per kg. BR96 sFv-PE40 induced a mild increase in the liver enzymes serum glutamic–pyruvic transaminase (SGPT) and serum glutamic–oxaloacetic transaminase (SGOT) by 6- and 3-fold, respectively, as well as a decrease in both albumin and total protein (Table 4). Rats pretreated with Dex followed by BR96 sFv-PE40 were found to have extremely elevated SGPT and SGOT levels (36- to 50-fold) as well as slightly elevated albumin and total protein levels, all of which are secondary to Dex treatment. Rats treated with Dex only had slightly elevated transaminases (71 and 153 international units/liter for SGPT and SGOT, respectively). Thus, Dex appears to exacerbate the hepatotoxicity of BR96 sFv-PE40 by increasing the transaminase levels.

Histologic Examination of Dex-Pretreated Lungs. The effects of Dex pretreatment on rats receiving 2 mg of BR96 sFv-PE40 per kg were evaluated histologically 24 hr after immunotoxin administration. Animals pretreated with Dex had only slightly vascular edema or were indistinguishable from the control animals receiving no immunotoxin, whereas unprotected rats exhibited marked accumulation of fluid in the perivascular and surrounding parenchymal spaces (Fig. 2). Thus, pretreatment of rats receiving 2 mg of BR96 sFv-PE40 per kg and 1 mg of Dex per kg blocks both gross and microscopic evidence of pulmonary edema.

Dex or DSG Does Not Abrogate the Antitumor Activity of BR96 Immunotoxin on Tumor Xenografts in Rats. Dex and DSG are both immunomodulating agents. Having determined that Dex and, to a lesser extent, DSG, inhibit VLS in BR96 sFv-PE40 (2 mg/kg)-treated rats, we next evaluated whether they would interfere with immunotoxin activity against tumors *in vivo*. The antitumor activity of BR96 sFv-PE40 has previously been measured against H3396 human breast carcinoma xenografts in rats (16). Since the rats used in the antitumor study were athymic, we also tested the ability of athymic rats to develop dose-dependent hydrothorax. The accumulation of thoracic fluid in the athymic rats was found to be the same as that in the immunocompetent rats (data not shown). Using the H3396 model, rats were pretreated with

Table 4. CBC and chemistry screen

Parameter	Untreated	IT only	Dex and IT	DSG and IT
		CBC		
WBC	13.0	13.8	16.9	9.2
Poly	1593	5032	13,130	3286
Lymph	8217	7322	2,584	5587
Mono	659	395	486	230
Hct	36.8	48.6	53.1	38.9
		Chemistry screen		
SGPT	56	301	5,790	113
SGOT	99	299	3,641	131
Albumin	3.6	2.8	4.5	3.2
Tot. prot	6.7	5.0	7.1	6.3

Data (average of two to four rats) were collected 24 hr after immunotoxin (IT) administration (2 mg/kg). White blood cell (WBC) count is in thousands/mm^3; polymorphonuclear cells (Poly), lymphocytes (Lymph), and monocytes (Mono) are in absolute numbers; hematocrit (Hct) is in %; SGPT and SGOT are in international units/liter; albumin and total protein (Tot. prot) are in g/dl.

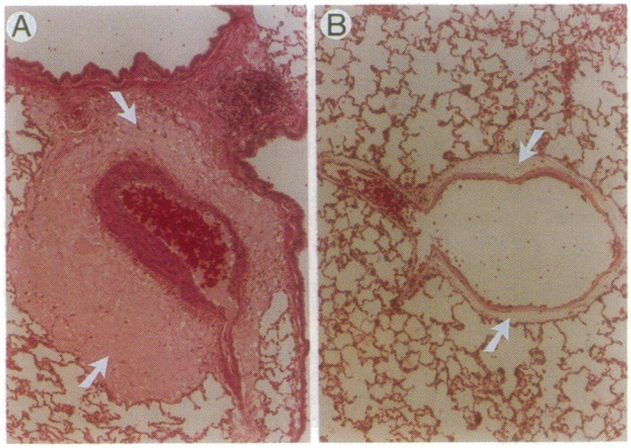

FIG. 2. Histopathology of rat lungs (with and without Dex pretreatment) following BR96 sFv-PE40 administration. (*A*) Section from rat lung taken 24 hr following treatment (BR96 sFv-PE40 at 2 mg/kg), with pink peribronchovascular edema fluid (arrows) surrounding a pulmonary blood vessel. (*B*) Histologic section of rat lung taken from a rat pretreated with 1 mg of Dex per kg for 2 days prior and on the same day as BR96 sFv-PE40 (2 mg/kg) administration. The perivascular space is slightly widened by edema fluid, demonstrating the ability of Dex pretreatment to eliminate or markedly reduce BR96 sFv-PE40-associated VLS. The thoracic cavity of this rat contained no free fluid. (×50.)

either Dex or DSG, followed by a single suboptimal i.v. dose of BR96 sFv-PE40 (0.5 mg/kg). Regressions of the H3396 tumor xenografts were observed in the immunotoxin-treated animals, with or without Dex or DSG pretreatment (Fig. 3). In the Dex-pretreated group, there was a slight reduction in the regression of the tumor xenograft in comparison with tumors in both the DSG or non-pretreated groups. Thus, pretreatment of rats with the immunomodulatory agent Dex or DSG does not block BR96 sFv-PE40 from regressing implanted tumor xenografts.

DISCUSSION

Immunotoxin therapy is a promising approach for the treatment of cancer (20, 21). However, dose-limiting side effects have prevented the use of sufficient amounts needed for the best therapeutic response. VLS has been the most limiting of these side effects (4, 22). For immunotoxins to become effective drugs for the treatment of human cancer, prevention of immunotoxin-induced VLS is necessary. Studies in nonhuman primates suggest that these models may be poor predictors of VLS toxicity in humans, as evidenced by the lack of VLS in monkeys and presence of VLS in humans treated with B3-LysPE40 immunoconjugates (23, 24).

No immunocompetent animal model of immunotoxin-induced VLS has been described to date. Administration of ricin A chain immunotoxins was reported to be unable to induce VLS in immunocompetent mice, rats, or guinea pigs (25). However, Vallera *et al.* (26) have reported that irradiated mice displayed VLS symptoms following administration of the pan T-cell immunotoxin anti-Thy 1.2-ricin A chain.

In vitro experimentation to investigate VLS has been performed because of the lack of an adequate *in vivo* model. Soler-Rodriguez *et al.* (25) demonstrated that ricin A chain could be directly cytotoxic to endothelial cells. However, it is not clear whether *in vivo* toxicity to endothelial cells is, in fact, the cause of immunotoxin-induced VLS.

We have focused on establishing an animal model that approximates VLS as seen in human clinical trials utilizing targeted immunotoxins. Rats administered with the single-chain immunotoxin BR96 sFv-PE40 responded with a dose-dependent VLS similar to that seen in humans. The VLS

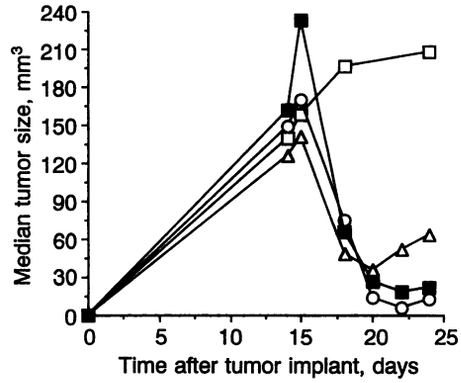

FIG. 3. Effect of Dex or DSG on BR96 sFv-PE40 antitumor activity in rats xenografted with H3396 human breast carcinoma. ○, BR96 sFv-PE40, 0.5 mg/kg (2.95 mg/m²); △, Dex, 1 mg/kg pretreatment, and BR96 sFv-PE40, 0.5 mg/kg (2.95 mg/m²); ■, DSG, 10 mg/kg pretreatment, and BR96 sFv-PE40, 0.5 mg/kg (2.95 mg/m²); □, nontreated control.

response was seen as an accumulation of thoracic fluid and pulmonary edema with a concomitant increase in hematocrit and body weight as well as a decrease in serum albumin levels (Table 1). The rats died within 48–72 hr after administration of BR96 sFv-PE40 as a result of fluid accumulation in the lungs and thoracic cavity.

In contrast, there were no VLS symptoms in mice administered with BR96 sFv-PE40 at doses up to 4 mg/kg, in which mice died due to hepatic toxicity (Table 2). Preliminary studies indicate that dogs do not respond to BR96 sFv-PE40 with any VLS-like symptoms at doses at least 6 times higher (on a mg/m² basis) than that causing a VLS response in a rat (data not shown). Additionally, there were no apparent VLS symptoms in monkeys following administration of immunotoxins prepared with both ricin A chain and PE40 (4, 23). Thus, rats represent the only species described so far to naturally respond to an immunotoxin like that seen in humans.

To accurately evaluate the toxicity of immunotoxins, standard screening of more than two species (often done with mouse and monkey) may be necessary. While rats provide a useful model for BR96 sFv-PE40-induced toxicity because they respond with a dose-limiting VLS, they may not be the optimal species for other immunotoxins with different specificities or with different toxins. Therefore, continued experimentation in evaluating the toxicity of other immunotoxins in the rat model, specifically focusing on VLS symptoms, is necessary.

Having a model for Lewis Y antigen (Le^y)-immunotoxin-induced VLS, we set out to search for inhibitors of VLS that could be combined with the immunotoxin without masking its antitumor activity. Of the four drugs tested, Dex totally blocked and DSG variably inhibited immunotoxin-induced VLS (Table 3). Additionally, Dex prevented death in rats treated with an otherwise lethal dose (2 mg/kg) of BR96 sFv-PE40 (Table 3), while DSG did not. However, Dex was unable to prolong survival in rats treated with 4 mg of BR96 sFv-PE40 per kg (data not shown). The mechanism whereby Dex suppresses VLS in this model system is unclear. Glucocorticoids are capable of exerting a multiplicity of antiinflammatory and immunosuppressive effects, including blockade of arachidonate metabolism, suppression of lymphocyte and macrophage functions with marked lympholysis in rodents, down-regulation of several proinflammatory cytokines, and stabilization of mast cells among others (18, 27). The absence of any modulating effects following CsA pretreatment and the presence of an effect in athymic rats suggest that, in this model, VLS is not mediated by classic

T-cell responses. The variable regulation of VLS associated with DSG pretreatment along with the potent effect of Dex suggest that the targets influenced by these immunosuppressive drugs may be critical to the pathogenesis of VLS in rats.

While VLS is the dose-limiting toxicity of many immunotoxins, it remained to be determined whether the same properties that mediate VLS are also responsible for tumor reduction. To address this issue, the effects of Dex or DSG prophylaxis on immunotoxin antitumor activity were studied in athymic rats carrying established H3396 human breast carcinomas. It was found that tumor xenografts in rats pretreated with either drug underwent regression similarly as compared to xenografts in rats treated with BR96 sFv-PE40 alone (Fig. 3), although xenografts in rats that received prophylactic Dex were regressed slightly less compared to xenografts in nonpretreated rats or those pretreated with DSG. One explanation for the slight reduction in antitumor activity is that Dex has been shown to decrease the delivery of a carcinoma-reactive monoclonal antibody to tumor xenografts in athymic rats (28). Thus, in our study, the immunotoxin may have been slightly retarded from leaving the vasculature in Dex-treated animals. However, since we specifically used a suboptimal treatment of immunotoxin and could only detect a slight difference between Dex pretreated and control groups, while the same level of Dex completely prevented all signs of VLS, it suggests that, at least in the rat model, antitumor activity and VLS occur by separate mechanisms.

DSG is currently undergoing clinical evaluation for suppression of human anti-mouse antibodies in cancer patients treated with an antibody or antibody-based imaging agent. Preliminary results from these studies suggest that DSG is effective at blocking HAMA. These results combined with our results showing that DSG inhibits VLS in rats without abolishing antitumor effects of BR96 sFv-PE40 support the further investigation of DSG in the clinic for combination therapy with immunotoxins.

IL-2 has also been found to cause VLS in humans (11, 12). Corticosteroids have been shown to reduce IL-2-mediated side effects (29, 30). However, corticosteroids also reduce the antitumor effect of IL-2 in mouse models of cancer since they are immunosuppressive and inhibit adoptive immunotherapy (31). Corticosteroids have been administered either concurrently or subsequent to use of anti-CD22-ricin A chain immunotoxin conjugates in humans, based on the ability of corticosteroids to inhibit IL-2-induced VLS (4, 9). These limited studies were unable to correlate the grade of toxicity in groups of patients who received corticosteroids in combination with immunotoxin, nor were they able to determine whether corticosteroids masked the ability of immunotoxins to regress tumors. Our data suggest that prophylactic treatment may be the key to blocking VLS induced by immunotoxin therapy. Further studies will be necessary to determine the optimal dose level and regime necessary to achieve maximal protection.

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