Immunotoxins Containing *Pseudomonas* Exotoxin That Target Le^Y Damage Human Endothelial Cells in an Antibody-specific Mode: Relevance to Vascular Leak Syndrome

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ABSTRACT

Vascular leak syndrome (VLS) was originally found to be a major dose-limiting toxicity in humans with cancer treated with several immunotoxins (ITs) containing ricin A chain or blocked ricin. Recently, VLS has also been observed in patients treated with an IT containing the murine monoclonal antibody (MAb) B3 coupled to LysPE38, a recombinant truncated form of *Pseudomonas* exotoxin (PE) A. Antibody B3 (IgG1k) recognizes $Lewis^Y$ and related carbohydrate epitopes present on many human solid tumors, and B3-LysPE38 showed excellent antitumor activity in nude mice bearing tumors that express the B3 antigen. In the clinical trial, the development of VLS has prevented the administration of the amount of IT necessary to achieve blood levels required for good therapeutic responses. We have now investigated the effects of several PE-based ITs on different human endothelial cell lines to elucidate the mechanism of VLS induced by ITs containing PE. To assess the cytotoxic effect of IT on endothelial cells, various ITs were incubated with cells for 2 or 20 h, and the incorporation of [^{3}H]leucine into protein was measured. The endothelial cells studied were human umbilical vein endothelial cells, human lung-derived microvascular endothelial cells (HUVECs), human adult dermal microvascular endothelial cells, human pulmonary artery endothelial cells, and human aortic endothelial cells. We found that both B3-LysPE38 (LMB-1), a chemical conjugate of MAb B3 with PE38, as well as B3(Fv)-PE38 (LMB-7), a recombinant single chain immunotoxin, inhibited protein synthesis, with 50% inhibitory concentrations between 600 and 1000 ng/ml for 20-h incubation in HUVECs, human lung-derived microvascular endothelial cells, and human adult dermal microvascular endothelial cells but not on human pulmonary artery endothelial cells. The cytotoxic effect was specific since PE38 itself or PE coupled to several other antibodies did not inhibit protein synthesis in these cells even at 10,000 ng/ml. Further evidence that the cytotoxicity of B3-containing ITs is due to specific B3 binding to endothelial cells comes from the fact that the cytotoxicity can be blocked by excess free MAb B3. HUVECs undergo overt morphological changes after treatment with B3-LysPE38 or B3(Fv)PE38. Gaps between the cells are formed after a 20-h exposure but not after 2 h. These studies suggest that VLS in patients is due to capillary damage caused by prolonged exposure to high concentrations of LMB-1.

INTRODUCTION

One evolving approach to cancer treatment is to attach plant or bacterial toxins to antibodies to make immunotoxins. Several different types of immunotoxins have been prepared, and some of these are now in clinical trials (1, 2). Some of the toxins that have been used to make immunotoxins are ricin A chain, blocked ricin, saporin, pokeweed antiviral protein, and mutant forms of PE[2] (3–6). Most of the ricin-containing immunotoxins have been directed at antigens present on the surface of lymphomas and leukemias (7, 8), but several have been developed against solid tumors (9–11). For solid tumor therapy, LysPE38, a recombinant truncated form of PE lacking the cell binding domain (12), has been chemically attached to murine monoclonal antibody B3, which reacts with a carbohydrate antigen in the $Lewis^Y$ family that is present on the surface of many solid tumors (13). This immunotoxin is termed B3-LysPE38 or LMB-1 (14) and is currently being evaluated in a Phase I clinical trial (15).

VLS has been shown to be one of the major side effects causing dose-limiting toxicity found in many clinical trials utilizing immunotoxins, including those prepared with ricin A chain, blocked ricin, and, more recently, PE (7, 8, 15). This syndrome is manifested by decreased serum albumin and accumulation of fluid in the interstitial space, leading to weight gain, edema, and, in the most severe cases, accumulation of excess fluid in the lungs and pericardium. The problem of capillary leak has prevented the administration of the amount of immunotoxin necessary to achieve maximum therapeutic responses.

VLS was not observed in the preclinical testing of PE-containing immunotoxins in mice or monkeys. Because of the lack of an appropriate mouse model, progress in our understanding of immunotoxin-mediated VLS has been delayed. Several hypotheses have been explored to explain the VLS. One of these hypotheses is direct damage of capillaries by the immunotoxin. Another hypothesis is an indirect effect due to the production of

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[2] The abbreviations used are: PE, *Pseudomonas* exotoxin; VLS, vascular leak syndrome; RTA, ricin A chain; HUVEC, human umbilical vein endothelial cell; HPAEC, human pulmonary artery endothelial cell; HAEC, human aortic endothelial cell; HMVEC-L, human lung-derived microvascular endothelial cell; HMVEC-dAd, human adult dermal microvascular endothelial cell; IL-2, interleukin 2; MAb, monoclonal antibody; IC_{50}, 50% inhibitory concentration.

cytokines or other vasoactive substances which damage capillaries. The VLS has been observed in patients receiving high-dose IL-2 therapy, and it has been suggested that in IL-2-treated patients, VLS is due to damage by IL-2-activated monocytes (16, 17).

To investigate the basis of VLS in patients receiving immunotoxins containing ricin A chain, Soler-Rodriguez *et al.* (18) studied the action of these immunotoxins on HUVECs. Their findings indicated that the HUVECS were sensitive to the action of these immunotoxins, and that the cytotoxic effect was due to a direct interaction of ricin A chain with the endothelial cell membrane and not due to the antibody to which it was attached. They observed that the HUVECs underwent rapid and dramatic changes in morphology followed by inhibition of protein synthesis (18).

A Phase I clinical trial in adult patients with solid tumors is currently being carried out using immunotoxin LMB-1 (B3-LysPE38) at the National Cancer Institute. The VLS appears to be the major toxicity observed in this trial (15). To investigate the mechanism of this VLS, we have exposed several different types of endothelial cells, including HUVECs, HMVEC-L, HMVEC-dAd, HPAECs, and HAECs, to B3-LysPE38 and other immunotoxins. The results indicate that the endothelial cell damage produced *in vitro* by this immunotoxin is due to the B3 antibody portion of the immunotoxin that reacts with an antigen present on the surface of endothelial cells and not due to an interaction of PE38.

MATERIALS AND METHODS

Cell Culture. HUVECs, HPAECs, HAECs, HMVEC-L, and HMVEC-dAd isolated from single donors were purchased from Clonetics Corporation EndoPack cell culture systems. They were grown at 37°C in 5% CO_2 in endothelial cell growth medium, which is a complete medium based on the MCDB 131 formulation, and supplemented with 10 ng/ml human recombinant epidermal growth factor, 1 μg/ml hydrocortisone, 2% fetal bovine serum, 50 μg/ml gentamicin, 50 ng/ml amphotericin-B, 10 μg/ml heparin, and 12 μg/ml bovine brain extract. Endothelial cells (ECs) in the third to fifth passage were used in these studies. ECs were harvested from the culture flasks by trypsinization in the presence of EDTA.

Toxins and Immunotoxins. The ricin A chain used in this work was purified by J. Fulton of Inland Labs., and it does not contain free ricin B chain. B3-LysPE38, B3(Fv)-PE38, MOPC-LysPE38, LysPE38, e23(dsFv)-PE38, anti-Tac(Fv)-PE38, PEΔ553, and MAb B3 were prepared as described elsewhere (13, 19–21). B3(Fv)-PE38 is a single-chain immunotoxin composed of the variable (Fv) region of MAb B3 fused to PE38 and is presently undergoing clinical testing (19).[3] e23(dsFv)PE38 is a recombinant toxin composed of the Fv region of anti-erbB-2 MAb 23 connected to PE38, in which the V_H and V_L are stabilized by a disulfide bond (22).[4] Anti-Tac(Fv)-PE38 is a recombinant immunotoxin containing the anti-Tac antibody variable region fused to PE38 (20).

[3] L. Pai, manuscript in preparation.
[4] Y. Reiter and I. Pastan, unpublished results.

Protein Synthesis and Inhibition Assays. ECs were plated at 3200 cells/well and cultured in 96-well plates for 96 h or until near confluency. Toxins or controls diluted in 0.2% human serum albumin-PBS were added to a final volume of 200 μl/well. After incubation at 37°C for the indicated time (usually 20 h), each well was pulsed for 4 h with [^{3}H]leucine (1 μCi). The cells were subjected to a freeze-thaw cycle to lyse the cells so that we could measure the incorporation of [^{3}H]leucine into protein. After freezing and thawing, the lysed cells were harvested on glass filters, and the incorporation of radioactivity into protein was quantitated by a Betaplate scintillation counter (LKB). Results were calculated as the percentage of incorporated cpm cells incubated without toxin and represent the average of experiments performed in triplicate. For competition with MAb B3, 1 mg/ml MAb B3 was added to each well and incubated at 37°C for 30 min prior to toxin addition. For time course study, the cells were treated with different concentrations of immunotoxins for 2, 4, 8, 12, or 24 h. Cells exposed to toxin for less than 24 h were washed to remove the toxin, and incubated further until 24 h. Then they were labeled with [^{3}H]leucine, frozen, thawed, washed, and counted (19).

Endothelial Cell Morphology Studies. HUVECs and HPAECs were grown in 35-mm dishes and treated with various concentrations of B3-LysPE38, B3(Fv)-PE38, and RTA. Morphology of the cells was observed after 2- and 20-h incubations using an Olympus IMT2 inverted phase-contrast microscope.

RESULTS

Specificity of B3 MAb on HUVECs. To assess the cytotoxic effect of immunotoxins on endothelial cells, various immunotoxins were incubated with HUVECs for 20 h. As shown in Fig. 1*A*, B3-LysPE38 inhibited protein synthesis in HUVECs with a IC_{50} of ~1000 ng/ml. Although a very high concentration of B3-LysPE38 is necessary to produce this toxic effect, the toxic effect is specific since it is blocked by excess MAb B3 (Fig. 1*A*). Furthermore, MOPC-LysPE38, an immunotoxin made with the MOPC antibody that has no known reactivity with tumor cells, had no cytotoxic effect on HUVECs (Fig. 1*C*). MAb B3 and MAb MOPC are both of the IgG1 isotype. B3-LysPE38 is composed of the entire B3 antibody coupled to LysPE38; B3(Fv)-PE38 is a much smaller recombinant immunotoxin in which the binding site of B3 in a single-chain form is fused to PE38 (19).[3] B3(Fv)-PE38 was also cytotoxic to HUVECs with a IC_{50} of ~600 ng/ml (Fig. 1*D*). MAb B3 blocked the cytotoxicity of B3(Fv)-PE38, indicating that it was also binding to HUVECs via the antibody portion of the immunotoxin (Fig. 1*B*). In support of this conclusion was the finding that anti-Tac(Fv)-PE38, which binds to the p55 subunit of the IL-2 receptor expressed on human monocytes, had no cytotoxic effect on HUVECs (Fig. 1*D* and Table 1). Also, e23(Fv)PE38, a recombinant immunotoxin that binds to the erbB-2 receptor, did not inhibit protein synthesis of HUVECs even at the extremely high concentration of 10,000 ng/ml (Table 1).

We investigated the cytotoxic effect of several other proteins and toxins on HUVECs as shown in Table 2. Native PE did show cytotoxicity on HUVECs with a IC_{50} of ~200 ng/ml. This cytotoxicity is due to the presence of α2-macroglobulin recep-

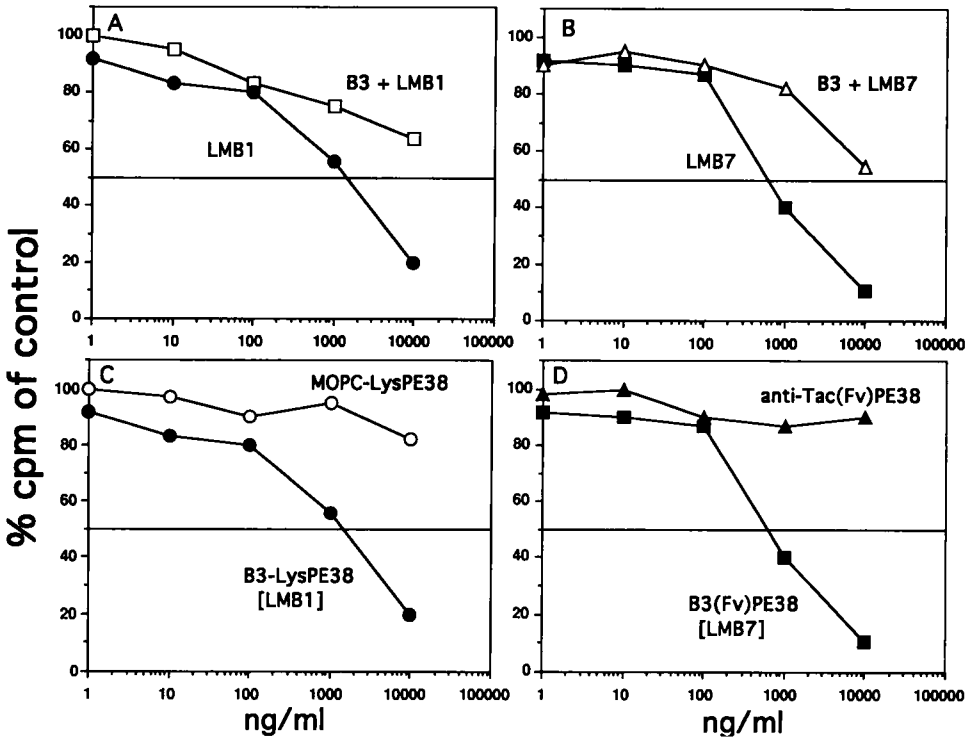

Fig. 1 Specificity of B3 antibody on HUVECs. HUVECs were incubated with immunotoxins for 20 h, and protein inhibition of the cells was measured. *A*, competition between B3-LysPE38 (LMB-1) and excess MAb B3 on HUVECs. *B*, competition between B3 (Fv)-PE38 (LMB-7) and excess MAb B3 on HUVECs. *C*, comparison of cytotoxicity toward HUVECs of MOPC-LysPE38 and B3-LysPE38. *D*, comparison of cytotoxicity toward HUVECs of anti-Tac(Fv)-PE38 and B3(Fv)-PE38.

Table 1 Cytotoxicity of immunotoxins on various human endothelial cells

Proteins	Time of incubation (h)	IC_{50} (ng/ml) A431	HUVEC	HPAEC	HAEC	HMVEC-L	HMVEC-dAd
B3-LysPE38	20	2	1,000	>10,000	>10,000	4,000	7,000
	2		2,000				
B3(Fv)PE38	20	0.5–1	600	>10,000	800	750	1,200
	2	2.5	2,300				
ATac(Fv)PE38	20		>10,000	>10,000	>10,000	10,000	>10,000
	2		>10,000				
e23(dsFv)PE38	20	3	>10,000	ND[a]	ND	>10,000	>10,000
	2		>10,000				

[a] ND, not done.

tors (23) on the surface of HUVECs. However, a truncated form of PE, LysPE38, had no cytotoxic activity on HUVECs even at 10,000 ng/ml (Table 2). This presumably is because LysPE38 does not have a binding domain to react with endothelial cells. The mutant PEΔ553, which is defective in ADP ribosylation activity, also did not show any activity in HUVECs. MAb B3 by itself had no effect on HUVECs. In confirmation of the findings of Soler-Rodriguez *et al.* (18), we found that RTA was cytotoxic to endothelial cells with a IC_{50} of ~500 ng/ml (Table 2). Taken together, the results in Fig. 1 and Table 2 indicate that the cytotoxic effects produced by B3-LysPE38 and B3(Fv)-PE38 are due to the B3 antibody portion reacting with an antigen on the surface of endothelial cells and not due to an interaction of PE38. Furthermore, the cytotoxicity required the ADP ribosylation activity of the toxin.

Time Course of B3-LysPE38- and B3(Fv)-PE38-induced Cytotoxicity on HUVECs. To determine the kinetics of inhibition of protein synthesis in HUVECs, the cells were treated with different concentrations of B3-LysPE38 and B3(Fv)-PE38 for various periods of time (Fig. 2), and inhibition of protein synthesis was measured. In all assays, [^{3}H]leucine was added 24 h after toxin addition. When cells were exposed for less than 24 h to immunotoxin, they were placed in immunotoxin-free medium until 24 h was reached, and then [^{3}H]leucine was added. These results show that the cytotoxic activity caused by B3-LysPE38, B3(Fv)-PE38, or RTA increased over the period of 2–24 h. For B3-LysPE38 and B3(Fv)-PE38, the cytotoxic effects on HUVECs increased approximately 2-fold between 2 and 24 h of incubation, whereas the cytotoxic effects of RTA increased more than 4-fold during this time period. Thus, the results show that the cytotoxic effect of B3-LysPE38, B3(Fv)-PE38, and RTA on HUVEC cells is time dependent.

Capillary Endothelial Cells (HMVEC-L and HMVEC-dAd) Are Sensitive to Immunotoxins Targeting Le^Y. In addition to testing the effects of B3-related immunotoxins on

Table 2 Inhibition of protein synthesis in HUVECs by various immunotoxins and toxins

Proteins	IC_{50}[a] (ng/ml)
B3-LysPE38	1,000
B3(Fv)-PE38	600
NLysPE38	>10,000
PEΔ553	>10,000
B3 MAb	>10,000
PE	210
RTA	500

[a] Average of three experiments. For each experiment performed in triplicate, the HUVECs were from an individual single donor.

HUVECs, we studied human endothelial cells from other sources. We measured the cytotoxic activities of B3-LysPE38, B3(Fv)-PE38, e23(dsFv)-PE38, and anti-Tac(Fv)-PE38 on four other human endothelial cells besides HUVECs. Anti-Tac(Fv)-PE38 reacts with the IL-2 receptor α subunit present on leukemias and lymphomas (20). The cells studied were HMVEC-L, HMVEC-dAd, HPAECs, and HAECs. HMVEC-L and HMVEC-dAd are primarily from capillaries and HUVECs and HAECs, or HPAECs may be considered as from large blood vessels. The data were collected from four single donors for HUVECs and two single donors for HMVEC-L, HMVECs, HPAECs, and HAECs, respectively.

The data in Table 1 summarize the results of experiments in which the type of cells were incubated with immunotoxins for 2 or 20 h. HUVECs were sensitive to B3-LysPE38 with IC_{50}s of 1000 and 2000 ng/ml at 20 and 2 h. They were also sensitive to B3(Fv)-PE38 with IC_{50} of 600 ng/ml after a 20-h exposure and 2300 ng/ml after a 2-h exposure. In parallel experiments, the cytotoxicity of these agents was examined on A431 cells that had abundant Le^Y, and the cells were very sensitive with IC_{50}s ranging from 0.5 to 2.5 ng/ml. Neither anti-Tac(Fv)-PE38 nor e23(Fv)-PE38 inhibited protein synthesis in HUVECs; their IC_{50}s were over 10,000 ng/ml.

HMVEC-L and HMVEC-dAd were also sensitive to B3-based immunotoxins with IC_{50}s of 4000 and 7000 ng/ml for B3-LysPE38 and 750 and 1200 ng/ml for B3(Fv)-PE38. The cells were unaffected by 10,000 ng/ml of anti-Tac(Fv)-PE38 or e23(Fv)-PE38.

Cells derived from HAEC were more resistant, with no detectable inhibition of protein synthesis by B3-LysPE38 at 10,000 ng/ml and a IC_{50} of 1000 mg/ml with B3(Fv)-PE38. Anti-Tac(Fv)-PE38 did not inhibit protein synthesis in these cells. Interestingly, HPAECs were completely resistant to B3-LysPE38 and B3(Fv)-PE38 as well as to anti-Tac(Fv)-PE38. The relative resistance of HPAECs and HAECs to immunotoxins suggests that the endothelial cells from the large vessels contain less Le^Y than endothelial cells from capillary beds and the umbilical vein.

Morphological Changes in B3-LysPE38- and B3(Fv)-PE38-treated HUVECs and HPAECs. To evaluate the effect of B3-containing immunotoxins on cell morphology, HUVECs were incubated with various concentrations of B3-LysPE38 and B3(Fv)-PE38. The untreated HUVEC monolayers consisted of tightly packed cells with elongated shapes. Initially, HUVECs were treated with either B3-LysPE38 or B3(Fv)-PE38 at 0.01, 0.1, or 1 μg/ml, but there were no morphology changes observed for 2-h or 20-h incubation at 37°C. Thereafter, when

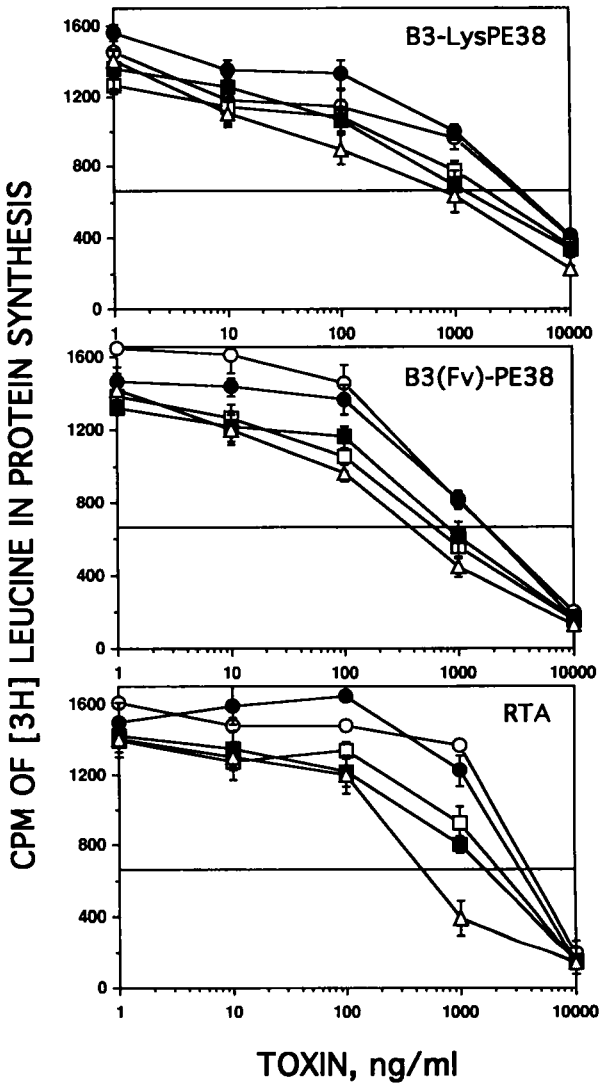

Fig. 2 Kinetics of inhibition of protein synthesis in HUVECs treated with B3-LysPE38, B3(Fv)-PE38, and RTA. HUVECs were treated with different doses of B3-LysPE38, B3(Fv)-PE38, or RTA for 2 h (○), 4 h (●), 8 h (□), 12 h (■), or 24 h (△). *Points*, mean from three experiments; *bars*, SDs.

HUVECs were treated with either B3-LysPE38 or B3(Fv)-PE38 at 10 μg/ml, no changes of cell shape were observed after 2 h (Table 3). However, after 20-h incubation with B3(Fv)-PE38 or B3-LysPE38, gaps appeared in the endothelial monolayers. In contrast, treatment with 10 μg/ml RTA induced cell rounding after only 2-h incubation, and huge gaps were found after 20-h exposure to RTA. Similar morphological changes were also observed when HUVECs were treated with a lower concentration of RTA at 1 μg/ml, which was very similar to the concentration that Soler-Rodriguez *et al.* (18) found to trigger rapid and dramatic changes in cell monolayers.

We then investigated the effects of B3-LysPE38, B3(Fv)-PE38, and RTA on the morphology of HPAECs. The results are summarized in Table 3. There were no effects of either B3-LysPE38 or B3(Fv)-PE38, at 100 μg/ml, on HPAEC morphology

Table 3 Effect of immunotoxins and toxins on cell shape

	Time (h)	B3-LysPE38[a]	B3(Fv)-PE38[a]	RTA[a]
HUVEC	2	No effect	No effect	Rounding cells
	20	Gaps	Gaps	Gaps
HPAEC	2	No effect	No effect	Rounding cells
	20	No effect	No effect	Gaps

[a] HUVECs and HPAECs were treated with 10 μg/ml B3-LysPE38 or B3(Fv)-PE38, or 1 μg/ml RTA.

after a 2-h or 20-h exposure. However, RTA at 1 μg/ml cell induced rounding in HPAECs after a 2-h incubation and caused the formation of gaps after 20 h. We also found that the IC_{50} of RTA on HPAECs was ~1000 ng/ml in the 20-h assay (data not shown).

Taken together, the HUVEC cell morphology changes mediated by B3(Fv)-PE38 or B3-LysPE38 were concentration dependent and occurred only at high concentrations of immunotoxins.

DISCUSSION

We have shown that immunotoxins B3-LysPE38 and B3(Fv)-PE38 specifically inhibit protein synthesis in cultured endothelial cells derived from human umbilical vein, human lung-derived and dermal-derived microvascular endothelium, as well as endothelial cells from human aorta. Competition experiments and comparison to other immunotoxins showed that the cytotoxic effects of B3-LysPE38 and B3(Fv)-PE38 on HUVECs are specific. These data show that the Le^y-related B3 antigen is present on endothelial cells and suggest this is the basis of the VLS found in patients treated with B3-LysPE38.

The cytotoxic effect of B3-LysPE38 and B3(Fv)-PE38 on HUVECs was time dependent. With B3-LysPE38 a maximum effect was observed after a 24-h continuous exposure. With B3(Fv)-PE38 a maximum effect was produced by an 8-h exposure. This is probably related to the instability of B3(Fv)-PE38 at 37°C where it has a $t_{1/2}$ of about 2 h (24). The morphological study showed that no dramatic change of the HUVECs was observed until 20 h of treatment with B3-LysPE38 or B3(Fv)-PE38. These results suggest that changes in cell shape are secondary to inhibition of protein synthesis caused by the exposure of cells to B3-containing immunotoxins. This is in contrast to results with immunotoxins containing ricin A chain, which induce rounding of cells before inhibition of protein synthesis is detected (18).

Variation of Response with Different Cell Lines. We analyzed the activity of B3-LysPE38 and B3(Fv)-PE38 on four other endothelial cell lines besides HUVECs. One line was derived from pulmonary arteries (HPAECs), one line from the aorta (HAECs), and the other two lines were derived from lung or dermal microvascular endothelium (HMVEC-L and HMVEC-dAd). Some cytotoxic activity was observed with B3(Fv)-PE38 on HAECs, but the concentration required for inhibition of protein synthesis was greater than that required on HUVECs. On HPAECs, very little inhibition of protein synthesis was observed. Three cell lines (HUVECs, HPAECs, and HAECs) are derived from large vessels. Two cell lines (HMVEC-L and HMVEC-dAd) are from capillary beds. Both cell lines derived for microvasculature were sensitive to both types of B3 immunotoxins. We assume that the cytotoxic activity of B3-LysPE38 on capillaries and not large vessels initiates the VLS. In immunohistochemical studies carried out with MAb B3 on humans before the clinical trials were initiated, no reactivity was detected in the capillaries. However, small amounts of antigen could escape detection by immunocytochemistry and yet be sufficient to mediate binding and cytotoxicity of B3-LysPE38 and B3(Fv)-PE38.

Prior to the clinical studies with B3-LysPE38, extensive toxicological studies were carried out on *Cynomolgus* monkeys. The maximum tolerated dose was found to be 4 mg/kg, which results in an initial blood level of approximately 125 μg/ml. Even at this high-dose level, no signs of the VLS were observed. It seems likely that the B3 antigen is not present on the capillaries of these animals, although it is present in stomach, trachea, bladder, and esophagus, which are also the major locations of the antigen in humans.[5] Toxicological studies were also carried out with B3-LysPE38 in mice where the maximum tolerated dose is 3 mg/kg. At this dose there is extensive damage to the liver, but no evidence of VLS (21).

Implications for Clinical Trials. In a Phase I study currently ongoing in patients with cancers expressing the B3 antigen, we have found that the VLS occurs in patients receiving B3-LysPE38 at doses ranging from 10 to 100 μg/kg (15). Measurements of blood levels in these patients indicate that shortly after administration of B3-LysPE38, blood levels of 500-1000 ng/ml are obtained.[6] B3-LysPE38 disappears from the blood with a $t_{1/2}$ of approximately 9 h. Therefore, endothelial cells are exposed to a very high concentration of B3(Fv)-PE38 for many hours. As shown in Fig. 2, an 8-h exposure to B3-LysPE38 is sufficient to cause significant inhibition of protein synthesis in HUVECs. Thus, the blood levels obtained in patients correlate fairly well with the concentrations of B3(Fv)-PE38 necessary to intoxicate HUVECs.

If the VLS is due to exposure of capillaries to high concentrations of B3-LysPE38, then several strategies are available to diminish this side effect. One strategy is to use a smaller molecular weight immunotoxin which escapes from the circulation more quickly, and therefore would be in contact with capillaries for a shorter time. B3(Fv)-PE38 has a M_r 62,000, and, in studies with monkeys and mice, its $t_{1/2}$ ranges from 20 to 40 min. Its smaller size allows it to escape from the circulation more rapidly, probably due to a combination of better penetration into tumors and tissues and more rapid excretion. Therefore, capillaries should be exposed to B3(Fv)-PE38 only for a short period of time and may not be damaged as they are with B3-LysPE38. If capillary leak syndrome is observed with B3(Fv)-PE38, it may be possible to give a mixture of B3(Fv)-PE38 and polyethylene glycol-modified MAb B3. Because the derivatized antibody will escape slowly from the circulation, it should prevent the binding of B3(Fv)-PE38 to capillaries, but have very little effect on binding of B3(Fv)-PE38 to tumor cells that are outside of the capillary bed.

Differences between Ricin- and PE-containing Immunotoxins. Studies by Soler-Rodriguez *et al.* (18) have shown that ricin A chain and ricin A chain-containing immunotoxins are cytotoxic to HUVECs. These effects appear to be due to an

[5] M. Willingham, L. Pai, and I. Pastan, unpublished data.
[6] L. Pai and I. Pastan, unpublished data.

interaction of ricin A chain with the cells. As shown in Fig. 2, we confirmed their findings that HUVECs are sensitive to ricin A chain with a IC_{50} at 24 h of 500 ng/ml. Furthermore, RTA has a rapid effect on the morphology of HUVECs which can be observed after incubation of that toxin with the cells for 2 h or less. It has been suggested that this effect is independent of protein synthesis (16). In contrast, a 20-h exposure of HUVECs to B3-LysPE38 or B3(Fv)-PE38 was required to observe changes in morphology, and these changes occurred at a time when protein synthesis was markedly inhibited. The mechanism of alteration of HUVECs by ricin-containing immunotoxins and PE-containing immunotoxins appears to be different.

We are currently conducting preclinical studies with recombinant immunotoxins containing the anti-Tac antibody variable region, anti-Tac(Fv)-PE38 (20), and an immunotoxin directed at erbB-2, e23(Fv)-PE38KDEL (25). Phase I clinical studies with these agents should begin within a year and clarify whether or not the VLS observed with B3-LysPE38 is due to an interaction of the antibody or the toxin with endothelial cells in patients.

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