

Anti-Tac(Fv)-PE40, a Single Chain Antibody *Pseudomonas* Fusion Protein Directed at Interleukin 2 Receptor Bearing Cells*

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Anti-Tac(Fv)-PE40 is a chimeric single chain immunotoxin in which anti-Tac variable heavy and light chains held together by a peptide linker are attached to PE40, a truncated form of *Pseudomonas* exotoxin. This molecule was shown to be extremely cytotoxic for interleukin 2 (IL2) receptor bearing cells in tissue culture (Chaudhary, V. K., Queen, C., Junghans, R. P., Waldmann, T. A., FitzGerald, D. J., and Pastan, I. (1989) *Nature* 339, 394–397). Here we describe various forms of anti-Tac(Fv)-PE40 protein in which the order of the variable domains of anti-Tac has been switched and also three different types of peptide linkers have been used. All these proteins were purified to near homogeneity and were found to have similar cytotoxic activities against various human cells expressing the p55 subunit of the IL2 receptor. Anti-Tac(Fv)-PE40 was also found to have a very potent suppressive activity against phytohemagglutinin-activated human lymphoblasts and in a human mixed lymphocyte reaction. Anti-Tac(Fv)-PE40 appeared in the blood rapidly in mice after intraperitoneal administration and could be detected in the blood for up to 8 h. Anti-Tac(Fv)-PE40 warrants evaluation as an anti-tumor and immunosuppressive agent in humans.

Immunotoxins are emerging as potential chemotherapeutic agents for the treatment of cancer, acquired immune deficiency syndrome, and immunological disorders (1, 2). Several different toxins have been employed to make immunotoxins. These include ricin, diphtheria toxin, *Pseudomonas* exotoxin, modecin, and saporin. We have been constructing immunotoxins using *Pseudomonas* exotoxin (PE).¹ PE is composed of three major structural domains each with a different function. Domain I is responsible for cell recognition, domain II for translocation across the cell membrane, and domain III for the ADP-ribosylation activity of the toxin, although recently the last five amino acids of the carboxyl end of domain III have also been shown to have a role in translocation (3–5). Immunotoxins are conventionally made by coupling monoclonal antibodies to toxins using chemical cross-linkers. Recently, it has been possible to produce immunotoxins in *Escherichia coli* by fusing DNA segments encoding the Fv

regions of antibodies to a truncated form of the toxin, thereby producing single chain antibody toxin fusion proteins (6, 7). We initially produced a molecule termed anti-Tac(Fv)-PE40 in which the variable region of the heavy chain of the anti-Tac antibody is attached through a peptide linker to the variable light chain which in turn is fused to PE40 (6). The latter is a truncated form of *Pseudomonas* exotoxin which lacks the binding domain of the toxin. Anti-Tac(Fv)-PE40 is cytotoxic to cells expressing the p55 subunit of the human IL2 receptor (6).

The affinity of anti-Tac(Fv)-PE40 for the p55 subunit of the IL2 receptor was found to be very close to that of native anti-Tac (6). To study the structural requirements for binding and to explore the possibility of creating a more active molecule, we have made several different anti-Tac(Fv)-PE40 constructions. In these, the order of the variable heavy and light chains has been switched and the role of the peptide linker joining the variable chains has been explored by using three different linkers. In addition, the activity of anti-Tac(Fv)-PE40 was assessed on various types of normal and malignant human T cells. The cytotoxicity was compared with that of two other chimeric toxins composed of IL2 linked to different mutant forms of PE. Anti-Tac(Fv)-PE40 was found to be more active than either IL2-PE40 or IL2-PE66^{4Glu} (8, 9), two other chimeric toxins directed at IL2 receptor-bearing cells.

MATERIALS AND METHODS

Construction of Plasmids—Polymerase chain reaction-based methods were used to amplify, create cloning sites, and assemble the plasmids containing the variable domains of the light and the heavy chains of anti-Tac joined together by a peptide linker. These variable domains are fused to DNA sequences encoding PE40. These fusion genes are under the control of a T7 promoter and an appropriate ribosome binding site has been included.

p7018—Fig. 1 summarizes the cloning strategy employed for this construction. The starting template material was cDNA of the light and heavy chain of anti-Tac cloned as an *EcoRI* insert in pUC18. Oligonucleotides VK73 and VK74 were used to amplify the variable domain of the heavy chain (V_H) using anti-Tac heavy chain cDNA as template. VK75 and VK76 were used to amplify the variable domain of the light chain (V_L) using the anti-Tac light chain cDNA as template. The conditions of the polymerase chain reaction have been described previously (7). For VK73, 23 nucleotides at the 3'-end were the same as the 5'-sequence of the cDNA encoding the mature heavy chain of anti-Tac. VK73 also introduced an *NdeI* site and an initiation methionine at the 5'-end. The 3'-end of the oligonucleotide, VK74, was complementary to the 3'-end of the variable domain of the heavy chain. VK74 also encoded the first 8 anticodons of the peptide linker followed by a *KpnI* site, and the nucleotides at its 5'-end were complementary to the sequences in the CH1 domain. VK75 carried nucleotides for a *KpnI* site and for the last 8 codons of a peptide linker. The last 23 nucleotides of VK75, at the 3'-end, were the same as that of the 5'-end of the mature light chain. The 3'-end of oligonucleotide VK76 contained 24 nucleotides which were complementary to the 3'-end of the variable domain of the light chain,

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¹ The abbreviations used are: PE, *Pseudomonas* exotoxin; IL2, interleukin 2; anti-Tac, monoclonal antibody to p55 subunit of human IL2 receptor; V_H, heavy chain variable region; V_L, light chain variable region; kb, kilobase(s); SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PHA, phytohemagglutinin.

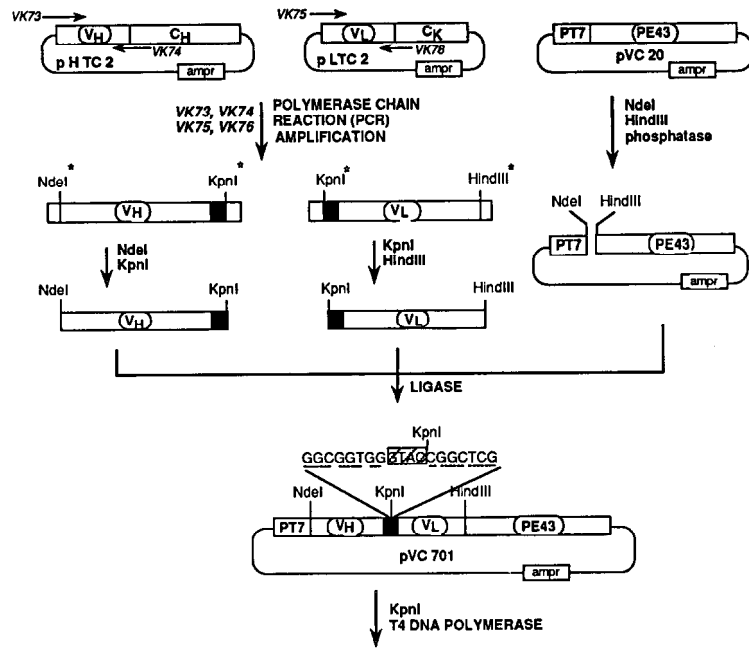
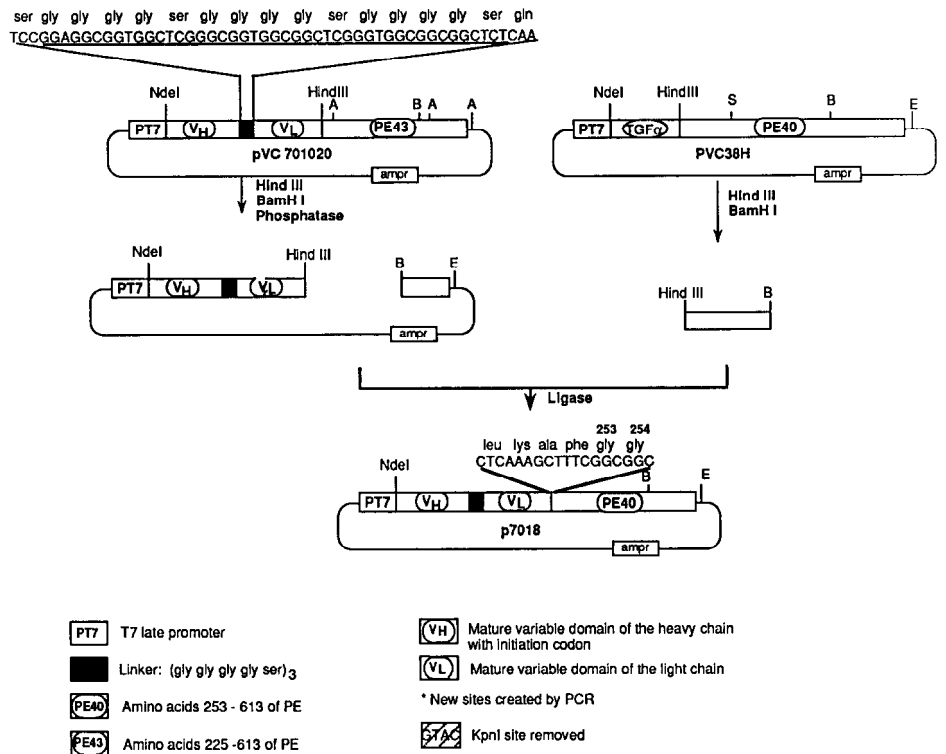


FIG. 1. Scheme for construction of plasmid p7018 encoding the anti-Tac(Fv)-PE40 fusion protein.



followed by nucleotides for a *HindIII* site. The 5'-end of VK76 was complementary to the constant domain of the κ light chain.

The amplified DNA containing V_H was digested with *NdeI* and *KpnI*, while that containing V_L was digested with *KpnI* and *HindIII*. The digested fragments were purified on 1.8% low melting point agarose (Sea Plaque GTG agarose, FMC). Separately, plasmid pVC20 was digested with *NdeI* and *HindIII* and dephosphorylated. pVC20 encodes a PE derivative of 43 kDa termed PE43 (10). A three fragment in-gel ligation was set up using two polymerase chain reaction-amplified, enzyme-digested fragments containing V_H and V_L with a 3.75-kb fragment of pVC20 carrying PE43 and promoter sequences. Correct clones were identified by restriction enzyme analysis. One of the right clones, pVC701, was digested with *KpnI* followed by T4 DNA polymerase to remove the 4-base overhang and ligated. The clones were screened for the loss of the *KpnI* site. These clones (pVC701020) produced a protein of approximately 66 kDa reacting with antibodies to PE. pVC701020 was digested with *HindIII* and

BamHI and dephosphorylated. Separately, pVC38H (7) was digested with *HindIII* and *BamHI* to isolate an 0.8-kb fragment which was ligated with the *HindIII*- and *BamHI*-restricted 2.9-kb fragment of pVC701020. The resulting clones contained V_H , linker L1, V_L , and DNA sequences for PE40 under the control of a T7 promoter (Fig. 2). The clones were checked by restriction enzyme digestions and, finally, by protein expression. One of the correct clones, p7018, which expressed a 63-kDa protein reactive with the antibodies to PE, was selected for large scale expression and purification of the protein.

p70528—This plasmid was constructed using a similar strategy as used for p7018, except that the oligonucleotide primers were different. In this plasmid, V_L is linked to the V_H through a 15-amino acid peptide linker L1, and V_H is linked to PE40 (Fig. 2). Accordingly, oligonucleotides VK77 and VK78 were used to amplify V_L . These oligonucleotides introduced an *NdeI* site, initiator methionine at the 5'-end, the first 7 codons of the peptide linker, and a *KpnI* site at 3'-end. VK79 and VK89 introduced a *KpnI* site, the last 8 codons of the

- | | | | |
|-----|--|-------|---|
| PT7 | T7 late promoter | V_H | Mature variable domain of the heavy chain with initiation codon |
| | Linker: (gly gly gly ser) ₃ | V_L | Mature variable domain of the light chain |
| | Amino acids 253 - 613 of PE | * | New sites created by PCR |
| | Amino acids 225 - 613 of PE | | $KpnI$ site removed |

peptide linker at the 5'-end of V_H , and a *Hind*III site at the 3'-end of V_H . Other steps were the same as described above for the construction of p7018.

p70538—This plasmid is similar to p70528 but contains a 16-amino acid peptide linker termed L2 which has a different amino acid composition (Fig. 2). p70538 was derived from p70528 using two oligonucleotides, VK93 and VK89. The 5'-end of the VK93 is the same as the J region of V_L in p70538 followed by codons for the new peptide linker, and the 3'-end is the same as the 5'-end of the V_H . A polymerase chain reaction was set up with VK93 and VK89 using p70528 as template. The amplified product was digested with *Sst*I and *Hind*III and ligated with a 3.95-kb fragment of *Sst*I-*Hind*III-digested/dephosphorylated p70528. The correct clones were identified by restriction enzyme digestion followed by expression of protein in BL21 (λ DE3).

p70548—This was created using a strategy identical with that described for p70538 except using oligo VK94 and VK89, which introduced a 14-amino acid linker L3 (Fig. 2).

The DNA sequences in the peptide linker region were confirmed by dideoxy sequencing.

Expression and Purification of Proteins—To express and localize anti-Tac(Fv)-PE40, *E. coli* BL21 (λ DE3) cells were transformed with the appropriate plasmids and cultured at 37 °C in super broth supplemented with ampicillin (100 μ g/ml). At an OD_{600} of 0.8, isopropyl-1-thio- β -D-galactopyranoside was added to a final concentration of 1 mM, and the culture was further incubated for 90 min. Cells were harvested and protein was localized as described previously (11). The fusion proteins were mostly contained in the inclusion bodies so these were used as the source for further purification. Proteins were denatured in 7 M guanidine hydrochloride and renatured by rapid dilution in phosphate-buffered saline (12). The renatured protein was applied onto a fast protein liquid chromatography Mono Q 10/10 column in 0.02 M Tris-HCl, pH 7.4, and eluted by a linear gradient of 0–0.5 M NaCl in the same buffer. Fractions containing the fusion protein were detected by SDS/PAGE and cytotoxic activity on HUT102 cells, pooled, and concentrated. For further purification, chimeric protein was applied to a TSK 250 gel filtration column and fractions corresponding to the monomeric form were pooled, quantitated, and used for further characterization.

Protein Synthesis Inhibition Assay—Cytotoxic activity of the fusion protein was determined by assaying the inhibition of protein synthesis on HUT102, Cr11.2, MT-1, ELT5, and EL4J3.4. Cells were washed twice with RPMI 1640, plated in 24-well plates, and incubated with several dilutions of the toxin for 16–20 h at 37 °C. Protein synthesis was assayed by labeling the cells with [³H]leucine for 90 min and counting the trichloroacetic acid-precipitable protein as described (6).

Determinations of Blood Level and Toxicity of Anti-Tac(Fv)-PE40 in Mice—Anti-Tac(Fv)-PE40 was injected intraperitoneally into five groups of female BALB/c mice in doses ranging from 5–100 μ g. Animals were observed for 72 h for signs of toxicity and death. LD₅₀ is the dose of toxin able to kill 50% of the animals in a group in 48 h. For blood level analysis, female BALB/c mice were injected intraperitoneally with 10 μ g of the fusion protein 7018. Blood was drawn from the orbital vein at different times after injection, and the level of anti-Tac(Fv)-PE40 was assayed by incubating the serum with HUT102 cells and measuring inhibition of protein synthesis. A standard curve made with pure anti-Tac(Fv)-PE40 on HUT102 cells was used to determine the concentration in the serum.

Effect of Anti-Tac(Fv)-PE40 on Human Lymphocytes—Peripheral blood mononuclear cells were prepared from the blood of normal volunteer donors using the Ficoll and sucrose density gradient centrifugation. For PHA stimulation, peripheral blood mononuclear cells were cultured at a density of 5×10^5 cells/ml at 37 °C in 0.1% phytohemagglutinin-P (PHA-P) for 3 days. The culture was diluted 1:1 with fresh medium, and human rIL2 was added to a concentration of 50 units/ml. The culture was further incubated for 1–2 days, cells were then harvested, washed, and resuspended at 8×10^5 cells/ml in assay medium consisting of leucine-free Eagle's minimal essential medium with 5% fetal bovine serum, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids, 2 mM L-glutamine, 100 units/ml penicillin, 100 μ g/ml streptomycin, and 50 μ g/ml gentamycin. For two-way mixed lymphocyte reaction, 60 ml of heparinized blood was collected from two individuals and peripheral blood mononuclear cells were isolated. 5×10^6 cells from each individual were mixed in 10 ml of medium and incubated at 37 °C, rIL2 (50 units/ml) was added on day 3, and the culture was further incubated for 3 days. Cells were harvested and resuspended in the assay medium as described above.

To determine the effect of anti-Tac(Fv)-PE40, lymphoblasts were aliquoted into 96-well plates, and serial dilutions of the toxin were added along with rIL2 (10 units/ml). The culture was incubated for 2 days at 37 °C, pulsed for 6 h with [³H]leucine. Cells were harvested using a microplate harvester on glass fiber filters and counted.

Other Methods—Protein was measured by the method of Bradford using the Bio-Rad protein assay reagent. SDS/PAGE was done by using the method of Laemmli (13).

RESULTS

We have previously shown that a single chain anti-Tac(Fv)-PE40 molecule composed of the heavy and light chain variable regions of anti-Tac connected to PE40 makes a very active immunotoxin that inhibits protein synthesis in HUT102 cells with an ID₅₀ of ~0.15 ng/ml (6). To determine if the structure of the recombinant immunotoxin could be altered and its activity retained or improved, we made several new constructions in which the order of the V_L and V_H chains was reversed, or in which the amino acid sequence of the linking peptide was altered. The structures of these new constructions are illustrated in Fig. 2 and described under "Materials and Methods." All the chimeric proteins were expressed in *E. coli* using the T7 expression system, extracted from inclusion bodies by guanidine hydrochloride, and, after renaturation, purified to near homogeneity by successive ion exchange and size exclusion chromatography. In all cases, a highly purified chimeric toxin was obtained (Fig. 3), and the yields of toxin were approximately equal.

The purified toxins were tested on several different cell lines. These included HUT102 and CrII.2 which contain both

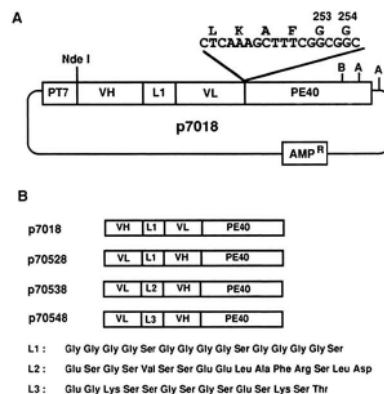


FIG. 2. Expression vector for various anti-Tac(Fv)-PE40 proteins. A, p7018 showing the basic plasmid used for expression of the chimeric proteins. B, order of variable domains in various constructions and linkers. V_H , variable heavy; V_L , variable light; L, linker; B, *Bam*HI; A, *Ava*I; *Amp*, β -lactamase gene.

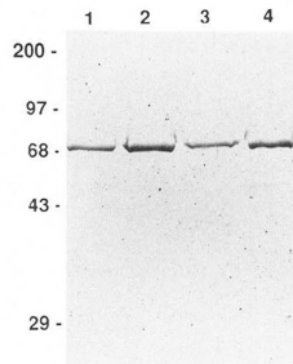


FIG. 3. SDS-PAGE of purified anti-Tac(Fv)-PE40 proteins. 10% SDS-polyacrylamide gels were run and stained with Coomassie Blue. Lane 1, 7018; lane 2, 70528; lane 3, 70538; lane 4, 70548. Molecular weight markers are shown $\times 10^{-3}$.

subunits of the human IL-2 receptor (p55 and p75), MT-1 which contains only human p55, ELT5 which has human p55 and mouse p75, and EL4J3.4 which has mouse p55 and p75. As shown in Fig. 4 and Table I, all four chimeric toxins were very active in inhibiting protein synthesis in HUT102 cells with ID₅₀ values ranging from 0.10 to 0.15 ng/ml. The agents also had similar action against the other cell lines which contained the human p55 subunit (CrII.2, MT-1, and ELT5) but not on cells containing the mouse p55 subunit reflecting the species specificity of the anti-Tac antibody.

Effect of Anti-Tac(Fv)-PE40 on Human T Lymphoblast Proliferation—One possible use of anti-Tac(Fv)-PE40 is in the treatment of autoimmune diseases or allograft rejection. Therefore, we tested the activity of one of the recombinant immunotoxins on human lymphoblasts which were activated either by the mitogen, PHA, or in a mixed lymphocyte reaction. The stimulated lymphoblasts express functional IL2 receptor on their surface. For this purpose, we used the protein encoded by plasmid p7018 (Fig. 2). As shown in Table II, anti-Tac(Fv)-PE40 inhibited the protein synthesis of both PHA and mixed lymphocyte reaction-stimulated human lymphoblasts with ID₅₀ values of 0.13 ng/ml and 0.05 ng/ml, respectively. As a control, we tested the toxin on K562 cells which do not express the IL2 receptors and found that high levels of the toxin were required to intoxicate these cells. The activity of anti-Tac(Fv)-PE40 on activated T lymphoblasts was compared with IL2-PE40 and IL2-PE66^{4Glu}, chimeric toxins that also bind to the IL2 receptor. IL2-PE40, although previously shown to be very active against mouse splenocytes (14), had very little activity toward the human cells (Table II). IL2-PE66^{4Glu}, a chimeric toxin in which the ability of domain I to bind to the *Pseudomonas* exotoxin receptor has been inactivated by mutations at positions 57, 246, 247, and

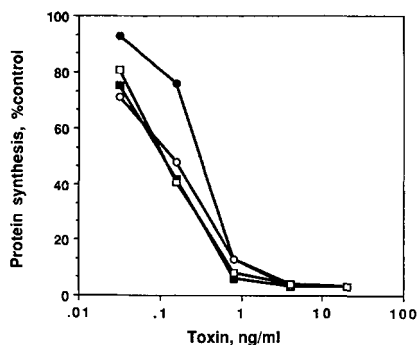


FIG. 4. Cytotoxicity of various fusion proteins on HUT102 cells. Cells were incubated with the respective toxin for 16–20 h and pulsed with [³H] leucine and protein synthesis is shown as percent of control where no toxin was added. ●, 7018; ■, 70528; ○, 70538; and □, 70548.

TABLE I
Cytotoxicity of anti-Tac(Fv)-PE40 proteins on various IL2-receptor-bearing cells

ID₅₀ is the protein concentration required to inhibit protein synthesis by 50%. H, human; M, mouse. Other details are given under "Materials and Methods."

Cell line	Subunit present		ID ₅₀			
	p55	p75	7018	70528	70538	70548
	ng/ml					
HUT102	H	H	0.15	0.11	0.13	0.10
CrII.2	H	H	1.7	1.1	1.8	1.6
MT-1	H	None	0.37	0.15	0.25	0.25
ELT5	H	M	0.56	0.32	0.54	0.45
EL4J3.4	M	M	>1,000	>1,000	>1,000	>1,000

TABLE II

Comparison of anti-Tac(Fv)-PE40, IL2-PE40, and IL2-PE66^{4Glu} for inhibition of human lymphoblast protein synthesis

ID₅₀ is the concentration of protein required to inhibit protein synthesis by 50%. K562 is an IL2 receptor-negative cell line.

	ID ₅₀	
	Activated T lymphoblasts	K562
	ng/ml	
Experiment 1, PHA lymphoblasts		
Anti-Tac(Fv)-PE40	<0.16	93
IL2-PE40	380	345
IL2-PE66 ^{4Glu}	15	910
Experiment 2, PHA lymphoblasts		
Anti-Tac(Fv)-PE40	0.13	91
IL2-PE40	>500	180
IL2-PE66 ^{4Glu}	72	1145
Experiment 3, MLR lymphoblasts		
Anti-Tac(Fv)-PE40	0.05	
IL2-PE40	83	
IL2-PE66 ^{4Glu}	12	

TABLE III

Toxicity of anti-Tac(Fv)-PE40 in mice

Female BALB/c mice were injected with a single indicated dose of the fusion protein. Number of deaths were recorded after 48 h of the toxin administration.

Amount injected	Number of deaths
μg	
5	0/3
10	0/3
25	2/3
50	3/3
100	3/3

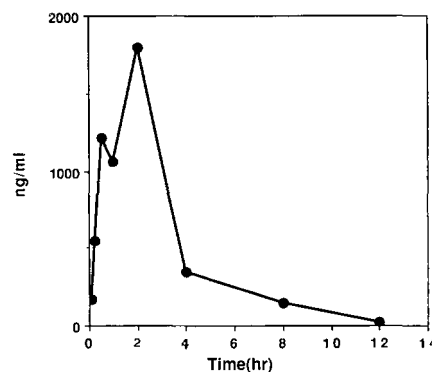


FIG. 5. Blood level of anti-Tac(Fv)-PE40. The experiment was performed as described under "Materials and Methods." Results are average of two experiments.

249, had more activity than IL2-PE40 against the activated human cells, but was nevertheless much less potent than anti-Tac(Fv)-PE40 (Table II). Clearly, anti-Tac(Fv)-PE40 was the most cytotoxic of the three molecules tested.

Blood Level and Toxicity of Anti-Tac(Fv)-PE40 in Mice—The extremely potent *in vitro* cytotoxicity of anti-Tac(Fv)-PE40 on IL2 receptor-bearing cells makes it a promising molecule for testing *in vivo* against IL2 receptor-bearing tumors or for immunosuppression. Accordingly, various amounts of anti-Tac(Fv)-PE40 were given intraperitoneally to mice to assess its toxicity. The LD₅₀ of a single dose was found to be between 10 and 25 μg (Table III). To determine the blood level of anti-Tac(Fv)-PE40, BALB/c female mice were injected intraperitoneally with 10 μg of protein. Blood was drawn at various times after the injection and assayed on HUT102 cells for immunotoxin activity. A peak blood level

of 1.9 $\mu\text{g}/\text{ml}$ was obtained 2 h after the injections (Fig. 5). Detectable levels of toxin were present for up to 8 h, but by 12 h, toxin could no longer be found in the blood (Fig. 5).

DISCUSSION

We have shown that anti-Tac(Fv)-PE40, a single chain immunotoxin, is very active in killing cells bearing the human p55 subunit of the IL2 receptor. In this type of chimeric toxin, the variable regions of the heavy and light chain are held together by a linking peptide. We have utilized several different peptide linkers containing 14, 15, or 16 amino acids and found all of them to produce active immunotoxins with about the same activity. Furthermore, we have changed the order of the variable regions without affecting activity (Table I). Stimulated by the finding of Ward *et al.* (15) that a single variable region can bind antigen, we made a chimeric toxin composed of the V_H of anti-Tac fused to PE40, but found it to be inactive (data not shown). Apparently, V_H by itself does not have a high affinity for the p55 subunit. However, it may be possible to make active immunotoxins with single variable regions with selected antibodies.

Anti-Tac(Fv)-PE40 has much greater activity on human cells than IL2-PE40, a molecule which also binds to the IL2 receptor (Table II). There are several reasons that may account for this difference which are related to the number of p55 subunits on the target cells and the relative affinities of the two ligands for p55. Anti-Tac binds to p55 with a $K_d \sim 10^{-9}$ M, whereas the affinity of IL2 for p55 is $\sim 10^{-8}$ M (16). Only when p55 is associated with p75 is the affinity increased to $\sim 10^{-11}$ M. In MT-1 cells where there are 100,000 p55 subunits and no p75 subunits, the ID_{50} of anti-Tac(Fv)-PE40 is <1 ng/ml and the ID_{50} of IL2-PE40 is >100 ng/ml. This probably reflects the affinities of the two ligands. On HUT102 cells, where there are many p55 subunits and a moderate number of p75 subunits, IL2-PE40 and anti-Tac(Fv)-PE40 are about equally active. It has been shown previously that the high affinity IL2 receptor composed of p55 and p75 is rapidly internalized when occupied by IL2 (or anti-Tac), whereas the low affinity p55 receptor is internalized much more slowly (17). Nevertheless, it appears that this slow entry route is compensated for by the fact that there are many more p55 molecules than high affinity receptors on the surface of target cells and also because the binding of anti-Tac to these molecules is much stronger than the binding of IL2 to p55.

Anti-Tac(Fv)-PE40 is very active on HUT102 cells that express both the low and high affinity human IL2 receptor. Moreover, the fusion protein is also highly active on cells with moderate or low numbers of receptors (Table I). In a mixed lymphocyte (leukocyte) reaction, which is an *in vitro* model system for alloreactive responses, anti-Tac(Fv)-PE40 inhibited the protein synthesis of T-lymphoblasts very efficiently. It has been shown that IL2 receptor-bearing cells play a very crucial role in graft rejection and the administration of anti-IL2 receptor antibody prolongs allograft survival (18–20). We have earlier made a chimeric toxin containing IL2 and PE40, which is active on mouse IL2 receptor-bearing cells (8, 14) and has been shown to significantly prolong the survival of vascularized heart allografts in mice (21). However, IL2-PE40 has low activity on human activated T cells. Anti-Tac(Fv)-PE40, being enormously more active, might prove to be a potent immunosuppressive agent in humans.

Another objective of creating chimeric toxins is their potential use as anti-tumor agents. Since anti-Tac(Fv)-PE40 ap-

pears to be a very active protein *in vitro*, we performed several experiments to investigate its activity *in vivo*. The LD_{50} of anti-Tac(Fv)-PE40 in mice was determined to be between 10 and 25 μg . The protein appears to be slightly more toxic than some other chimeric toxins produced in our laboratory ($LD_{50} \sim 20\text{--}50$ μg). However, because of the extremely high activity of anti-Tac(Fv)-PE40, it may not be required to use very high doses *in vivo*. Anti-Tac(Fv)-PE40 when injected intraperitoneally in mice shows a peak of activity in the blood after 2 h and detectable levels are present up to 8 h.

In summary, anti-Tac(Fv)-PE40 is a chimeric toxin which has been shown to be extremely cytotoxic to IL2 receptor expressing cells in tissue culture and in mixed lymphocyte reaction. The agent warrants evaluation as an anti-tumor and immunosuppressive agent in humans.

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