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Immunology

Cytotoxic activity of an interleukin 2–*Pseudomonas* exotoxin chimeric protein produced in *Escherichia coli*

(cancer/T cells/leukemia/diabetes/transplantation)

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ABSTRACT **A cDNA clone for human interleukin 2 (IL-2) has been fused to the 5′ end of a modified *Pseudomonas* exotoxin (PE) gene that lacks the sequences encoding the cell recognition domain. The chimeric protein IL-2–PE_{40} was produced in *Escherichia coli*. It was extremely toxic to IL-2 receptor-positive cells but had no measurable effect on cells lacking the IL-2 receptor. IL-2–PE_{40} might be a useful cytotoxic agent in the treatment of diseases involving IL-2 receptor-positive cells and in the treatment of allograft rejection.**

Bacterial and plant toxins have been chemically attached to monoclonal antibodies (1, 2), lectins (3, 4), and polypeptide hormones (5–7), to direct the action of the toxin toward specific eukaryotic cells. In our laboratory, *Pseudomonas* exotoxin (PE) has been used to construct specific cell killing reagents (8). X-ray diffraction analysis has shown that PE is structurally composed of three domains (9). Functional analysis of deletion mutations of the PE structural gene has shown that domain I of PE is responsible for cell recognition; domain II, for translocation of the toxin across membranes; and domain III, for ADP-ribosylation of elongation factor 2, the step actually responsible for cell death (Fig. 1 and ref. 10). A PE molecule from which domain I has been deleted (PE_{40}) has full ADP-ribosylating activity but extremely low cell killing activity because of the loss of the cell recognition domain. Thus it seemed worthwhile to use PE_{40}, which contains domains II and III, to construct chimeric toxins imparting specific cell recognition properties to the PE molecule. Initially a chimeric protein was constructed by fusing DNA sequences encoding PE_{40} to a cDNA encoding transforming growth factor α (TGF-α) (11). The fusion protein, PE_{40}–TGF-α, was specifically cytotoxic to cells expressing epidermal growth factor receptors.

Here we report the construction of a chimera in which the gene encoding PE_{40} has been fused to a cDNA encoding human interleukin 2 (IL-2). The lymphokine IL-2 is synthesized and secreted by activated T cells and plays a critical role in the proliferation and expression of T-lymphocyte effector cells during the immune response (12). Concomitant with the production of IL-2, T cells synthesize surface receptors for IL-2 that are not expressed by resting and memory T cells (13, 14). The chimeric protein, IL-2–PE_{40}, is produced in *Escherichia coli* and is extremely toxic to IL-2 receptor-positive (HUT 102) cells but has no effect on cells lacking the IL-2 receptor.

MATERIALS AND METHODS

Materials. Restriction endonucleases, T4 DNA polymerase, DNA polymerase I, Klenow fragment, and T4 DNA ligase were purchased from New England Biolabs, Bethesda Research Laboratories, or Pharmacia and used under conditions recommended by the supplier. Agarose (SeaKem) was supplied by FMC (Rockland, ME). Reagents for NaDodSO$_4$/PAGE were from Bio-Rad. The immunoblotting kit was from Vector Laboratories (Burlingame, CA). All other chemicals were of analytical grade. Nicotinamide [U^{14}-C]adenine dinucleotide and [^{3}H]leucine were purchased from Amersham and New England Nuclear.

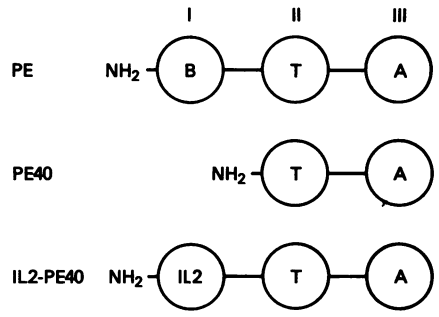

FIG. 1. Diagrammatic representation of the functional domains of PE, PE_{40}, and IL-2–PE_{40}. I, II, and III refer to the domains of PE determined from the crystal structure (9). B, T, and A refer to the functional domains determined by deletion analysis (10). B, binding domain; T, translocation domain; A, ADP-ribosylation domain.

Bacterial Strains, Plasmids, and Cell Lines. *E. coli* strain HB101 was used for transformation and amplification of plasmids (15). BL21(λDE3), which carries a T7 RNA polymerase gene in lysogenic and inducible form (16), was used as the host for the synthesis of PE_{40} and IL-2–PE_{40}. pJH4, which carries the PE gene under the phage T7 promoter control, was constructed in this laboratory (10). The pGEM3 vector was from Promega Biotec (Madison, WI). Plasmid PST-5, which carries a cDNA for human IL-2 inserted at the *Pst* I site in pBR322, was obtained from F. Wong-Staal through Biotech Research Laboratories. HUT 102 and CEM cells were gifts of T. A. Waldmann (National Cancer Institute).

Nucleic Acids Preparation and Oligonucleotide Synthesis. Plasmid DNAs were prepared by the alkaline lysis method and purified on cesium chloride/ethidium bromide gradients (17). Restriction fragments were analyzed by electrophoresis on horizontal agarose or vertical polyacrylamide gels in TBE buffer (0.089 M of Tris borate, pH 8.0/2 mM EDTA). Synthetic oligonucleotides were synthesized on an Applied Biosystems DNA synthesizer and purified by electrophoresis on 20% polyacrylamide gels. Oligonucleotides were cloned into plasmid vectors by standard methods (17).

Plasmid Construction. A plasmid for the expression of the IL-2–PE_{40} fusion protein under the T7 promoter was constructed as shown in Fig. 2. pJH4 [5.9 kilobases (kb)], which

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Abbreviations: IL-2, interleukin 2; rIL-2, recombinant IL-2; PE, *Pseudomonas* exotoxin.

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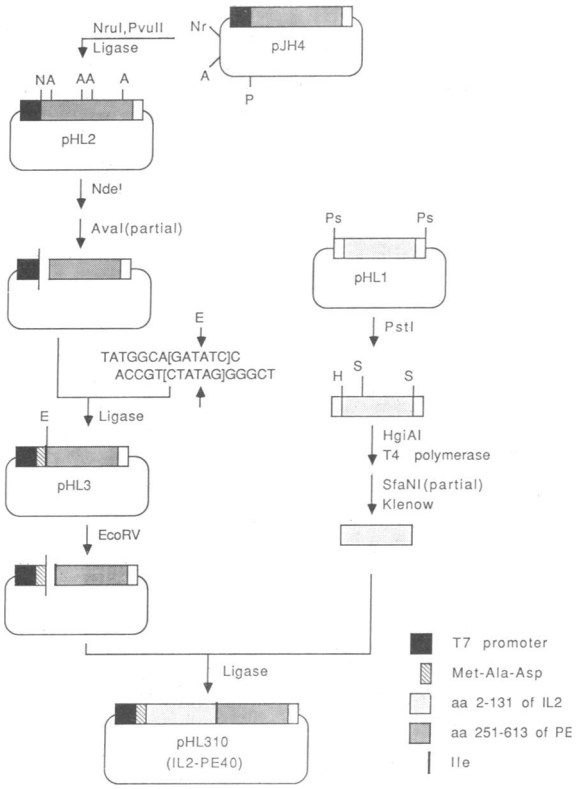

FIG. 2. Scheme for the construction of expression plasmid pHL310, which encodes the IL-2–PE_{40} fusion protein. Nr, *Nru* I; P, *Pvu* II; A, *Ava* I; N, *Nde* I; E, *Eco*RV; Ps, *Pst* I; H, *Hgi*AI; S, *Sfa*NI.

carries a full-length PE gene (10), was cut with *Nru* I/*Pvu* II and the 4.8-kb fragment was eluted and self-ligated. The resulting plasmid (pHL2) was used to insert the IL-2 gene in place of domain I of the PE gene. pHL2 was cut with *Nde* I and the linearized fragment was partially cut with *Ava* I. The largest fragment, 4 kb long, was eluted and ligated to a synthetic oligonucleotide containing a unique *Eco*RV site (GATATC) to generate pHL3, which was then linearized with *Eco*RV.

The IL-2 cDNA was subcloned as a *Pst* I insert into the *Pst* I site of the vector pGEM3. The resulting plasmid (pHL1) was cut with *Pst* I to yield a 1.2-kb fragment containing the IL-2 insert. After separation on an agarose gel the 1.2-kb fragment was eluted and cleaved with *Hgi*AI; the resulting fragments were treated with T4 DNA polymerase and a 0.68-kb blunt-ended fragment was isolated. The 0.68-kb fragment was partially cut with *Sfa*NI and a 0.38-kb fragment was separated. The ends of that fragment were filled in with Klenow fragment and inserted into the *Eco*RV site of pHL3. The resulting plasmid had a 390-base-pair gene in two orientations at the 5′ end of the PE gene. Restriction analysis with *Xba* I/*Bam*HI or *Xba* I/*Eco*RI was used to identify a plasmid with the IL-2 gene in the proper orientation with respect to the PE gene. The resulting plasmid, pHL310, was examined for its size and the expression of the IL-2–PE_{40} fusion protein in BL21(λDE3) cells.

Expression of the Chimeric Fusion Protein. For the expression of IL-2–PE_{40}, BL21(λDE3)/pHL310 cells were cultured in LB broth with ampicillin (50 μg/ml) at 37°C. When the A_{650} value reached 0.3, isopropyl β-D-thiogalactoside was added to a final concentration of 1 mM. Cells were harvested 90 min later.

Gel Electrophoresis and Immunoblotting. $NaDodSO_4$/PAGE on gradient gels (10–15%) was performed by using a Phast gel system (Pharmacia). Samples were added with sample buffer and treated as described by Laemmli (18). The gels were stained with Coomassie blue or a silver staining reagent. For immunoblotting, electrophoresed samples were transferred from the gels to nitrocellulose paper (11), and the nitrocellulose paper was processed using antibodies to native PE or to human recombinant IL-2 (rIL-2). Vector staining kits were used as recommended by the manufacturer. The antibody to native PE has been described (10). A rabbit anti-human rIL-2 antibody and rIL-2 were gifts of Maurice Gately (Hoffmann-La Roche).

Protein and ADP-Ribosylation Assays. Protein concentration was measured by a Bradford protein assay kit (Bio-Rad) using bovine serum albumin as standard. ADP-ribosylation activity of tested samples was measured using wheat germ extracts enriched in elongation factor 2 (19). Samples were incubated for 30 min at 37°C.

Extraction and Partial Purification of IL-2–PE_{40}. *E. coli* BL21(λDE3)/pHL310 was grown and induced, and cells from 500 ml of medium were collected by centrifugation at 4000 × *g* for 15 min at 4°C. The cell pellet was treated with 7.5 ml of extraction buffer (50 mM Tris·HCl/5 mM EDTA/3 M guanidine hydrochloride, pH 7.0), and the suspension was sonicated (three 20-sec bursts, at 100 W), stirred for 1 hr at 4°C, and centrifuged at 15,000 × *g* for 15 min. Then, an equal volume of cold water was added to the supernatant and the solution was dialyzed for 24 hr against 50 vol of dialysis buffer (10 mM Tris·HCl, pH 8.0) with four changes. After dialysis, the supernatant was centrifuged at 15,000 × *g* for 15 min. The resulting supernatant (whole cell extract, guanidine hydrochloride treated) was used as the source of the IL-2–PE_{40} fusion protein.

Gel Filtration Chromatography on TSK G250. A sample of the whole cell extract (guanidine hydrochloride treated) (6 ml, 10 mg of protein) was loaded onto a TSK G250 (21.5 × 600 mm) column (Bio-Rad) attached to a Pharmacia FPLC column and eluted with 0.2 M sodium phosphate, pH 7.0/1 mM EDTA. Fractions of 4.0 ml were collected at a flow rate of 4.0 ml/min and analyzed for ADP-ribosylation activity (cpm × 10^{-6}/ml), for protein content (mg/ml), for the presence of the IL-2–PE_{40} fusion protein by $NaDodSO_4$/PAGE and immunoblotting, and for ability to inhibit protein synthesis on HUT 102 cells.

Protein Synthesis Inhibition Assay. The activity of the IL-2–PE_{40} fusion protein was tested on HUT 102 cells. To reduce the amount of IL-2 present, the cells were washed three times with RPMI 1640 medium/10% fetal bovine serum, seeded into 24-well tissue culture dishes in 1 ml medium at 4–5 × 10^5 cells per well, and kept at 4°C. Various concentrations of IL-2–PE_{40} diluted with 0.2% bovine serum albumin in phosphate-buffered saline were added. For competition experiments, 10 μg of purified rIL-2 or anti-Tac or another antibody was added prior to addition of IL-2–PE_{40}. After 1 hr at 4°C, the dishes were placed at 37°C; 15–16 hr later, the cells were incubated with 5 μCi (1 Ci = 37 GBq) of [^{3}H]leucine for 1.5 hr at 37°C. The cells were collected, washed with 0.5 ml of PBS, and dissolved in 0.25 ml of 0.1 M NaOH, and cellular proteins were precipitated with cold 12% trichloroacetic acid. Incorporation of [^{3}H]leucine into proteins was measured by liquid scintillation counting. To test the biological activity of IL-2–PE_{40} on KB, OVCAR3, or Swiss 3T3 cells, the cells were seeded 16–20 hr before the experiment into 24-well dishes in 1 ml of medium at 1–2 × 10^5 cells per well. Then, IL-2–PE_{40} and other reagents were added at room temperature and the cells were incubated at 37°C for 15–16 hr, labeled with [^{3}H]leucine as described above, washed with two 0.75-ml portions of PBS, and harvested by addition of 0.5 ml of 0.1 M NaOH/0.1% bovine serum albumin. Incorporation of [^{3}H]leucine into cellular protein was measured as described

above. Results are expressed as percent of control experiments in which cells were not exposed to IL-2–PE_{40}.

RESULTS

Construction of IL-2–PE_{40}. A cDNA clone for human IL-2 was fused to the 5′ end of a DNA fragment encoding domains II and III of PE (Fig. 1). The fusion gene is expressed under the control of the bacteriophage T7 late promoter (10). The expression plasmid, pHL310, directs the synthesis of the fusion protein IL-2–PE_{40}, in which an IL-2 sequence of 130 amino acids (amino acids 2–131 of IL-2) is preceded by the 3 amino acid sequence Met-Ala-Asp (Fig. 2). The threonine residue at position 131 of IL-2 is connected to the amino terminus of domain II of the PE gene (amino acid 251 of PE) by an isoleucine residue encoded by the synthetic linker used (Fig. 2). The calculated molecular mass of the IL-2–PE_{40} fusion protein is 54 kDa. The sequences of the 5′ end of the IL-2–PE_{40} gene and of the junction between the IL-2 and the PE_{40} genes were confirmed by DNA sequence analysis (data not shown).

Expression of IL-2–PE_{40}. To express IL-2–PE_{40}, *E. coli* BL21(λDE3) cells were transformed with plasmid pHL310 or with plasmid pHL3, which carries the PE_{40} gene alone. After induction with isopropyl β-D-thiogalactoside, the cells were collected by centrifugation and an aliquot was suspended in $NaDodSO_4$/PAGE sample buffer (whole cell extract). The remainder of the cells was suspended in Tris buffer (50 mM Tris, pH 8.1/1 mM EDTA), sonicated, and centrifuged at 10,000 × *g* for 30 min at 4°C. The supernatant and the pellet were analyzed by $NaDodSO_4$ gel electrophoresis and immunoblotting. As shown in Fig. 3*A*, the cellular fractions from pHL310-carrying cells contain a band with a molecular mass of 54 kDa (lane 2) that is not detected in extracts of cells carrying pHL3 (lane 5). Immunoblots showed that the 54-kDa protein reacts with antibodies to both rIL-2 and PE (Fig. 3 *B* and *C*). Blotting with antibodies to PE also detected a minor band that comigrated with PE_{40} (Fig. 3*B*) and has a molecular mass of 40 kDa. The 40-kDa protein is probably a degradation product from which the IL-2 segment has been removed by proteolytic cleavage, because it does not react with antibodies to IL-2. Thus, the 54-kDa protein is identified as the fusion protein IL-2–PE_{40}.

The whole cell extract as well as the soluble and insoluble fractions prepared from sonicated B121(λDE3)/pHL310 cells exhibited ADP-ribosylation activity. The insoluble fraction exhibited most of this activity and also contained most of the IL-2–PE_{40} fusion protein, as shown by the immunoblots (Fig. 3 *B* and *C*). To study the biological activity of IL-2–PE_{40}, the cells were collected by centrifugation and the cell pellet was sonicated in 3 M guanidine hydrochloride and then renatured. This whole cell extract was used as the source of IL-2–PE_{40} for the experiments described below. To determine the amount of IL-2–PE_{40} present, various amounts of this preparation and a purified preparation of PE_{52} (amino acids 135–613 of PE; T. Idziorek and I.P., unpublished data) were subjected to immunoblot analysis using antibodies to PE. From comparison of the intensities of the reactions, we estimate that the concentration of IL-2–PE_{40} is 100 μg/ml (data not shown) and that the total amount of fusion protein produced is ≈4 mg/liter.

Cytotoxic Activity of the IL-2–PE_{40} Fusion Protein. The cytotoxic activity of the IL-2–PE_{40} fusion protein was tested on HUT 102 cells (20), which contain both the Tac antigen (p55) and the p70 protein components of the IL-2 receptor (21–23), by a quantitative assay in which its ability to inhibit protein synthesis on the target cells was measured. As shown in Fig. 4*A*, HUT 102 cells are very sensitive to IL-2–PE_{40}, with an apparent ID_{50} of 10–20 ng/ml.

The toxic effect of IL-2–PE_{40} on HUT 102 cells was completely blocked by rIL-2 at 10 μg/ml (Fig. 4*A*), establishing the specificity of the response. It was also completely blocked by anti-Tac (10 μg/ml; Fig. 4*B*), a monoclonal antibody to the p55 component of the IL-2 receptor that is known to compete with IL-2 for binding to p55 (20). As a control, three different antibodies were tested for their ability to reverse the toxicity of IL-2–PE_{40} on HUT 102 cells: HB21 is a monoclonal antibody to the human transferrin receptor (24), OVB3 is a monoclonal antibody reacting with human ovarian cancer cells (25), and RPC-5 is an IgG of unknown reactivity. As shown in Fig. 5, of these antibodies, only anti-Tac reversed the effect of IL-2–PE_{40}, a result that further demonstrates the specificity of its action.

The effect of IL-2–PE_{40} was also tested on various human and mouse receptor negative cells: CEM cells (a T-cell line lacking the IL-2 receptor), KB cells, OVCAR3 cells (an ovarian cancer cell line), and Swiss 3T3 cells (a mouse cell line having high sensitivity to the native PE toxin). As shown in Table 1, all of these cell lines were unaffected by the IL-2–PE_{40} fusion protein. Together these data show that the toxic effect of IL-2–PE_{40} is a highly specific, receptor-mediated response.

Partial Purification of the IL-2–PE_{40} Fusion Protein. We have partially purified the IL-2–PE_{40} fusion protein from the whole cell extract (guanidine hydrochloride) by gel filtration on a TSK 250 column. Measurement of the ADP-ribo-

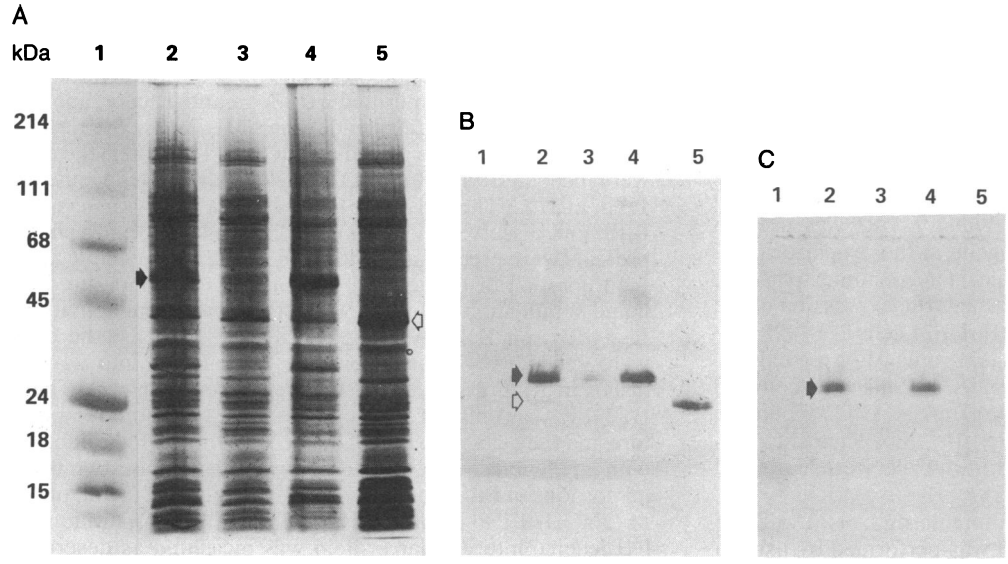

FIG. 3. $NaDodSO_4$/polyacrylamide gel electrophoresis analysis of cell fractions containing the IL-2–PE_{40} fusion protein. Samples were mixed with Laemmli sample buffer (18) and kept at 100°C for 3 min. Samples of 1 μl were loaded onto a 10–15% polyacrylamide gradient gel using a Pharmacia Phast system. (*A*) Silver-stained protein gel. (*B*) Immunoblotting with antibody to PE. (*C*) Immunoblotting with antibody to human rIL-2. Lanes: 1, markers; 2, whole cell extract; 3, soluble fraction (cell supernatant); 4, insoluble fraction (cell pellet); 5, whole cell extract containing PE_{40}. ◗, IL-2–PE_{40}; ◊, PE_{40}.

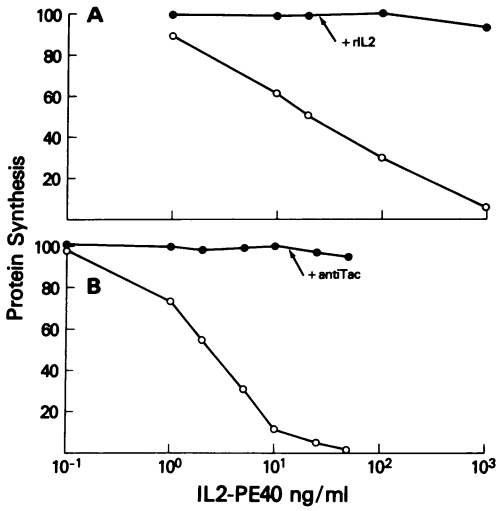

FIG. 4. Toxic activity of the IL-2–PE_{40} fusion protein on HUT 102 cells and competition by human rIL-2 or anti-Tac. IL-2–PE_{40} from whole cell extracts (*A*) or after purification on a TSK 250 column (fraction 33) (*B*) was added at various concentrations to HUT 102 cells in the absence (○) or presence (●) of rIL-2 (10 μg/ml; *A*) or anti-Tac (10 μg/ml; *B*). [^{3}H]Leucine incorporation into cellular protein was measured as described in *Materials and Methods*. Results are expressed as percent of control cells not exposed to IL-2–PE_{40}.

sylation activity, as well as the immunoblots of the column fractions, showed that the IL-2–PE_{40} fusion protein eluted as a broad peak (Figs. 6 and 7). However, when the fractions were tested for inhibition of protein synthesis on HUT 102 cells, only fractions 32–34 exhibited high toxic activity with fractions 21–33 having very low toxicity. When compared to standards run under similar conditions, fractions 21–33 were found to contain very high molecular weight proteins, suggesting that they probably contain aggregated and inactive forms of the IL-2–PE_{40} protein. Fractions 35–39 exhibited high ADP-ribosylation activity yet had no effect on HUT 102 cells. As shown by the immunoblots (Fig. 7) these fractions contain mostly degradation products. The proteins that eluted in fractions 32–34 and exhibited high toxic activity are in the

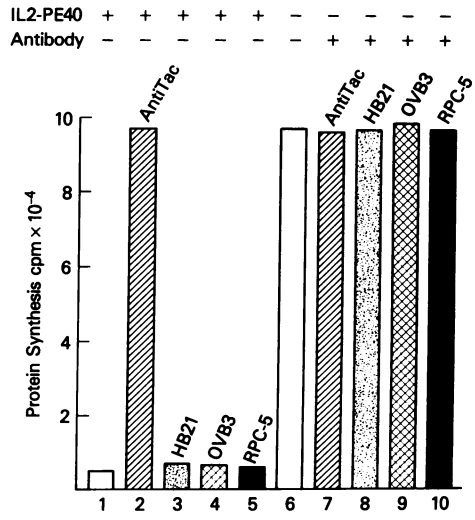

FIG. 5. Effects of various antibodies on reversal of toxicity of IL-2–PE_{40} (100 ng/ml) on HUT 102 cells. Bars: 1, no antibody added; 2, excess anti-Tac; 3, excess HB21; 4, excess OVB3; 5, excess RPC-5; 1–5, presence of IL-2–PE_{40}; 6–10, absence of IL-2–PE_{40}. All antibodies were added to a final concentration of 10 μg/ml. Results are expressed as cpm × 10^{-6} per ml (total counts).

Table 1. Cytotoxic activity of the IL-2–PE_{40} fusion protein and PE on various cell lines

Cell line	ID_{50}, ng/ml	
	IL-2–PE_{40}	PE
HUT 102	20	20
CEM	>1000	100
KB	>2000	20
SWISS 3T3	>2000	1.5
OVCAR3	>1000	30

A whole cell extract treated with guanidine hydrochloride was used as the source of IL-2–PE_{40}. The concentration of IL-2–PE_{40} was calculated from the intensity of the IL-2–PE_{40} band detected by immunoblotting with anti-PE.

position expected for a protein of M_r 54-kDa. These results indicate that only the monomeric form of the fusion protein has high biological activity. The effect of the IL-2–PE_{40} contained in fraction 33 on HUT 102 cells is shown in Fig. 4*B*. The partially purified material was very active in inhibition of protein synthesis, with an ID_{50} of ≈2.5 ng/ml.

DISCUSSION

We have constructed a fusion protein in which the cell recognition domain of PE has been deleted and replaced by IL-2 (Fig. 1). In native PE the cell recognition domain lies at the 5′ end of the gene and the amino terminus of the protein. IL-2 was placed at the amino terminus of PE_{40}, so that it occupied the same position as the cell recognition domain of PE. It is known that an intact amino terminus is required for full biological activity of the IL-2 molecule (26). We reasoned that, so long as the IL-2 sequences at the amino terminus of the chimeric protein were left freely exposed, they would be capable of binding to the receptor of the target cells. We have also constructed a fusion protein in which IL-2 is placed at the carboxyl terminus of PE_{40}, PE_{40}–IL-2. This fusion protein had no cytotoxic activity (data not shown).

When IL-2–PE_{40} was tested on various cell lines it was found to be extremely cytotoxic to HUT 102 cells (Fig. 4) but not to receptor-negative cell lines (Table 1). The toxic effect was easily detected in unpurified whole cell extracts, indicating its high biological activity, and was completely blocked by excess anti-Tac (Figs. 4*B* and 5) but not by other antibodies tested (Fig. 5). The cytotoxic effect was also reversed by excess rIL-2 (Fig. 4*A*), further establishing the specificity of IL-2–PE_{40} response. A single step of purifica-

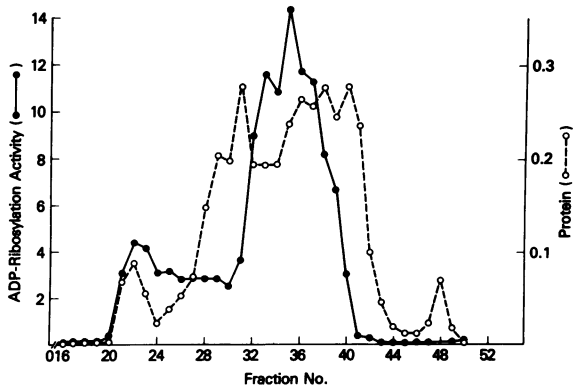

FIG. 6. Partial purification of the IL-2–PE_{40} fusion protein. A whole cell extract prepared by guanidine hydrochloride extraction and dialysis was purified by TSK 250 gel filtration chromatography. A 6-ml sample (10 mg of protein) was applied to a 21.5 × 600 mm column and 4-ml fractions were collected. ADP-ribosylation activity is expressed as cpm × 10^{-6}/ml and protein is expressed as mg/ml.

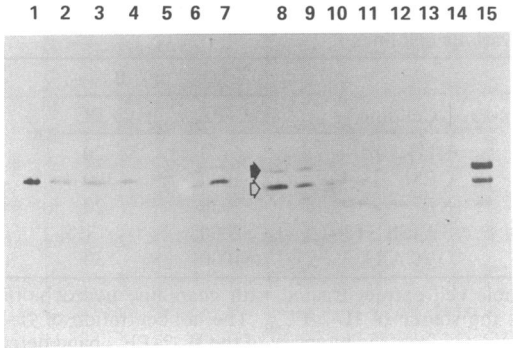

FIG. 7. Immunoblots of gel filtration column fractions. Samples were treated and run as described in the legend to Fig. 3. Immunoblotting was performed with anti-PE. ♦, IL-2–PE_{40}; ◊, PE_{40}. Lanes: 1, purified PE_{52}; 2–14, fractions 26–39 from the gel filtration column; 15, whole cell extract containing IL-2–PE_{40}.

tion increased the specific activity of the fusion protein 5- to 10-fold (Fig. 4*B*). In the solubilized whole cell extract (Fig. 6) most of the fusion protein exists in a nonactive aggregated form. Using a gel filtration purification step, we separated the monomeric active fusion protein from the inactive material, thus increasing its specific activity. Using a protein synthesis inhibition assay, we calculated an ID_{50} of 2–3 ng/ml for partially purified IL-2–PE_{40}. The IL-2–PE_{40} fusion protein is approximately as active as another chimeric protein recently constructed with PE_{40}, PE_{40}-TGF-α, which kills cells bearing epidermal growth factor receptors (11).

Studies in our laboratory have shown PE_{40}, PE_{40}-TGF-α, and antibodies chemically coupled to PE_{40} have considerably less nonspecific toxicity in mice than molecules coupled to native PE (ref. 11; unpublished data). These results predict that IL-2–PE_{40} should also have low nonspecific toxicity in mice. In preliminary experiments, we have found that 6 μg of IL-2–PE_{40} can be administered to a mouse in a single i.p. dose without killing the animal.

Activated IL-2 receptor-positive lymphocytes have been found to accumulate as cellular infiltrates in many diseases, including autoimmune thyroid disease (27), type 1 diabetes (28), graft rejection (29), rheumatoid arthritis (30), and Crohn disease (31). IL-2 receptor-positive lymphocytes are considered to play a crucial role in the pathogenesis of these conditions (32, 33). Treatment of allograft rejection remains the major problem in clinical organ transplantation. Recently, it was shown that administration of a monoclonal antibody to the IL-2 receptor prolongs cardiac allograft survival in mice (32), spares suppressor T-cells, and prevents or reverses acute allograft rejection in rats (33, 34). These data indicate that IL-2 receptor-bearing cells are an important target for immunosuppression therapy. Because the IL-2–PE_{40} fusion protein is a potent cytotoxic agent for cells bearing the IL-2 receptor and has relatively low nonspecific toxicity, it may be useful in the treatment of diseases involving IL-2 receptor-positive cells.

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