DYNAMICS OF TOXIN AND LECTIN RECEPTORS ON A LYMPHOMA CELL LINE AND ITS TOXIN-RESISTANT VARIANT USING FERRITIN-CONJUGATED, $^{125}$I-LABELED LIGAND

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ABSTRACT

The dynamics of the toxin *Ricinus communis* agglutinin II (RCA$_{II}$ or ricin) on cells of a murine lymphoma line (BW5147) and a toxin-resistant variant line (BW5147Ric R. 3) that is 200 times more resistant than the parent to direct RCA$_{II}$ cytotoxicity were examined using ferritin-conjugated, affinity purified, $^{125}$I-labeled RCA$_{II}$ (ferritin-$^{125}$I-RCA$_{II}$). Ferritin-$^{125}$I-RCA$_{II}$ was indistinguishable from native RCA$_{II}$ in quantitative binding and cytotoxicity experiments. When RCA$_{II}$-sensitive BW5147 and -resistant BW5147Ric R. 3 cells were labeled with ferritin-$^{125}$I-RCA$_{II}$ at various toxin concentrations (1-10 μg/ml), no differences in toxin binding were observed. These same cells were examined by electron microscopy. At low ferritin-$^{125}$I-RCA$_{II}$ concentrations (1-3 μg/ml RCA$_{II}$) where only the parental BW5147 cells were significantly more sensitive to RCA$_{II}$, toxin receptors were internalized by ferritin-$^{125}$I-RCA$_{II}$-induced endocytosis. In parallel experiments, ferritin-$^{125}$I-RCA$_{II}$ that bound to the resistant BW5147Ric R. 3 cells remained relatively dispersed or clustered, and there was little evidence of transport into cells via endocytosis. At higher ferritin-$^{125}$I-RCA$_{II}$ concentrations (>7 μg/ml RCA$_{II}$) where both parental and resistant variant cells are sensitive to the cytotoxic effects of RCA$_{II}$, more ferritin-conjugated toxin was bound, and subsequent endocytosis occurred to a similar degree in both cell types. Endocytosis of ferritin-conjugated concanavalin A was indistinguishable on RCA$_{II}$-sensitive parental and resistant variant cells at all concentrations tested. The results suggest that a specific defect on the selected BW5147Ric R. 3 cells prevents RCA$_{II}$ entry into these cells at low toxin concentrations, rendering them more resistant to the cytotoxic effects of RCA$_{II}$.

KEY WORDS toxin · lectin · membrane · endocytosis · lymphoma variants · receptor · ricin · concanavalin A

Certain proteins and glycoproteins isolated from plants are extremely toxic to animals and man (2, 19, 37). An ~60,000 mol wt glycoprotein (*Ricinus communis* agglutinin II [RCA$_{II}$ or ricin]) that...
has been isolated and purified from the castor plant (*Ricinus communis*) (11, 24, 25, 35, 38, 49) inhibits protein synthesis in intact cells (12-14, 27, 38) and in cell-free protein synthesis systems (17, 27, 32-34, 43). The toxicity of RCA~ or ricin has been utilized in experiments to differentially kill tumor cells in vitro (27) as well as in animals (12, 41) and man (8, 50).

The cellular toxicity of RCA~ (ricin) is generally thought to occur via the following sequence of events: Toxin molecules bind to the cell surface and induce redistribution of their receptors. Some of the aggregated toxin-receptor complexes are endocytosed into the cell where after a period of time a proportion of the endocytotic vesicles break down, releasing toxin molecules into the cell cytoplasm where they irreversibly inactivate protein synthesis (18, 27, 32, 38, 40, 43, 45). To study this sequence in more detail, several somatic cell mutants have been isolated that are more resistant to the direct toxic effects of RCA~ or other lectins (7, 9, 16, 17, 44, 46-48). Although many of these toxin- and lectin-resistant mutants possess cell surface changes leading to large decreases in toxin or lectin binding (6, 7, 17), certain resistant variants are capable of binding substantial quantities of toxin or lectin molecules to their cell surfaces (9, 16, 44). One such variant cell line which was selected from a murine lymphoma cell line by direct RCA~ cytotoxicity is ~200 times more resistant compared to its parental line in the absence of serum, although it has lost only ~30-40% of its total cell-binding sites for RCA~ (44). Cell-free protein synthesis studies suggest that the ribosomes of the RCA~ resistant variant line are just as sensitive to toxin inactivation as parental ribosomes, indicating that the properties of resistance must reside at a site(s) other than the level of protein synthesis (43). In addition, although most surface proteins on the resistant variant cells are similar to parental cell surface proteins as judged by electrophoresis after lactoperoxidase-catalyzed iodination or affinity chromatography of membrane glycoproteins, a surface glycoprotein of ~80,000 mol wt on the surfaces of parental cells is altered on the variant cells to a lower apparent molecular weight component (44). In preliminary nonquantitative experiments, it appeared that the resistant variant cells might not transport ferritin-RCA~ at the same rate as the parental sensitive cells (28). We now report in detail, using a fully biologically active, quantitative, electron-dense probe consisting of ferritin-conjugated, ~125I-labeled-RCA~ (ferritin-~125I-RCA~), that the RCA~ toxin molecules bind to cell surfaces of both resistant and sensitive cells. However, at low RCA~ concentrations where the maximum differential toxic effects occur, the ricin molecules only enter at significant levels into the sensitive cells.

**MATERIALS AND METHODS**

**Biochemicals and Chemicals**

Bovine serum albumin (BSA, three times crystallized) was obtained from Armour Pharmaceuticals (Chicago, Ill.). Ferritin was purchased from Immuno-Diagnostics (Solana Beach, Calif.) and Na~125I (~17 Ci/mg) from International Nuclear Corp. (Irvine, Calif.). ~15PH)leucine (20 Ci/mmol) was bought from New England Nuclear (Boston, Mass.), and osmium tetroxide and glutaraldehyde, EM grade, were obtained in sealed vials from Polysciences, Inc., (Warrington, Pa.). Saccharides were products of Calbiochem (San Diego, Calif.), and all inorganics were obtained from Mallinckrodt, Inc. (St. Louis, Mo.).

**Cell Lines and Growth**

The BW5147 cell line is a spontaneous AKR/J lymphoma that has been adapted to tissue culture and grows in suspensions with Dulbeco modified minimal essential medium (DMEM) plus 2 mM glutamine and 10% horse serum. A thioguanine-resistant subclone (BW5147- ~G. 1) is defined here as the parental BW5147 cell line (10) from which an RCA~ resistant variant line (BW5147Ric~) was obtained by stepwise selection over 1 yr against a series of RCA~ concentrations increasing from ~5 x 10^{-3} ~g/ml to 1 ~g/ml. The BW5147Ric~ variant cell line is ~200 times more resistant compared to the parental BW5147 line, as measured by the ability of the cells to survive a 60-min exposure to RCA~ in serum-free medium. These experiments were repeated with ferritin-RCA~ conjugates as follows: UV-sterilized toxin or ferritin-toxin conjugate was diluted in DMEM plus 0.1% crystalline bovine serum albumin (DMEM-BSA) in place of DMEM plus horse serum. The cells were washed once in DMEM-BSA, counted, and suspended in DMEM-BSA to 2 x 10^6 cells/ml. Ferritin-~125I-RCA~ or RCA~ (1 ml) and cells (1 ml) were mixed on ice and then transferred to a humidified tissue culture incubator at 37°C. After a 60-min incubation with agitation every 15 min, the tubes...
were placed on ice, and 2 ml of cold DMEM plus 10% horse serum was added, and the cells were washed twice by centrifugation (600 g for 5 min at 4°C). The cells were then resuspended in 2 ml of DMEM containing 10% horse serum and inoculated into 35-mm dishes. The dishes were sampled initially and at 48 and 72 h to determine the number of live/dead cells by trypan blue dye exclusion.

Cell protein synthesis was measured as described previously (27), using log phase cells. Cultures treated with an inhibitory concentration of RCA\textsubscript{a} or ferritin-\textsuperscript{125I}-RCA\textsubscript{a} for various times in DMEM-BSA were washed in DMEM minus t-leucine plus 5% horse serum and incubated in the same medium at 37°C in a CO\textsubscript{2} incubator for 60 or 120 min with added \textsuperscript{3}H]leucine (3 \textmu Ci/ml). After the incubation, the cells were washed three times with phosphate-buffered saline and dissolved in 1 ml of NaOH containing 0.1% BSA. Protein was precipitated by addition of 4-5 vol of cold 10% TCA, and after a 60-min incubation at 4°C, the precipitates were filtered through Whatman GF/C glass filters. Filters were washed twice with 5% TCA, once with 95% ethanol, dried, and \textsuperscript{3}H was determined in a scintillation counter.

**Lectins and Ferritin-\textsuperscript{125I}-RCA\textsubscript{a} Lectin Conjugates**

Concanavalin A (Con A) obtained from Calbiochem was affinity purified according to the procedures of Agrawal and Goldstein (1). RCA\textsubscript{a} was affinity purified from *Ricinus communis* extracts by the method of Nicolson and Blaustein (24). RCA\textsubscript{a} was labeled with \textsuperscript{125I} by the McFarlane (15) procedures as described previously (25). Ferritin conjugates were synthesized and purified by a modification of the procedures of Nicolson and Singer (29). \textsuperscript{125I}-RCA\textsubscript{a} was added to purified ferritin (Immuno-Diagnostics) in a ratio of 1:5 wt/wt in 0.2 M sodium chloride-0.005 M sodium phosphate buffer, pH 7.2 (PBS 1) containing 0.2 M D-galactose. The reaction mixture was split into two equal portions, and 10 \mu l of 1% glutaraldehyde in distilled water was added to one of the samples. 10 min later and at each successive 10-min interval, 10 \mu l of 1% glutaraldehyde were added to both reaction mixtures. When the sample containing the additional 10 \mu l of glutaraldehyde displayed a slight turbidity under strong beam of visible light, the reactions in both samples were terminated by addition of 0.25 vol of 1 M glycine in PBS 1. The samples were centrifuged at 27,000 g for 15 min at 4°C and the small pellets were discarded. The supernates were pooled and centrifuged at 160,000 g for 120 min onto a 0.5-ml cushion of Sepharose 4B beads. After removal of the supernates containing unconjugated \textsuperscript{125I}-RCA\textsubscript{a}, the pellet of ferritin and ferritin-\textsuperscript{125I}-RCA\textsubscript{a} was gently resuspended in PBS 1 and purified by affinity chromatography on a 3 x 90 cm column of Sepharose 4B (Pharmacia Inc., Piscataway, N. J.) or Bio-Rad A 1.5 m (Bio-rad Laboratories, Richmond, Calif.) as previously described (29).

Ferritin-conjugated concanavalin A (Ferritin-Con A) was synthesized using the same procedures, except that the conjugation buffer was 1 M sodium chloride-0.05 M sodium phosphate buffer, pH 6.8 (PBS 2), containing 0.2 M \alpha-methyl-p-mannopyranoside, and the affinity column was made with Sephadex G-75 beads (29).

**Labeling Procedures**

Cells were labeled with ferritin-\textsuperscript{125I}-RCA\textsubscript{a} or ferritin-Con A by the following procedures: BW5147 or BW5147Ric\textsuperscript{a}-3 cells were incubated for 10 min at 22°C with the ferritin conjugates at the appropriate concentration in PBS 1 (ferritin-\textsuperscript{125I}-RCA\textsubscript{a}) or 1 M sodium chloride-0.05 M sodium phosphate buffer (PBS 2) (ferritin-Con A) plus 0.1% BSA. The cells were washed twice in PBS + 0.1% BSA and either fixed in phosphate-buffered 1% glutaraldehyde for 5 min at 22°C or incubated in DMEM plus 5% horse serum for an additional 60 min at 37°C in a humidified CO\textsubscript{2} incubator. At the end of the 60-min incubation, the cells were fixed in phosphate-buffered 1% glutaraldehyde for 5 min at 22°C and washed in 0.1 M cacodylate buffer before postfixation in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h at 22°C. The fixed cells were centrifuged into soft agar, and the hardened pellets were processed as tissue blocks (30). The agar blocks were washed and dehydrated in ethanol and finally propylene oxide before embedding in Spurr resin (Electron Microscopy Sciences, Fort Washington, Pa.). Thin sections were cut on a Reichert model OMU-3 ultramicrotome C. Reichert, sold by American Optical Corp., Buffalo, N. Y. with a diamond knife (Rondiken, Honolulu), dried, and observed unstained in a Hitachi model HU-12 electron microscope at 75 kV.

**RESULTS**

**Properties of Ferritin-\textsuperscript{125I}-RCA\textsubscript{a}**

Before performing the ultrastructural experiments, we examined the biological activities of the ferritin-\textsuperscript{125I}-toxin conjugates. In one experiment, we monitored the effectiveness of ferritin-\textsuperscript{125I}-RCA\textsubscript{a} in inhibiting the growth of the murine lymphoma cell lines. Growth of the parental BW5147 line is completely inhibited at RCA\textsubscript{a} concentrations >0.01 \mu g/ml and partially inhibited at RCA\textsubscript{a} concentrations >0.001 \mu g/ml when toxin is continuously present in the cultures for 48–72 h. In contrast, the Ric\textsuperscript{a}-3 variant line shows only slight inhibition of growth at RCA\textsubscript{a} concentrations of 0.25 \mu g/ml, and some cells survive even at 2.5 \mu g/ml RCA\textsubscript{a} (44). When this experiment was performed with RCA\textsubscript{a} and ferritin-\textsuperscript{125I}-RCA\textsubscript{a} in parallel, no difference in biological activity (measured by cell growth) was ob-
served at equivalent toxin concentrations. These effects were specific and could be blocked by inclusion of 0.1 M α-gal or lactose in the incubation medium. Similar experiments were performed by pulse labeling with $^{125}$I-RCA$_h$ or ferritin-$^{125}$I-RCA$_h$ for 10 min at 22°C or 60 min at 37°C. Since RCA$_h$ binds rapidly to cells (in most cases to plateau values in under 10 min [c.f. references 25 and 26], short incubations with toxin or ferritin-toxin followed by removal of excess toxin and incubation for 24–72 h gave essentially the same results (Fig. 1 a and b). For the ultrastructural experiments, concentrations of ferritin-$^{125}$I-RCA$_h$ (3–10 μg/ml toxin) were chosen that resulted in significant amounts of cell surface bound ferritin-$^{125}$I-RCA$_h$ in each experiment so that the location of a reasonable number of toxin molecules could be easily determined on each thin-sectioned cell. At the lowest dose (3 μg/ml), ferritin-$^{125}$I-RCA$_h$ inhibits protein synthesis >80% in intact parental BW5147 cells within 90 min but has no effect on protein synthesis in the BW5147Ric$^+$-3 variant cells. However, after 48–96 h, BW5147Ric$^+$-3 variant cells are growth-inhibited at 3 μg/ml ferritin-$^{125}$I-RCA$_h$ (Fig. 1).

Quantitative binding of ferritin-$^{125}$I-RCA$_h$ was assessed with BW5147 or BW5147Ric$^+$-3 cells. Although the amounts of ferritin-$^{125}$I-RCA$_h$ bound per cell were RCA$_h$ dose-dependent and usually slightly below the levels of unconjugated toxin, little difference was found in specific binding of ferritin-$^{125}$I-RCA$_h$ between the sensitive parental BW5147 cells and the Ric$^+$-3 variant, and sometimes the resistant Ric$^+$-3 variant cells bound slightly more ferritin-$^{125}$I-RCA$_h$ (Fig. 1 c). Ferritin-$^{125}$I-RCA$_h$ labeling could be blocked by 0.1 M α-gal or lactose in the labeling and wash solutions as found previously (19, 27). In other experiments the ability of ferritin-$^{125}$I-RCA$_h$ to inhibit cell protein synthesis was monitored at 60 and 120 min after toxin binding to BW5147 cells. Ferritin-$^{125}$I-RCA$_h$ (3 μg/ml RCA$_h$) almost completely inhibited cell protein synthesis (>90%) within 60 min in BW5147 cells, confirming our previous results with fibroblastic cells (19, 26), and there was little difference between ferritin-conjugated and unconjugated toxin.

**Labeling and Dynamics of Ferritin-$^{125}$I-RCA$_h$**

The binding and dynamics of ferritin-$^{125}$I-RCA$_h$ on RCA$_h$-sensitive BW5147 and -resistant BW5147Ric$^+$-3 cells were determined by gamma isotope counting and electron microscopy. Cells were pulsed with ferritin-$^{125}$I-RCA$_h$ for 10 min at 22°C, washed, fixed with glutaraldehyde, and the radioactivity in each sample was determined (Fig. 1 c). Then, these same samples were postfixed, dehydrated, and embedded for electron microscopy. When BW5147 and BW5147Ric$^+$-3 cells were labeled with ferritin-$^{125}$I-RCA$_h$ containing 3 μg/ml RCA$_h$ for 10 min, ~10$^6$ ferritin-toxin molecules bound specifically to either cell type. Ultrastructurally, the ferritin-$^{125}$I-RCA$_h$ molecules were found at the cell surface in a more or less dispersed distribution (Fig. 2). Inclusion of 0.1 M lactose in the incubation and wash medium prevented binding of ferritin-$^{125}$I-RCA$_h$ (Fig. 3). When parental BW5147 cells were pulsed for 10 min at 22°C with ferritin-$^{125}$I-RCA$_h$ containing 1–3 μg/ml

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FIGURE 2 BW5147 cells were labeled with ferritin-125I-RCA<sub>a</sub> (3 μg/ml RCA<sub>a</sub>) for 10 min at 22°C, washed twice in PBS + 0.1% BSA, and fixed for electron microscopy. Bar, 0.2 μm. × 63,000.

FIGURE 3 Same as in Fig. 2, except that the samples contained 0.1 M lactose in the labeling and wash solutions. Bar, 0.2 μm. × 63,000.
RCAII and then washed and incubated for 60 min at 37°C before fixation, many of the ferritin-conjugated toxin molecules did not remain at the cell periphery, and endocytosis of the ligand was observed (Fig. 4). Not all of the ferritin-125I-RCAII molecules were endocytosed after the 60-min incubation, and the remaining surface-bound molecules appeared to be present in clusters and patches on the cell surface. At these dose levels of RCAII, the variant BW5147RicA-3 cells are more resistant to RCAII toxic effects, and therefore the experiment was repeated with the resistant cells. Although differences in ferritin-125I-RCAII binding and location on parental and RicA-3 variant cells were not discernible immediately after the 10-min labeling incubation, by 60 min after the ferritin-125I-RCAII pulse (1–3 μg/ml RCAII) there was little evidence of toxin transport via endocytosis in the BW5147RicA-3 cells (Fig. 5). However, at higher toxin concentrations where the parental and RicA-3 variant cells are equally sensitive to the cytotoxic effects of RCAII (7–10 μg/ml RCAII), toxin-mediated endocytosis occurred in both cell types (Figs. 6 and 7 at 7 μg/ml RCAII).

**Dynamics of Ferritin-Con A**

The apparent defect in the RicA-3 cells which prevents extensive endocytosis of ferritin-125I-RCAII at concentrations where the ferritin-conjugated toxin enters parental BW5147 cells could have been due to a general depression in the ability to transport low concentrations of surface-bound polyvalent ligands. Therefore, we examined BW5147 and the RicA-3 variant line for their abilities to bind and endocytose another ligand, ferritin-Con A. Cells were pulsed with several concentrations of ferritin-Con A for 10 min, washed, and incubated for an additional 60 min at 37°C as in the ferritin-125I-RCAII experiments. At low concentrations of ferritin-Con A (3–10 μg/ml Con A), both BW5147 and RicA-3 cells bound similar amounts of ferritin-Con A or 125I-Con A (Table 1) and transported the ligand into cells via endocytosis (Figs. 8 and 9). Although there appeared to be a slight enhancement in ferritin-Con A transport in the parental BW5147 cells, the variant BW5147RicA-3 cells were fully capable of endocytosis of this ligand.

**DISCUSSION**

Quantitative ultrastructural analyses of cell surface receptor distribution and dynamics are difficult to determine and are affected by a variety of variables. These include the specific activity and valence of the probe, its size, charge, binding strength, and other parameters (see references 3 and 23). In some studies the substitution of ferritin-lectin reagents for native lectins did not result in a quantitatively similar degree of labeling (4) or ultrastructural distribution of lectin-binding sites (3). These problems were probably attributable to the purity of the starting materials, methods of probe synthesis, and subsequent purification, the large size of the ferritin molecule (~150 Å, hydrated) and the potential problem that some saccharide-binding sites may be blocked during conjugation. We have used highly purified reagents and very careful conjugation conditions which have been continually modified over the years to synthesize a ferritin-125I-RCAII probe. The probe was then purified by affinity chromatography to yield a potent biologically active label. Comparison of the ferritin-125I-RCAII probe to RCAII in a variety of experiments indicated that the ferritin-toxin conjugate was unchanged in cell binding and inhibition of cell growth and protein synthesis, important biological activities of RCAII (for reviews see references 20 and 37).

RCAII Exerts its toxic effects by a sequence of events that results in inhibition of cell protein synthesis. First, the toxin binds rapidly to the cell surfaces of susceptible cells (almost all cells are susceptible [37]) through D-gal or D-galNAc-terminal membrane oligosaccharide residues (26, 38, 39). This binding is very specific and can be blocked or reversed by D-gal or lactose (5, 19, 26, 27, 38–40). Some cells fail to bind RCAII or ricin, and these are resistant to toxin-mediated cytotoxicity (7, 16, 17). RCAII contains two separable polypeptides (25, 42): One of these is thought to be involved in cell binding (35, 36), while the other is thought to inactivate cell protein synthesis. Only intact toxin molecules are cytotoxic in animals and unbroken cells.

RCAII must enter cells to act on protein synthesis, and its entry mechanism has been the subject of several studies. The first clue to the entry of RCAII or ricin into cells came from kinetic studies on inhibition of protein synthesis. Although toxin molecules bind rapidly to cells, a lag time of at least 30–60 min occurs before onset of inhibition of protein synthesis (27, 42). This lag period corresponds nicely with the time required for aggregation of surface toxin-receptor complexes, endocytosis, and escape of some toxin molecules from endocytic vesicles into the cytoplasm (19,
FIGURE 4 BW5147 cells were labeled with ferritin-^{125}I-RCA_{3} (3 μg/ml RCA_{3}) for 10 min at 22°C, washed, and incubated for 60 min at 37°C before fixation. Extensive endocytosis of the ferritin-toxin occurred. Bar, 0.2 μm. × 63,000.

FIGURE 5 Same as in Fig. 4, except that BW5147Ric^{R} 3 variant cells were labeled with ferritin-^{125}I-RCA_{3} (3 μg/ml RCA_{3}). Bar, 0.2 μm. × 63,000.
FIGURE 6 Same as in Fig. 4, except that BW5147 cells were labeled with ferritin-125I-RCAII (7 μg/ml RCAII). Bar, 0.2 μm. × 63,000.

FIGURE 7 Same as in Fig. 4 except that BW5147Ric9-1 cells were labeled with ferritin-125I-RCAII (7 μg/ml RCAII). Bar, 0.2 μm. × 63,000.
TABLE I

| Cell Line   | 125I-Con A | cpm Bound* |
|-------------|------------|------------|
|             | µg/ml      |            |
| BW5147      | 1.0        | 3,300 ± 250|
|             | 3.0        | 7,800 ± 400|
|             | 10         | 20,100 ± 900|
|             | 10 + α-methyl-d-mannoside | 1,100 ± 400 |
| BW5147Ric8.3| 1.0        | 3,800 ± 300|
|             | 3.0        | 8,300 ± 500|
|             | 10         | 22,100 ± 1,100|
|             | 10 + α-methyl-d-mannoside | 1,600 ± 400 |

* 4 x 10⁶ cells were labeled with 125I-Con A in PBS + 0.1% BSA for 10 min at 22°C and washed twice with PBS + 0.1% BSA.

27). The 30- to 60-min lag time can be almost completely eliminated by using lipid vesicles with encapsulated toxin capable of directly introducing RCA II into the cytoplasm after fusion of the lipid vesicle membrane with the cell plasma membrane.2 Experiments using reticulocytes that have diminished endocytotic activities also indicate that toxin entry occurs most likely via endocytosis. Refsnes et al. (42) found that ricin bound very rapidly to reticulocytes, but protein synthesis was not inhibited for at least 4 h under conditions where it rapidly stopped protein synthesis in Ehrlich ascites and HeLa cells.

Once the toxin molecules have gained entry into susceptible cells, ribosome-dependent protein synthesis is catalytically and irreversibly inactivated (18, 32-34). Inhibition of cell-free protein synthesis was effective when ribosomes but not supernates of cell-free systems were treated with toxin (31), and Sperti et al. (45) found that separate in vitro treatment of the 40S and 60S ribosome subunits with toxin resulted in identification of the 60S ribosome subunit as the site of toxin action. In addition, isolation and separation of ribosome subunits from toxin-treated cells revealed that the 60S, but not the 40S, subunits were inactivated (40).

Toxin-resistant tumor cell variants have proven useful in studying the various events leading to toxin-mediated cellular death (see Introduction). Since there are several possible mechanisms whereby tumor cells can escape RCA II cytotoxic effects, this could explain why toxin therapy has not proven universally effective (see review by Olsnes and Pihl [37]). In the present study, an RCA II toxin-sensitive murine lymphoma cell line and its toxin-resistant variant line were compared for RCA II binding, transport, and cytotoxicity at low toxin concentrations where only the parental line is sensitive, and at higher toxin concentrations where both parental and resistant variant lines are susceptible to RCA II killing. Although similar amounts of ferritin-125I-RCA II are bound to the parental and Ric8.3 variant cells at low toxin concentrations, the ferritin-toxin molecules are only transported at significant levels into the sensitive parental cells. At higher toxin concentrations where both parental and Ric8.3 cells are sensitive, ferritin-125I-RCA II molecules are bound and transported into both cell lines. The lack of endocytotic transport of ferritin-125I-RCA II in toxin-resistant Ric8.3 cells at low RCA II concentrations is not simply a generalized defect in cellular endocytosis, because these cells are capable of transporting low concentrations of ferritin-Con A.

The cell surface components of BW5147 and BW5147Ric8.3 cells have recently been analyzed using surface labeling techniques and affinity chromatography (44). Of the many cell surface components identifiable by these techniques, two surface-exposed proteins of ~80,000 and 35,000 mol wt are iodinatable by lactoperoxidase techniques on the parental but not on the Ric8.3 cells. The larger of these proteins is a glycoprotein-binding site for RCA II, while the smaller 35,000 mol wt component could not be identified as a binding site for RCA II in affinity chromatography experiments. Ric8.3 cells that do not possess the 80,000 mol wt component have instead a component of lower molecular weight (~70,000) which can be isolated by affinity chromatography or precipitation by anti-BW5147 in NP-40 cell membrane extracts (44).

A hypothesis for RCA II resistance in this system has been proposed by Robbins et al. (44) that depends on the endocytotic transport of toxin molecules into sensitive cells mediated by the 80K component. This hypothesis presumes that the toxin molecules bind to a variety of cell surface binding sites, but that only the 80K components are productive receptors, and that their 70K form is not capable of mediating endocytotic transport of the toxin at low toxin concentrations. Presumed genetic mutation(s) in the Ric8.3 variant cell line responsible for the above-described alteration could be in the genes coding for the 80K component, glycosyltransferases that specifically glycosylate this component, or in genes that code for or control a degradative enzyme that alters the 80K component.
Figure 8  BW5147 cells were labeled with ferritin-Con A (10 μg/ml Con A) for 10 min at 22°C, washed, and incubated for 60 min at 37°C before fixation. Bar, 0.2 μm. × 63,000.

Figure 9  Same as in Fig. 8, except that BW5147Ric³ variant cells were labeled with ferritin-Con A (10 μg/ml Con A). Bar, 0.2 μm. × 63,000.
component before insertion into the cell membrane. The data in this paper are consistent with this hypothesis but provide no direct evidence on the role of the 80K component in endocytosis of ricin. The experiments presented here suggest that either these or other changes in the variant could disrupt the normal transmembrane communication (21, 22) that is required to initiate a specific endocytotic process resulting in toxin sensitivity.

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