EVIDENCE FOR PLEIOTROPIC CHANGES IN LINES OF CHINESE HAMSTER OVARY CELLS RESISTANT TO CONCANAVALIN A AND PHYTOHEMAGGLUTININ-P

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ABSTRACT

Lines of Chinese hamster ovary cells resistant to the lectins concanavalin A (Con A) and phytohemagglutinin-P (PHA-P) have been isolated and characterized. Lines were isolated by a stepwise, a single-step, or a cycling single-step procedure, from both mutagen-treated and untreated cultures. The resistant lines showed a higher efficiency of colony formation in the presence of the appropriate lectin than did the wild-type parental line. The cell lines resistant to Con A did not exhibit any detectable cross resistance to PHA-P, nor did the PHA-resistant cells exhibit cross resistance to Con A. The toxicity of Con A from the wild-type and Con A-resistant lines was reduced in the presence of methyl α-D-glucopyranoside; this effect was not seen with the PHA-resistant line. Using ^{125}I-labeled Con A, it was found that Con A was bound preferentially to the surface of intact cells, and that the amount of labeled Con A bound to intact cells was similar for the wild-type and lectin-resistant lines. The Con A-resistant lines were found to be more susceptible to the toxic effects of a number of different compounds, including cyclic AMP and its dibutyryl derivative, sodium butyrate, high concentrations of glucose, phenethyl alcohol, phenol, ouabain, and testosterone. It appears that, in these lines, acquisition of resistance to Con A gave rise to pleiotropic effects which were detected by changes in the sensitivity of the cells to a variety of agents.

There is a considerable body of evidence which supports the view that changes in reguiatory processes involved in the growth and differentiation of mammalian cells usually result in an alteration of the surface membrane (1). Much of this information comes from the in vitro study of viral oncogenesis (2–4) but it is likely that changes in the composition or organization of the cell surface occur in a variety of other systems such as cells of the immune system (5, 6) and cells responsive to hormones (7, 8).

By the use of appropriate selection procedures it should be possible to isolate mammalian cells with alterations in the surface membrane, with the idea of studying the changes that have occurred in the regulatory processes associated with the cell surface (9, 10). The lectin concanavalin A (Con A) appears to be a suitable selective agent for this purpose. It has been used to detect changes in cell surface properties associated with the malignant transformation induced by tumor viruses (4, 11, 12); in addition, cell lines resistant to Con A have been isolated and reported to exhibit modified growth and cell surface properties (13).

In this paper, we report studies on the characteristics of Chinese hamster ovary (CHO) cell lines that were selected for resistance to Con A. The properties of these lines have been compared to those of wild-type CHO cells, and of lines selected for resistance to another lectin, phytohemagglutinin-P (PHA-P). Acquisition of resistance to Con

A was found to be associated with changes in the response of the cells to several different agents.

MATERIALS AND METHODS

Cells

CHO cells (14), derived from a stock originally provided by Dr. W. C. Dewey, were grown in suspension or as monolayer cultures at 34°C in alpha medium (15), supplemented with 10% (vol/vol) fetal bovine serum (Flow Laboratories Inc., Rockville, Md.). A higher temperature was not chosen since it would select against the possibility of isolating lines that were temperature sensitive for cell division or lectin resistance in future experiments (see reference 10). The doubling time of wild-type CHO cells in suspension culture was approximately 15 h.

Isolation of Membranes

A membrane isolation procedure was used to study the binding of Con A to surface membranes. Cells were grown at 34°C to a concentration of about 1.5×10^5 cells/ml in a Spinner flask, and treated with 0.2 μCi/ml of [^{125}I]Con A (Frosst) in the presence of 30 μg/ml unlabeled Con A in alpha medium with 10% serum, for periods of 45, 75, 100, and 120 min. The cells were spun down and washed twice with 0.15 M NaCl solution, and the surface membranes were isolated using an aqueous two-phase polymer system (16). In brief, the cells were suspended in 1 mM $ZnCl_2$ for 20 min and ruptured in a Dounce homogenizer. The homogenate was centrifuged for 15 min at 1,400 rpm, and the resulting pellet (pellet I) was suspended in a dextran-polyethylene glycol two-phase system (16). The system was centrifuged at 8,500 rpm for 10 min, and phase-contrast microscopy was used to determine the percent of unruptured cells in the pellet (pellet II). The supernatant (supernatant II) consisting of the two phases and the material at the interface was subjected to another cycle of mixing and centrifugation, and the pellet was discarded. The surface membranes at the interface of the two-phase system formed after the second cycle were collected with a Pasteur pipette and tested for ^{125}I radioactivity using a Nuclear-Chicago model C120 radiation analyzer (Nuclear-Chicago Corp., Des Plaines, Ill.).

Binding of Con A to Intact Cells

A double-label procedure (17) was used to estimate the amount of Con A bound to whole cells. Cells were grown in suspension culture to a concentration of about 2×10^5 cells/ml, centrifuged, and washed three times in calcium- and magnesium-free phosphate-buffered saline (PBS). These cells were incubated at $3–4 \times 10^6$ cells/ml in calcium- and magnesium-free PBS containing [^{125}I]Con A together with free ^{131}I (Frosst, as sodium iodide, at an activity equivalent to the activity of [^{125}I]Con A) and varying amounts of unlabeled Con A. After a 30 min incubation at room temperature to allow binding of Con A, the cells were sedimented at low speed, the supernatant was carefully removed, and the pellet of cells was resuspended in buffer for determination of bound radioactivity, using a Nuclear-Chicago model 4224 automatic gamma counter (Nuclear-Chicago Corp.). The binding of Con A to whole cells was determined on the basis of the increase in the ratio of ^{125}I radioactivity to ^{131}I radioactivity in the pellet as compared to the supernatant.

Determinations of Plating Efficiencies in the Presence or Absence of Various Agents

Colony-forming efficiencies was determined in Falcon plastic Petri dishes (Falcon Plastics, Div. of Bioquest, Oxnard, Calif.) containing alpha medium, 10% fetal bovine serum, and various concentrations of a variety of agents. The cell numbers tested varied from 10^2 cells/60 mm Petri dish to 10^6 cells/100 mm Petri dish. The dishes were incubated at 34°C for 10 days in a CO_2 and humidity controlled incubator.

The agents tested included Con A, glucose, and methyl α-D-glucopyranoside (Me α-D-glu, Calbiochem, La Jolla, Calif.); PHA-P (Difco Laboratories, Detroit, Mich.); adenosine 3′,5′-cyclic monophosphoric acid (cAMP, titrated to pH 6.8 with NaOH before use), dibutyryl cAMP, adenosine 5′-monophosphoric acid (5′-AMP), ouabain, testosterone, progesterone, gramicidin J, and digitonin (Sigma Chemical Co., St. Louis, Mo.); phenethyl alcohol (Matheson, Coleman and Bell Manufacturing Chemists, Norwood, Ohio); butyric acid and isobutyric acid (ICN Nutritional Biochemicals Div., International Chemical and Nuclear Corp., Cleveland, Ohio, titrated with NaOH of pH 6.8 before use); phleomycin (Bristol Laboratories, Div. of Bristol-Myers Co., Syracuse, N. Y.); phenol and ethanol.

A precipitate was observed when alpha medium containing Con A was supplemented with 10% fetal bovine serum. Therefore medium containing the various concentrations of Con A was incubated for 24 h at 34°C in the presence of serum before addition of cells, and centrifuged to remove the precipitate. When medium prepared at an initial concentration of 50 μg/ml of Con A was freed of precipitate and diluted to one-half of its initial concentration, it had approximately the same effect on the plating efficiency of the various cell lines as medium containing 25 μg/ml Con A. This result which suggests that the precipitate did not contain an appreciable proportion

of the Con A initially added to the medium containing serum is supported by a finding that less than 5% of the ^{125}I-labeled Con A added with 50 μg/ml cold Con A is recovered in this precipitate.

When 570 μg/ml PHA-P was added to alpha medium supplemented with 10% fetal bovine serum, a precipitate formed within 24 h. Again the medium was centrifuged to remove the precipitate before use.

RESULTS

Isolation of Resistant Lines

Con A and PHA-P were used as selective agents to obtain resistant lines. Cultures were treated with a mutagen by exposing cells grown exponentially in suspension at 34°C, at a concentration of 2×10^5 cells/ml, to 0.2 μg/ml of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) for 60 min. The fractional survival of colony-forming ability after treatment with mutagen was 0.01. The mutagen-treated cells were washed once with PBS, resuspended in fresh medium, and incubated at 34°C for 7 days to permit regrowth of the surviving cells. Mutagen-treated cultures were used in the selection of lectin-resistant cell lines on the assumption that the number of lectin-resistant mutants may be low. Whether MNNG treatment actually increased the number of lectin-resistant mutants in a population is not known. Some preliminary data suggest that MNNG may not be very effective.

For the selection of lectin-resistant mutants, three different procedures were used. For stepwise selection of mutants, 2×10^6 cells of an exponentially growing nonmutagenized or mutagen-treated culture were added to a 16 ounce Brockway bottle (Brockway Glass Co., Inc., Brockway, Pa.) containing growth medium (alpha medium and 10% fetal bovine serum), along with 10 μg/ml of Con A. Cells surviving the initial selection were grown to confluence at 34°C. This population was then subjected to increasingly higher concentrations of Con A (20, 40, and 60 μg/ml) for a period of 2–3 wk at each concentration. When the cells were cultured in the presence of Con A, the medium was changed frequently to remove any precipitate which may have formed when Con A was added to medium containing fetal bovine serum. The cells able to grow in 60 μg/ml of Con A were cloned by distributing the cells at limiting dilution into the wells of a Linbro plastic tray (IS-FS96-TC). Nine clones were picked from wells containing only one colony and were cultured in the absence of Con A to obtain clonal lines; all exhibited resistance to Con A when retested. From these nine clones, three were selected for further investigation. Con A^R-1 and Con A^R-2 were isolated from a mutagen-treated culture, and Con A^R-3 was obtained from a nonmutagenized population.

Isolation of Con A-resistant mutants from a nonmutagenized culture by a cycling single-step procedure was carried out by adding 4×10^6 wild-type cells to a 32 ounce Brockway bottle containing growth medium and 40 μg/ml of Con A. The culture was incubated at 34°C, and fresh medium was added every 24–48 h for a period of 3–4 wk. Since the medium was replaced at regular intervals during the selection period the word "cycling" was added to describe this type of single-step isolation procedure. The surviving cells (approximately $1:10^5$) were grown out to a confluent monolayer and were used in the selection of a cloned line, Con A^R-4, as described above.

Single-step isolation of PHA-P-resistant mutants was carried out by adding 5×10^5 nonmutagenized or mutagen-treated cells to a Brockway bottle containing growth medium and 180 μg/ml of PHA-P. The culture was incubated at 34°C for 10 days and the surviving cells (approximately $1:10^4$ for both nonmutagenized and mutagen-treated cultures) were grown out and cloned. PHA^R-1 was obtained from the mutagen-treated culture and PHA^R-2 was isolated from a nonmutagenized population.

Sensitivity of Lectin-Resistant Cells to Con A

The effects of various concentrations of Con A on the plating efficiency of wild-type and lectin-resistant CHO lines were determined by incubating various numbers of cells for 10 days at 34°C in the presence of different concentrations of Con A, and counting the number of colonies formed. These experiments were performed on a pseudodiploid line of wild-type cells, and on resistant lines that had been cultured in the absence of a selective agent for at least 3 mo.

It is apparent from Fig. 1 that the various Con A-resistant lines showed a similar reduced sensitivity to the cytotoxic effects of Con A, whereas the PHA-P-resistant lines exhibited as sensitivity which was similar to that of the wild-type cells. These results clearly demonstrate the reduced sensitivity of the Con A-resistant lines to the toxic effects of Con A, and also that the cell lines isolated

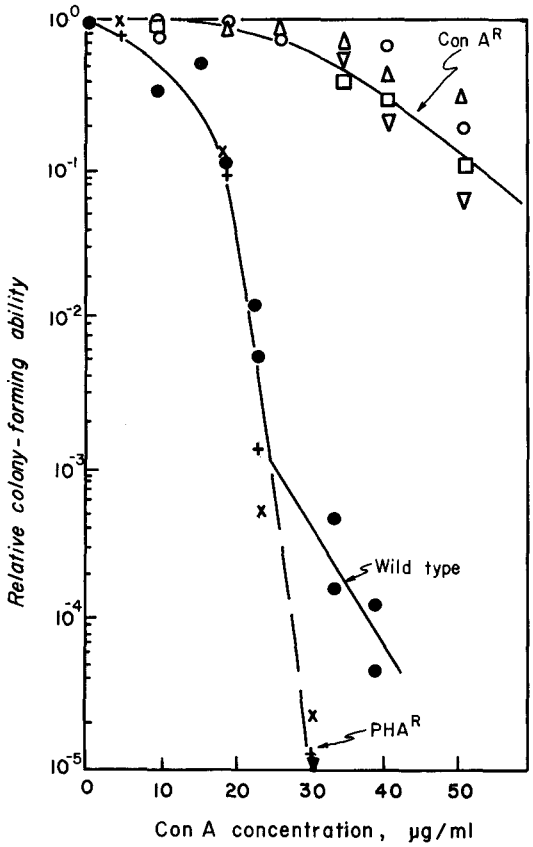

FIGURE 1 Effects of various concentrations of Con A on the colony-forming ability of wild-type and lectin-resistant CHO cells. ●, wild type; ○, Con A^R-1; △, Con A^R-2; ▽, Con A^R-3; □, Con A^R-4; ×, PHA^R-1; +, PHA^R-2.

on the basis of resistance to PHA-P had not acquired any detectable cross resistance to Con A.

Effect of Me α-D-Glu

We next investigated the specificity of the toxic effects of Con A on the different cell lines. If these toxic effects are a result of the ability of Con A to bind to polysaccharides and glycoproteins having α-D-glucopyranosyl, α-D-mannopyranosyl, or related terminal structures (18), then the toxicity of Con A should be reduced in the presence of competing compounds with structures of this kind. The results of such an experiment are shown in Fig. 2. It was found that in the presence of 10^{-2} M Me α-D-glu, the toxicity of Con A for the wild-type and Con A-resistant lines was markedly reduced, indicating that binding of Con A to structures related to Me α-D-glu is involved in the toxic effects of Con A on wild-type and Con A-resistant cells. In contrast, the sensitivity of the PHA-resistant lines to Con A was not affected by the addition of 10^{-2} M Me α-D-glu. Thus, although wild-type and PHA-resistant cells show a similar sensitivity to Con A in the absence of Me α-D-glu, the difference in their response to added Me α-D-glu provides evidence that the basis for the toxicity of Con A may be different in wild-type and PHA-resistant cells.

Sensitivity to PHA-P

Experiments analogous to those described above were also carried out to test the relative sensitivities of the different cell lines to various concentrations of PHA-P. The results are shown in Fig. 3. These results demonstrate the markedly reduced sensitivity of the PHA-resistant cells to PHA-P, com-

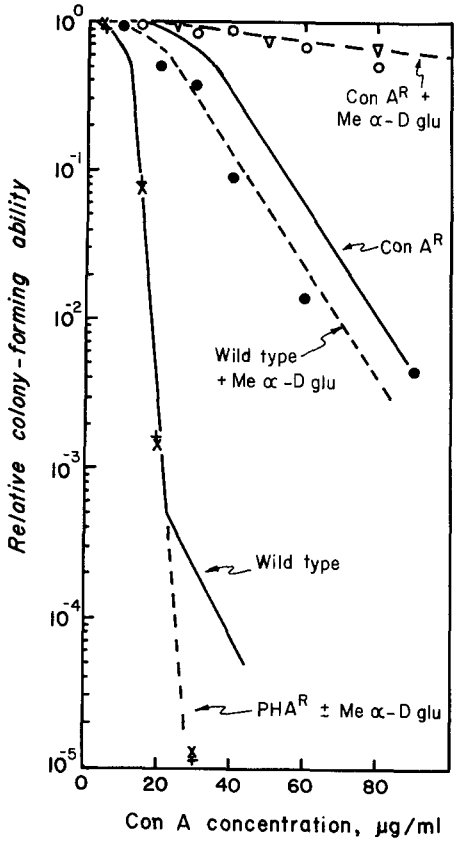

FIGURE 2 Toxicity of Con A in the presence of 10^{-2} M Me α-D-glu. The symbols used to designate the different cell lines are the same as in Fig. 1. The solid lines are taken from Fig. 1.

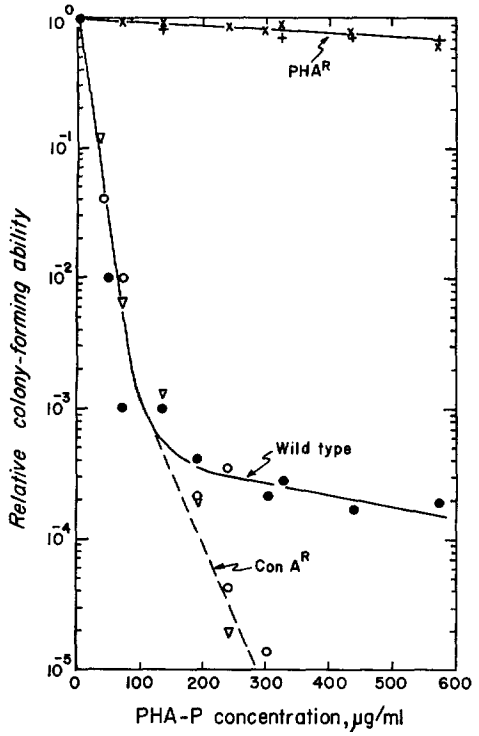

FIGURE 3 Sensitivity of the different cell lines to various concentrations of PHA-P. The symbols for the different lines are the same as in Figs. 1 and 2.

pared with the wild-type or Con A-resistant lines. They also indicate that the Con A-resistant lines, like the PHA-resistant lines, had not acquired any detectable cross resistance to the other lectin.

At concentrations of PHA-P above 200 μg/ml, the wild-type cells showed a reduced sensitivity to PHA-P, in comparison with the Con A-resistant lines. The basis for this reduced sensitivity has not been examined; it is likely that the surviving colonies were derived from a resistant subpopulation analogous to the PHA-resistant lines.

Binding of Con A to Intact Cells

The marked reduction in sensitivity of the Con A-resistant cells to the toxic effects of Con A (Fig. 1) led us to ask whether or not this reduced sensitivity of Con A-resistant cells was the result of a reduction in the ability of the cells to bind Con A. As a preliminary to these studies, an experiment was done to determine the proportion of ^{125}I-labeled Con A bound to the surface membrane of wild-type CHO cells. Intact cells were incubated in the presence of [^{125}I]Con A for various periods of time and then surface membranes were isolated as outlined in Materials and Methods.

The percent of the ^{125}I activity in the surface membrane fraction, relative to the activity in supernatant II, was calculated. If a correction is made for the proportion of cells not ruptured in the Dounce homogenizer, 93, 90, 89, and 88% of the ^{125}I activity bound to the cells at the first stage of the purification procedure was recovered in the surface membrane fraction after incubation periods in the presence of ^{125}I-labeled Con A for 45, 75, 100, and 120 min, respectively. This is a minimum estimate of the fraction of cell-bound [^{125}I]Con A associated with the surface membrane, since any labeled Con A eluted during the isolation of the surface membrane was not taken into account. It appears that ^{125}I-labeled Con A binds primarily to the surface of intact cells under the conditions of this experiment.

Experiments were next carried out to compare the ability of intact wild-type and lectin-resistant cells to bind [^{125}I]Con A. It was found that repeated washing appeared to remove some loosely bound Con A from the cells, so the double-label technique described in Materials and Methods was used in subsequent experiments. With the double-label method, the second label, free ^{131}I, was used to detect contaminating trapped material, and repeated washing of the labeled cells was unnecessary. It was found that when intact cells were incubated with [^{125}I]Con A for various periods of time, maximum binding occurred within 30 min (results not shown).

The amount of labeled Con A bound as a function of Con A concentration during a 30 min incubation is shown in Fig. 4. No clear-cut differences in the ability of the wild-type and the lectin-resistant lines to bind labeled Con A were observed. Since the cells were incubated in the presence of [^{125}I]Con A for 30 min some differences in the binding properties of the various cell lines may be masked by such processes as pynocytosis.

Response to cAMP and Related Compounds

The lines resistant to Con A appear to be analogous to the "colicin-tolerant" mutants of *Escherichia coli* (19), in that their ability to bind Con A may not be modified compared with wild-type cells (Fig. 4), but their sensitivity to the toxic effects of Con A is decreased (Fig. 1). Because colicin-tolerant mutants appear to show pleiotropic changes in their responses to a variety of agents

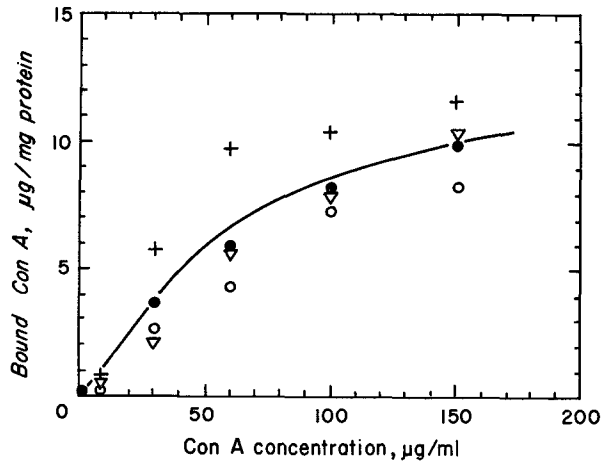

FIGURE 4 Amount of ^{125}I-labeled Con A bound to intact cells during a 30 min incubation, as a function of Con A concentration. A double-label method, with ^{131}I (as sodium iodide) as the second label, was used to determine the amount of contaminating trapped activity (see text). ●, wild type; ○, Con A^{R}-1; ▽, Con A^{R}-3; +, PHA^{R}-2.

TABLE I

Response of CHO Lines to cAMP and Related Compounds

Agent	Concentration	Percent survival*					
		W.T.‡	PHA^{R}-1	PHA^{R}-2	Con A^{R}-1	Con A^{R}-3	Con A^{R}-4
	M						
cAMP	5×10^{-3}	97	98	85	0.6	0.7	4.3
5′-AMP	5×10^{-3}	92	91	97	107	92	98
Dibutyryl cAMP	10^{-3}	85	48	60	0.02	0.02	0.07
Na butyrate	10^{-3}	69	4.3	6.5	1.0	0.9	4.4
Na isobutyrate	10^{-3}	98	95	87	88	97	103
Plating efficiency of untreated cells		69	77	71	70	70	61

* Percent survival equals the plating efficiency of treated cells relative to that of untreated cells times 100.
‡ W.T. = wild type.

(20), we tested for a differential effect of various compounds on the colony-forming ability of the lectin-resistant lines.

One compound chosen for such studies was cAMP, because of its apparent role in cell surface-mediated regulatory processes (7). It was found that the Con A-resistant lines were more susceptible to the toxic effects of cAMP and certain related compounds than were the wild-type or PHA-resistant lines.

The results of a representative experiment are presented in Table I. In this particular experiment, cAMP, at a concentration of 5×10^{-3} M, reduced the plating efficiency of all three of the Con A-resistant lines tested to less than 5%, while the same concentration of cAMP had no significant effect on the plating efficiency of the wild-type or PHA-resistant lines. These latter lines did, however, show changes in cellular morphology in the presence of cAMP, of the kind previously described (21–23). An equivalent concentration of 5′-AMP (5×10^{-3} M) had no detectable effects on either the wild-type or the lectin-resistant lines (Table I).

The dibutyryl derivative of cAMP also showed a selective toxicity for Con A-resistant cells, and was effective at lower concentrations than cAMP. As

may be seen from Table I, dibutyryl cAMP at 10^{-3} M reduced the colony-forming efficiency of the three different Con A-resistant lines to less than 0.1%. In this case, however, one of the compounds tested as a control, the sodium salt of butyric acid, was also toxic for the Con A-resistant lines. A closely related compound, sodium isobutyrate, was not effective at the same concentration. These results show that acquisition of resistance to Con A may be associated with an increase in the sensitivity of the cells to the toxic effects of other agents, such as cAMP or sodium butyrate.

It should also be noted that, in the presence of 10^{-3} M sodium butyrate, the plating efficiency of the PHA-resistant lines was reduced to about 5% of the value obtained for wild-type cells. Thus, although it was less effective on PHA-resistant lines, this compound showed a differential toxicity for both classes of lectin-resistant cells.

Response to Other Agents

The results presented above raised the possibility that the Con A-resistant cells might be sensitive to the toxic effects of other agents in addition to cAMP and sodium butyrate. A survey of a variety of other compounds revealed this to be the case. The results are summarized in Table II.

The colony-forming efficiency of Con A-resistant cells was reduced in comparison with wild-type cells in the presence of high glucose concentrations, phenethyl alcohol, phenol, oaubain, testosterone, and progesterone. The PHA-resistant cells showed a slightly higher plating efficiency than wild-type cells in the presence of 5×10^{-4} M ouabain. In preliminary studies the PHA-resistant lines have also shown an enhanced agglutinability compared with wild-type cells, in the presence of various concentrations of Con A between 20 and 400 μg/ml.

The different lines were also tested for their ability to form colonies at 38.5°C, compared with the lower temperature of 34°C used in the majority of this work. It was found that the colony-forming efficiency of two of the four Con A-resistant lines at the higher temperature was only about 5% of their colony-forming efficiency at the lower temperature (last line of Table II).

These results demonstrate that the lectin-resistant lines exhibited a modified response to a number of different agents, in comparison with wild-type cells.

TABLE II

Summary of Response of CHO Cells to Various Agents

Agent	Concentration	Relative sensitivity*		
		W.T.	PHA^R	Con A^R
Glucose	4×10^{-2} M	R	R	0.8–5
Me α-D-glucose	4×10^{-2} M	R	R	R
Phenethyl alcohol	0.05%	R	R	0.01–0.05
Ethanol	0.05%	R	R	R
Phenol	0.03%	R	R	0.02–0.07
Ouabain	5×10^{-4} M	15	R	0.07–0.8
Testosterone	2×10^{-4} M	R	R	0.1–0.8
Progesterone	10^{-4} M	R	R	0.02–0.7
Digitonin §	4.8×10^{-6} M	R	R	R
Gramicidin J§	2.7×10^{-6} M	R	R	R
Phleomycin§	1.5×10^{-5} g/ml	R	R	R
Temperature (38.5°C)	—	R	R	5‡

* Relative sensitivity expressed as percent survival of colony-forming ability. The range shown for Con A^R cells encompasses the values obtained using the Con A^R-1, Con A^R-3, and Con A^R-4 lines. R = resistant, percent survival 50% or greater.

‡ Con A^R-2 and Con A^R-3 only.

§ The concentrations used were those that reduced the plating efficiency of wild-type cells to about 50% of control values.

DISCUSSION

The results presented in this paper support the view that Con A is a suitable selective agent for the isolation of variant cell lines with altered membranes and membrane-associated properties. Con A binds selectively to the surface membrane of intact cells, as expected from its affinity for cell surface-associated carbohydrate or glycoprotein (24, 25). Intact cells are agglutinated by Con A (4), with an efficiency that appears to depend on the spatial organization rather than simply the total number of binding sites for Con A on the cell surface (26–28). In preliminary studies not reported here, we have found, as have Ozanne and Sambrook (13), that Con A-resistant cells show a reduced agglutination in the presence of Con A, even though their capacity to bind Con A may not be significantly affected (Fig. 4). It is possible that the spatial organization of Con A-binding sites is altered in the Con A-resistant cells.

The observation that the plating efficiency of wild-type and Con A-resistant cells is enhanced in the presence of Me α-D-glu (Fig. 2) supports the view that binding of Con A to carbohydrate-containing structures is involved in the toxic effects of Con A. It is also clear that Con A binds to the surface membrane of CHO cells in the presence of medium supplemented with serum. Furthermore, the number of Con A sites per cell estimated from this experiment (4×10^7) agreed with the values obtained for the binding of Con A to intact cells in the absence of serum (Fig. 4) and compared favorably to the estimates of lectin-binding sites per cell reported for other cell lines (27). Therefore, it is clear that during the isolation of Con A-resistant lines the lectin is bound to the cells effectively, but we cannot rule out the possibility that some of the toxicity of Con A in our system is due to the lectin-binding, carbohydrate-rich components in the serum.

The mechanisms responsible for the multiple changes in response of the Con A-resistant and PHA-resistant cells to a variety of agents remain to be determined. Although the effects apparent in the data shown in Tables I and II were detected using assays of colony-forming ability, the basis for the reduced survival of colony-forming ability has not been examined. It is possible that some reduction in colony formation is the result of a reduced growth rate of the cells in the presence of the agent, rather than an all-or-nothing effect on the ability of the cells to proliferate.

We cannot rule out the possibility that the pleiotropic effects of acquisition of lectin resistance were the result of an accumulation of several different mutations in the resistant lines. It seems unlikely that the same pattern of pleiotropic effects would be seen in independently isolated Con A-resistant lines as a result of accumulation of mutations, unless the different lines were all derived from a common progenitor preexistent in the wild-type parental line. Experiments designed to test this possibility are in progress (J. E. Till and R. M. Baker, personal communication). A preliminary analysis of the karyotypes suggests that the different lines arose independently; Con A^R-1, Con A^R-2, and PHA^R-1 exhibited modal chromosome numbers per cell approximately twice the value of 21 characteristics of the wild-type line, while Con A^R-3, Con A^R-4, and PHA^R-2 all resembled the wild-type in modal chromosome numbers.

It is unlikely that all the modified characteristics of the lectin-resistant cells were the result of some relatively nonspecific change in membrane properties, such as a gross alteration in membrane permeability. An attempt to detect such a gross permeability change, using fluorescein diacetate (29), did not reveal any differences between the different cell lines. Moreover, the enhanced toxicity of some compounds for Con A-resistant cells was not seen with other closely related compounds; for example, high concentrations of glucose were toxic, but not similar concentrations of Me α-D-glu (Table II). Also, the modified properties of these lectin-resistant lines are not shared by cell lines that were isolated for resistance to phleomycin or gramicidin J (unpublished observations). We conclude that Con A and PHA-P select for mutations giving rise to pleiotropic effects of a kind that have not, to our knowledge, been described previously for mammalian cells in culture.

The basis for these pleiotropic effects remains to be determined. It seems possible that the various changes observed in the lectin-resistant lines are due to composition or conformation alterations of the cell surface, analogous to the modification assumed to be responsible for the multiple differences in properties between virus-transformed and normal cells (8). This possibility is being investigated.

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