Detection of H-Y antigen in lymphocytes from 21-hydroxylase deficient patients

	Group	Sex	Age (years)	Number of patients	% Ig+ cells[d]	Percentage of positive cells reacting with: H-Y antiserum + FITC-anti IgG	FITC-anti IgG (control)[e]
Patients	1	Male 46, XY	3–13	5	19.1 ± 2.5	20.7 ± 5.5[a]	3.0 ± 2.8
	2	Female 46, XX	2– 5	3	22.0 ± 2.2	20.6 ± 1.3[a, b]	5.2 ± 0.3
	3	Pseudohermaphrodite 46, XX	40–50	2	21.3 + 0.5	17.5 + 1.3[a, b]	2.0 + 2.8
	3′	Pseudohermaphrodite 46, XX	41	1	21.5	4	1
	4	Female 46, XX	20–39	5	17.7 ± 2.5	12.2 ± 2.3[c]	3.1 ± 1.1
Controls	5	Female 46, XX	18–60	n	19.7 ± 3.0 (n = 59)	9.3 ± 2.1 (n = 70)	4.1 ± 2.5 (n = 54)
	6	Male 46, XY	18–60	n	20.9 + 4.7 (n = 77)	20.6 + 3.7 (n = 89)	4.0 + 2.8 (n = 63)

Values are mean ± SD. [a] Not significantly different from group 6 H–Y+ cells by χ^2 test; [b] significantly different from group 5 H–Y+ cells by χ^2 test ($p < 0.0005$); [c] significantly different from group 5 H–Y+ cells ($p < 0.05$) and group 6 H–Y+ cells ($p < 0.005$); [d] no significant difference by χ^2 test between any Ig+ cells percentages; [e] no significant differences by χ^2 test between any control percentages.

($p < 0.0005$ by χ^2 test). The 3rd case of female pseudohermaphroditism (case 3′) was H–Y negative[12]. Because he was obese and somewhat mentally deficient he seemed different from the group 3, and other disorders have been suspected. The percentages of H–Y+ cells from the group 4 (late forms of 21-hydroxylase deficiency) were significantly different from the female controls and from the male controls' percentages of H–Y+ cells.

In the present experiments, H–Y antigen was found to be lightly increased in the lymphocytes from 5 late 21-hydroxylase deficient women, and very much increased in the lymphocytes from 5 congenitally 21-hydroxylase deficient female patients. First, these findings suggest that a correlation exists between the degree of virilization of these female patients and their proportions of H–Y+ lymphocytes. There is also evidence that females 46,XX can produce excess of H–Y antigen in some circumstances like 21-hydroxylase deficiency.

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0014-4754/84/030273-02$1.50 + 0.20/0

Selection for resistance to *Bacillus thuringiensis* δ-endotoxin in an insect cell line *(Choristoneura fumiferana)*

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25 March 1983

Summary. A cell line of the spruce budworm (FPMI-CF1) consists of a mixed population of cells that possess variable sensitivity to δ-endotoxin from crystals of *Bacillus thuringiensis*. A cell strain was selected from FPMI-CF1 which was resistant to the entomocidal protein extracted from *B. thuringiensis* crystals. The resistant character was unstable, however, and could not be maintained in the absence of toxin during growth.

Protein isolated from parasporal crystals of the bacterium *Bacillus thuringiensis* var. *kurstaki* is toxic for cultured insect tissue from the spruce budworm *(Choristoneura fumiferana)*[2,3]. The cytological response of the insect cells to activated δ-endotoxin protein is progressive with time and eventually leads to cellular lysis[4]. Dissolved crystal protein possesses only slight activity toward spruce budworm (FPMI-CF1) cells, but becomes highly toxic (50% lethal concentration, LC_{50}, approximalety 4–6 μg toxin protein/ml) once activated by insect gut juices or by purified alkaline proteases. The cytological response of FPMI-CF1 cells to activated δ-endotoxin protein is sensitive and specific, and has been recommended for use as an alternative in vitro bioassay method for the determination of *B. thuringiensis* parasporal crystal toxicity[4–6].

Normal populations of FPMI-CF1 contain cells which are resistant to concentrations of activated toxin in excess of 10 times the usual LC_{50}[4]. These cells persist in the cultivation of the cell line and neither do they overcome the sensitive population nor do they disappear. Their presence interferes with precise measurement of toxic response, and contributes to poor statistical inference. Attempts to estab-

lish clones of the sensitive cell type have been unsuccessful, but it was possible to select for the resistant cell type by subculture in the presence of toxin protein.

Materials and methods. FPMI-CF1 is a cell line of the spruce budworm, *C. fumiferana* (Clemens) established from 1st instar larvae by S.S. Sohi at the Forest Pest Management Institute, Sault Ste. Marie, Ontario, Canada. The cells were maintained in 5 ml of modified Grace's medium[4] in 25-cm^2 plastic tissue culture flasks at 28 °C, and were routinely subcultured every 4–5 days at an inoculum level of 1.5×10^5 cells/ml.

Dissolution and activation of parasporal crystal protein was performed as before[4] using crystals isolated from *B. thuringiensis* var. *kurstaki* and purified by buoyant density centrifugation[7]. Activated crystal protein solution was filter-sterilized and added to the growth medium as required to yield the desired concentration. Protein concentration was determined by the method of Lowry et al.[8] using crystalline bovine serum albumin as a standard. The determination of toxicity was performed upon washed tissue cells by the method previously published[5], except for the addition of 5 mM $MgCl_2$ and 1 mM EDTA to the boiling buffer used for reaction sequence termination.

Results and discussion. The percentage of FPMI-CF1 cells that were normally resistant to high concentrations of δ-endotoxin protein in a 4–5-day-old population of cells ranged from 20–30%. Cells were spindle-shaped, uniform in size, and resistance was not a function of age. The resistant cells could, however, be readily selected from a mixed cell population by cultivation in the presence of δ-endotoxin protein. Propagation was in normal culture medium supplemented with increasing amounts of toxin (0.6 µg/ml, 3 µg/ml, 6 µg/ml, and finally 30 µg/ml). Cells surviving this treatment rapidly adapted to the presence of toxin protein in the growth medium and cell density approached normal levels after several passages. The toxicity of the standard δ-endotoxin preparation toward 4–5-day-old FPMI-CF1 cells grown in the presence and absence of δ-endotoxin is shown in the figure. Although not completely resistant to high levels of δ-endotoxin protein, the LC_{50} for the treated cell strain increased 89-fold, to an extrapolated LC_{50} of approximately 412 µg protein/ml. The resistant cell strain (designed CF1-R) responded linearly to toxin dose, unlike the curvilinear response obtained with the mixed cell population.

In order to determine the stability of toxin resistance, the CF1-R cell strain was propagated in the absence of toxin for extended lengths of time. Periodically, the parent cell line and the resistant cell strain were bioassayed for sensitivity to the standard δ-endotoxin preparation. The results over a 4-month period (24 passages) are presented in the table. In the absence of toxin in the culture medium resistance was slowly lost with time. Eventually, the cell strain returned to a stable but mixed population of cells that exhibited a sensitivity ratio to toxin similar to that of the original cell line.

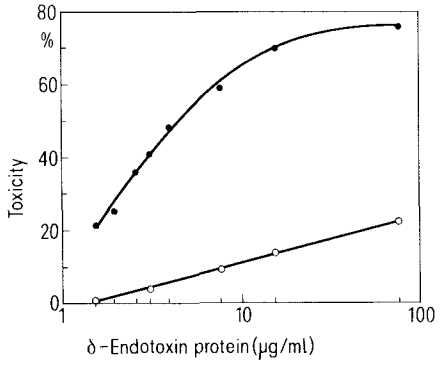
Similar instances of acquired resistance have been reported in several cell lines. Constant exposure of a tobacco hornworm cell line *(Manduca sexta)* to β-ecdysone produced a resistant cell population which later diminished after removal of the hormone from the growth media[9]. Likewise, Couregeon[10] was able to obtain a subline of *Drosophila* cells that no longer responded to β-ecdysone by growing the cells in the continuous presence of the hormone for several passages. The data presented here indicate that spruce budworm cells can be similarly selected for resistance to δ-endotoxin protein. Since the resistant cell strain was unstable in toxin-free media, resistance did not appear to be inherited. Elimination of the physiological stimulus (toxin) during subsequent cultivation resulted in a return to the original level of toxin sensitivity. Apparently a low level of resistance (20–30%) is a stable genetic trait in the FPMI-CF1 cell line. This conclusion is supported by data from a related cell line, IPRI-CF 124.0, in which a higher proportion (50%) of resistant cells were consistently produced during cultivation[2].

The resistant cell strain (CF1-R) should prove valuable in related investigations designed to elucidate the biochemical mode of action of δ-endotoxin at the cellular level. Structure-composition studies of the cell membranes of resistant and sensitive cell types may lead to a better understanding of the physiological basis of activity of this important biological insecticide.

Loss of resistance of CF1-R cells to δ-endotoxin after subculture in non-supplemented growth media[a]

Days in subculture	LC_{50}[b] CF1	CF1-R
0	4.5	412.7
30	5.2	103.4
60	4.9	18.6
90	5.3	10.2
120	5.8	8.4

[a] Growth medium free of δ-endotoxin protein used to condition the CF1-R cell line. [b] 50% lethal concentration, µg toxin protein/ml.



The response of CF1 cells to various concentrations of activated δ-endotoxin protein. Response (% toxicity) is a function of cell viability resulting from treatment of either control (parent) cells or conditioned (resistant) cells with δ-endotoxin. ●, CF1; ○, CF1-R.

1 The author wishes to thank S.S. Sohi (Forest Pest Management Institute, Canadian Forestry Service, Sault Ste. Marie, Ontario, Canada) for the *C. fumiferana* cell line, and L.I. Davidson (USDA) for his able technical assistance.

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