antibody that are produced following i.o. immunization. In these experiments, HRBC inoculated into the AC never hemolyzed, but were slowly absorbed over a period of 3 days. Note that the AC is in virtually direct contact with the venous system. The anterior ciliary vein of the eye is in direct contact with the canal of Schlemm which is separated from the spaces of Fontana and the AC by a single, very porous layer of endothelial cells. HRBC were apparently able to readily escape the AC and enter the venous circulation. Absorption must be passive as HRBC are not motile, as are lymphocytes. Active egress from the AC, as suggested by Kaplan and Streilein is not necessary. Kaplan and Streilein used trypsinized epithelial cells and whole skin grafts as AC implants, but were unable to measure hemagglutinating antibody using a relatively insensitive microtiter method of analysis. In my methods, HRBC were used as immunogens and as the test cell for antibody titration, and a highly sensitive microagglutination assay (100 times more sensitive than microtiter agglutination) was used. It is well established the i.v. route is superior for humoral immunization as compared to the i.m. route. This is supported by these results. Although the experiments were not designed as quantitative studies of antibody production, it appears that the i.o. route is at least as effective, and possibly more effective than the i.v. route for sensitization and stimulation of systemic antibody production. Such results are surprising, but may be due to an antigen depot effect.

Conclusion. The interpretation of these results are clear. Inoculation of HRBC into the AC vigorously stimulates systemic antibody production. This interpretation is not compatible with the theory that the AC of the eye is an immunologically privileged site.

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Increase of lymphocytic H-Y antigen in female 21-hydroxylase deficiency

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Summary. H-Y antigen was found to be increased in lymphocytes from 10 female 21-hydroxylase deficiencies, suggesting a correlation between the degree of virilization of these patients and their H-Y+ lymphocytes proportions. Furthermore, these findings demonstrate the ability of a 46,XX female subject to produce, in some circumstances, an excess of H-Y antigen.

H-Y antigen is a minor histocompatibility antigen which is detectable on male cells, at least in species where the male is the heterogametic sex[1-3]. Although its role in testicular organization seems to be very important[4], it has not clearly been demonstrated how H-Y antigen can act on gonadal organogenesis. Erickson suggested that androgens could easily play an important role in the expression of H-Y antigen[5]. Therefore we studied lymphocytic H-Y antigen in adrenal hyperplasia due to 21-hydroxylase deficiency. This deficiency results in decreased cortisol synthesis and secondary increased production of adrenocorticotrophic hormone (ACTH). In its turn, excess ACTH secretion leads to overproduction of androgens and consequent virilisation[6-8].

Materials and methods. The study was performed on 16 21-hydroxylase deficient patients. 11 had congenital adrenal hyperplasia with increased hydroxyprogesterone and testosterone levels (group 1, 2, 3 and 3'): 5 boys (3 with pubic hair and 2 with salt wasting crises), 3 little girls (1 with pubic hair and 2 with salt wasting crises) and 3 adults 46,XX with female pseudohermaphroditism[9]; 5 female cases (group 4) were late forms of adrenal hyperplasia (hirsutism in early adulthood associated with increased hydroxyprogesterone, testosterone and delta-4-androstenedione levels). H-Y control values were obtained from 159 healthy volunteers (89 male and 70 female). H-Y antigen was studied in human peripheral blood lymphocytes by indirect immunofluorescence[10]. Anti H-Y serum was raised in (C_{57}Bl/6 × DBA/2) F_1 female mice by i.p. inoculations of spleen cells from inbred males. Pooled sera were absorbed with human female blood buffy-coat cells before testing. The anti-mouse immunoglobulin was a fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ fragment goat anti-mouse IgG (Cappel) absorbed with pooled human buffy coat cells. Since a close correlation between the male B lymphocyte counts and the male H-Y+ cell counts had been shown, and not between female counts[11], Ig+ cells (B lymphocytes) counts were used as control for each patient. B lymphocytes were identified by direct immunofluorescence with FITC-conjugated $F(ab')_2$ fragment goat antiserum to human immunoglobulins (polyvalent) (Meloy).

Results and discussion. A correlation was found between the proportion of H-Y+ cells from the little boys (group 1), from the little girls (group 2), from 2 pseudohermaphrodites (group 3) and that from the male controls (table). Moreover the proportions of H-Y+ cells were significantly greater in the little girls (group 2) and in 2 female pseudohermaphrodites (group 3) than in the female controls

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

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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-