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Sex-dependent induction of human suppressor T cells by chorionic gonadotropin

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Human chorionic gonadotropin (hCG) in physiological retroplacental concentration has been shown to induce human female lymphocytes which suppress the proliferation and differentiation of B cells stimulated by purified protein derivative of tuberculin. To test whether the hCG-induced suppression was sex dependent parallel experiments using female and male peripheral lymphocytes were performed. hCG did not induce cells capable of suppressing purified protein derivative induced B cell proliferation in lymphocytes from males, which was in contrast to lymphocytes from females where a statistically significant suppression was found. A similar hCG-induced suppressive effect was found before menarche, after the menopause and in two patients with Turner's syndrome, suggesting that a gene(s) on the Y chromosome exerts a regulatory function and thus prevents the hormone from inducing suppressor T cells.

Introduction

Allogeneic pregnancy is an instance of successful transplantation across major histocompatibility barriers. Many theories have been put forward to explain this biological and immunological enigma but the exact mechanism still remains elusive (see Beer and Billingham, 1971). Endogenous immunosuppression has recently been proposed as the main reason for the successful outcome of pregnancy and chorionic gonadotropin (hCG), a glycoprotein hormone secreted by the trophoblastic cells of the placenta, is suggested to be an important factor modulating immunological responsiveness. This hormone has previously been shown to be capable of depressing mitogen-induced responses of T lymphocytes (Kaye and Jones, 1971; Adcock et al., 1973) and B lymphocytes (Hammarström et al., 1979) in the female via the induction of suppressor T cells (Fuchs et al., 1980; Fuchs et al., 1981) capable of suppressing T-helper cell-dependent B cell responses (Fuchs et al., 1981). Since pregnancy occurs only in females it is of interest to determine whether this mechanism is operative in both sexes or restricted to females. The subsequent experiments indicate that suppressor T cells could not be induced by hCG in Y chromosome-bearing individuals, thus constituting the first example of sex-dependent suppression.

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Materials and Methods

Experimental procedure

Human peripheral blood was obtained from males and females and separated on a Ficoll–Isopaque density gradient. The cells were either cultured untreated or in the presence of hCG (Gonadex®: phenol and preservative-free, containing 6000 IU hCG and 50 mg mannitol per ampulla (Leo AB, Hälsingborg, Sweden) — isoelectric focusing (PAG plates, pH 3–9) after coumassine blue staining gave rise to 5 bands focused between pH 4.5 and 6) or 50 μg/ml of concanavalin A (Con A; Pharmacia, Uppsala, Sweden). Culture was performed in 50 ml tissue culture flasks (Falcon 3013, Falcon Plastics, Oxnard, Calif.) at a density of $5 \cdot 10^6$ cells/5 ml of RPMI 1640 medium supplemented with 10% heat-inactivated unabsorbed human AB-serum. After 2 days the cultures were harvested and 10^6 pre-cultured cells added to $4 \cdot 10^6$ fresh autologous lymphocytes (prepared by Ficoll–Isopaque separation). The admixture was cultured in 50-ml tissue culture flasks in 5 ml of RPMI 1640 medium supplemented with 10% heat-inactivated unabsorbed human AB-serum, with or without the addition of purified protein derivative of tuberculin (PPD; Statens Serum-Institut, Copenhagen), a T cell-dependent human polyclonal B cell activator. After 7 days the cultures were harvested and assayed for plaque-forming cell numbers using the indirect haemolytic *Staphylococcus aureus* protein A plaque assay. Cell viability was determined by trypan blue dye exclusion.

Coupling of protein A to erythrocytes

Staphylococcus aureus protein A (Pharmacia) was coupled to sheep red blood cells (SRBC) using $CrCl_3$ as described by Gronowicz et al. (1976).

Plaque assay

100 μl of cells suspended in BSS were directly added together with 25 μl of protein A-coupled erythrocytes (diluted 1 : 3), 25 μl of the IgG fraction of anti-serum, diluted 1 : 30 (anti-human, μ, γ, α-chain Ig produced in rabbit; DAKO-Immunoglobulins Ltd., Copenhagen, Denmark), and 25 μl of SRBC-absorbed guinea-pig complement (diluted 1 : 4) into 700 μl 0.5% agar in BSS containing 0.45 mg/ml DEAE-dextran. Two 0.2 ml drops of the mixture were placed on a plastic 9 cm petri dish and a $24 \cdot 32$ mm glass cover-slip was immediately placed on each drop. Dishes were incubated for 4 h at 37°C, and plaques were counted using indirect light.

Assay for DNA synthesis

Proliferation was determined by culturing a triplicate of 0.2 ml of the total (5 ml) cell suspension subsequently to be tested for immunoglobulin production. [^{3}H]Thymidine (1 μCi/well, Amersham, U.K.) was added and the plates were harvested after 4 h of incubation. After drying, the radioactivity of the filters was counted in a scintillation spectrophotometer.

Statistical analysis

Results represent mean suppressive effect of the different Ig classes. Plaque-forming cell (PFC) numbers cultured in groups containing precultured cells without any

pre-activating substance added represent 0% suppression. Statistical analysis of suppression was performed using the matched Student's t-test. PFC numbers in cultures with cells pre-cultured in the absence of hCG or Con A $= a$. PFC numbers in cultures with cells pre-cultured in the presence of hCG or Con A $= b$. Variables were tested on the assumption that no significant difference existed. Comparison was made between $(a-b)/[(a+b)/2]$ and 0.

Results

As shown in Table 1, 2000 IU/ml of hCG did not induce cells capable of suppressing PPD-induced B cell differentiation in male lymphocytes after 48 h of precultivation ($P > 0.05$) which is in contrast to female lymphocytes where a statistically significant suppression was found ($P < 0.001$). This difference was maintained using different hCG concentrations (250–2000 IU/ml). When the pre-cultivation time was reduced (4–24 h), no suppressive effect was induced in either sex (data not shown). Two patients suffering from Turner's syndrome (sex chromosomal constitution of X0 *without* mosaicism), one of whom was treated with oestrogen and progesterone, were tested for suppressive capacity. In both these patients hCG-induced suppression of the immunoglobulin response was equal to that of normal females (Table 1). The hCG-induced suppression was independent of the age of the females. When tested before menarche, hCG diminished IgM, IgG and IgA responses by 83, 73 and 16%, respectively. When tested after the menopause, 75% suppression was found in the IgG response.

TABLE 1

hCG-induced suppression of immunoglobulin response in female lymphocytes using PPD as B-cell activator [a]

Cell source	No. of expts.	Mean suppression (% ± SE)					
		Con A			hCG		
		IgM	IgG	IgA	IgM	IgG	IgA
Male cells	9	92±2	89±2	80±5	−9±7	−15±10	−16±9
Female cells	8	94±2	92±2	81±5	52±5	55± 2	46±2
Turner (×0) cells	2	98±2	93±3	80±1	61±1	52±12	70±2

[a] Peripheral human blood lymphocytes were cultured in the presence of 50 μg Con A/ml, 2000 IU/ml hCG or devoid of pre-activating substance. After 48 h the cells were harvested and 10^6 pre-cultivated cells were mixed with $4 \cdot 10^6$ autologous fresh lymphocytes. The admixture was cultured in the presence of 100 μg PPD/ml and harvested on day 7. Results represent mean suppressive effect of the different Ig classes, backgrounds subtracted. PFC numbers/culture in groups containing 10^6 pre-cultivated cells without any pre-activating substance represent 0% suppression. Mean PFC value/culture in groups representing 0% suppression when all 19 groups were pooled were 33732 IgM PFC, 24337 IgG PFC and 20719 IgA PFC/culture.

TABLE 2

Suppression of proliferative responses measured by DNA synthesis [a]

Cell source	Female cells						Male cells					
Pre-act. subst.:	0		Con A		hCG		0		Con A		hCG	
Mitogen:	0	PPD	0	PPD	0	PPD	0	PPD	0	PPD	0	PPD
No. viable cells ($\times 10^{-6}$)	1,8	2,2	1,6	2,2	1,0	2,0	1,0	1,6	1,4	1,0	1,0	2,6
[^{3}H]dThd uptake (cpm/culture)	3694 ±81	62367 ±1636	1903 ±144	9267 ±145	180 ±74	26220 ±433	8185 ±331	64955 ±2259	9440 ±316	12915 ±434	7263 ±46	76990 ±363
% Suppression		0		88		56		0		94		−22

[a] Peripheral human blood lymphocytes were cultured as described in Table 1. Immediately after harvesting of the cells subsequently to be tested for PFC, 0.2 ml of the mixture were set up in triplicate microculture plates and [^{3}H]thymidine was added. The plates were harvested after 4 h. Results of one representative experiment are expressed as mean cpm of triplicate cultures (±S.E.).

Pure mannitol, when added in doses equimolar to those present in Gonadex®, failed to induce any suppressive effect. No sex difference in suppressive capacity could be detected when Con A was utilized as inducing agent (Table 1). Suppression of proliferative responses, as measured by DNA synthesis, also exhibited a similar sex dependency and parallelled that of the differentiative response, as measured by immunoglobulin production (Table 2). Cytotoxicity did not seem to be the cause of suppression either in the hCG or the Con A system since the number of viable cells was the same in suppressed and control cultures (Table 2). Freeze-killed cells mixed with hCG did not diminish the PFC response of secondary cultures, indicating the formation of true suppressor cells and not merely a transfer of hCG to the secondary cultures. This was also demonstrated by experiments showing that the induction of suppression was a time-dependent phenomenon, female cells requiring more than 24 h of cultivation with hCG before suppression could be demonstrated (data not shown).

In order to establish whether male lymphocytes fail to be induced by hCG or fail to respond to hCG-induced suppressive cells, HLA-identical (A, B, C and DR) sisters and brothers were tested. Preliminary data indicate that male lymphocytes can neither be induced by hCG to form suppressor cells nor respond to suppressor cells.

Discussion

The present results indicate that hormonal differences between males and females are not the cause of the sex-dependent hCG induction of suppressor cells, since the suppressive effect is similar with cells taken from females before menarche, after the menopause or from a Turner patient without hormonal treatment, as compared to women in their fertile period. It would appear that a gene(s) on the Y chromosome exerts a blocking or regulatory function and thus prevents the hormone from inducing suppressor T cells. One possible mechanism would be regulation of the expression of cell surface hCG receptors on male and female lymphocytes. So far, however, no hCG receptors have been found on human lymphocytes (Siebers et al., 1978), but only on the 'target' organs, that is ovaries and testes (Catt et al., 1974). Siebers et al. (1978), however, tested male lymphocytes only and it is not yet established whether female lymphocytes possess hCG binding receptors. Even if they do, the number of cell surface binding sites does not necessarily parallel the biological effects induced by hormones (Fauci et al., 1980). Thus, although female lymphocytes may be endowed with binding sites for hCG and suppression may be conveyed via these receptors, other mechanisms are equally plausible and similar findings of discrepancies in immunological reactivity between the sexes in normal subjects (non-pregnant females) have been reported previously (see Eidinger and Garret, 1972).

The possible clinical relevance for these in vitro findings remains to be determined. However, it is tempting to speculate that a defect in the system for the induction of suppressor cells by hCG could be the underlying cause of certain cases

of habitual spontaneous abortions. Suppressor cells have been described in a vast number of experimental systems and have been proposed to play an important role in the regulation of the immune response (Möller, 1975). In this report we have presented data indicating that genes in the Y chromosome may influence the induction of suppression and this is to our knowledge the first demonstration of a sex-dependent suppressor cell phenomenon and, as such, may contribute to an understanding of the function of the immune system.

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