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The Effects of Crude and Purified Human Gonadotropin on *in Vitro* Stimulated Human Lymphocyte Cultures[1]

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Crude human chorionic gonadotropin (hCG) was found to be several fold more immunosuppressive than purified hCG in human peripheral blood lymphocyte cultures stimulated by phytohemagglutinin, pokeweed, purified protein derivative and allogeneic cells *in vitro*. Immunosuppression by crude hCG was consistently noted at levels less than 1000 IU/ml and usually 80% inhibition was achieved with doses of 5000–10,000 IU/ml, whereas 40–50% inhibition or less was observed by purified hCG at 10,000 IU/ml. In two crude hCG preparations subjected to Sephadex G-100 chromatography, the fractions that inhibited lymphocyte cultures appeared in the eluate after the major peak of hCG activity. These data indicate that inhibitory substance(s) other than hCG are responsible for most of the immunosuppressive properties of first trimester pregnancy urine. Both crude and purified hCG were stimulatory to human lymphocytes when used alone without mitogens when cultured in fetal calf serum.

INTRODUCTION

The factors that protect the implanted, fertilized ovum from immunologic attack are not well understood, but suppression of the maternal immune response has been implicated. Clinical observations in support of this hypothesis include a weakened host defense during pregnancy to viral infections such as hepatitis (1), influenza (2), and poliomyelitis (3, 4). A decreased incidence of positive delayed hypersensitivity skin tests (5) as well as prolonged skin allograft survival have been reported (6, 7). The lymph nodes and thymus during pregnancy are atrophic (8, 9), and *in vitro* assays of cell mediated immunity have implicated both plasma (10–13) and cellular defects (14, 15).

Recently, evidence that human chorionic gonadotropin (hCG) is immunosuppressive was suggested by the finding that material extracted from pregnancy urine inhibited phytohemagglutinin (16–18) and antigen induced lymphocyte stimulation (19, 20) as well as mixed lymphocyte reactivity (20, 21). However, these studies employed only partially purified preparations of hCG. In an effort to define the structural elements necessary for these phenomena a study of the immunosuppressive effects of highly purified hCG and its subunits was initiated. While the results confirmed that partially purified hCG markedly suppressed the response of

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peripheral blood lymphocytes (PBL) to phytohemagglutinin, purified hCG preparations were much less inhibitory than the crude [2] preparations suggesting that molecules other than hCG were responsible for most of the observed immunosuppressive effect (22). Recently, Caldwell *et al.*, employing some of the same reagents used here have found similar results (23).

The present report describes studies in which the effects of crude and purified hCG have been compared in the response of PBL to other nonspecific mitogens, to specific antigens (PPD) and to allogeneic cells (mixed lymphocyte reaction). In each instance the crude preparations of hCG were more inhibitory than the purified hormone and the major inhibitory factor or factors in crude hCG have been found to be distinguishable from the native hormone following gel filtration. It is also of interest that both the crude and the purified hCG preparations, in the absence of mitogens, stimulated ^{3}H-thymidine incorporation several-fold above background.

MATERIALS AND METHODS

Lymphocyte Culture Methods

Short term lymphocyte cultures were prepared by a modification of the method of Boyum (24). Forty to fifty milliliters of blood were obtained from normal individuals in phenol-free heparin and allowed to sediment at 37°C for 1–2 hours. The plasma was expressed from the top of the syringe and gently mixed with an equal volume of sterile isotonic saline. Two volumes of the saline-plasma solution were layered over a third volume of Ficoll-Conray solution (10 ml 33.4% Conray + 24 ml 9% Ficoll) and centrifuged for 30 min at 4°C (Ficoll was from Pharmacia Fine Chemicals, Piscataway, N.J. and 60% Conray was from Mallinckrodt Pharmaceuticals, St. Louis, Mo.). The buffy coat was removed and washed three times with minimal essential medium (MEM) and containing 50 units/ml penicillin, 50 μg/ml streptomycin, 2 m*M* L-glutamine and the cells cultured in this medium with either 15% fetal calf serum (Grand Island Biological Co., Grand Island, N.Y.), pooled human (AB) plasma or autologous plasma. Unless otherwise specified, the serum or plasma was heated to 56°C for 30 min prior to use.

A few initial experiments were performed using the lymphocyte culture method of Valentine (25), but the majority of experiments used microtiter plates (Linbro IS FB 96, Linbro Chemicals Co., New Haven, Conn.). Triplicate cultures of 0.25 ml containing 1 or 2 $\times$ 10^5 lymphocytes per well were incubated at 37°C in 5% CO_2: 95% air for 5 days and incubated overnight with 1 μCi/well of ^{3}H-thymidine, 6.7 Ci/m mole (New England Nuclear Corp., Boston, Mass.). The microtiter cultures were harvested and washed extensively with saline on glass fiber filters (grade 934 AH, Reeve Angel, Clifton, N.J.) using a Mash II Harvester (26). The filters were dried and placed in 12 ml of Instabray (Yorktown Research, Hackensack, N.J.). ^{3}H content was determined in a liquid scintillation spectrometer (Packard model 3320). The results were expressed as counts per minute (cpm) of ^{3}H-thymidine per culture or as percentage of inhibition of thymidine incorporation of the mitogen control.

[2] The term "crude hCG" is used here to designate commercially available extracts from first trimester pregnancy urine. HCG comprises only approximately 25% of the material in these preparations.

Mitogens

Dose response curves with all mitogens, antigens and allogeneic lymphocytes were performed several times prior to studies with hCG. The cultures contained either 0.001 or 0.005 ml stock/ml PHA-P (Difco Laboratories, Detroit, Mich.) or 1000, 2500 or 6000 μg/ml pokeweed (PWM) (Grand Island Biological Co., Grand Island, N.Y.). Preservative free tuberculin purified protein derivative (PPD) (Connaught Laboratories Ltd., Willowdale, Ontario, Canada) was used in 1 μg and 10 μg/ml concentrations with leukocyte cultures obtained from tuberculin positive individuals. One-way mixed lymphocyte cultures (MLR) were prepared by incubation of the stimulating lymphocytes at 2×10^6 cells/ml for 1 hr at 37°C with 25 μg/ml mitomycin C (Sigma Chemicals Co., St. Louis, Mo.) followed by washing three times with large volumes of MEM. Dose response cultures were done with unrelated donors and usually the most stimulatory ratios of stimulator to responder cell were selected for use with hCG i.e., 1.6×10^6 of mitomycin-C treated lymphocytes to 8×10^5 responder lymphocytes. Trypan blue dye viability studies showed about 82–88% dye exclusion occurred in most experiments.

hCG Preparations

Crude preparations of hCG (purchased from Organon, West Orange, N.J.) were batches #91685, #91930, #91843, #92145 and #92666, for which the estimated potencies were 2900 IU/mg, 3900 IU/mg, 3810 IU/mg, 2700 IU/mg and 2680 IU/mg respectively. The bioassay results, as well as the subsequent yields of purified hormone, indicate that approximately 75% of the bulk material in these crude preparations was not hCG. The purified hCG employed in these studies was prepared from several of these batches and the methods of purification have been described previously (27, 28). Each batch of purified hCG appeared to contain less than 5% contamination with other material, and biological potency estimates, obtained by the rat ventral prostate weight bioassay (29), ranged from 8600 IU/mg to 13,450 IU/mg. It is known that the biological potency of different preparations of purified hCG can vary significantly as a function of sialic acid content (30), and this range of bioassay results is consistent with other chemical data indicating that the preparations were greater than 95% hCG. The purified hCG preparations described here were CR116, prepared from batch #91843, CR118 and CR119 prepared from batch #91930, and CR121 prepared from batch #92145. The hCG alpha and beta subunits were purified as previously described and had essentially no biological activity (31, 32). In each experiment the hCG preparation was millipore filtered prior to use.

RESULTS

Initial experiments were designed to study the inhibitory effects of crude and purified human chorionic gonadotropin on the stimulation of human peripheral blood lymphocytes by phytohemagglutinin-P (PHA-P). Figure 1 shows the results of a representative experiment comparing the inhibition of PHA stimulated lymphocytes by both hormone preparations. The concentrations of hCG are expressed in IU/ml. Note that at concentrations of 11,600 IU/ml (1 mg/ml) the purified hormone gave 23% inhibition of PHA stimulation compared with 85% inhibition by the crude material at 3900 IU/ml (1 mg/ml). Despite biologic

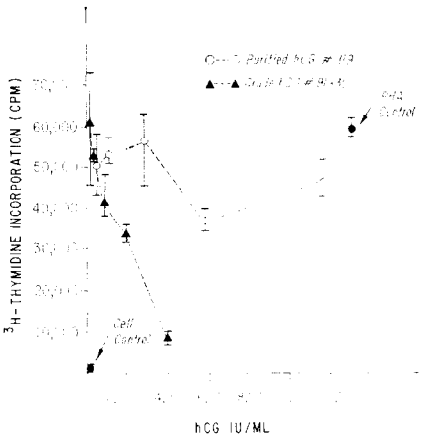

FIG. 1. Comparison of the inhibitory effect of two preparations of hCG containing equal quantities of hormone on human PBL stimulated by PHA-P. Purified hCG #119 was prepared from crude hCG #91930 and the concentrations are expressed in IU/ml. The 0.25 ml microtiter cultures contained 1×10^5 lymphocytes with 15% heated AB plasma. PHA-P was used at a concentration of 0.005 ml per ml of culture.

variability from experiment to experiment, comparable doses of the crude hormone preparation were consistently more effective than the purified hormone in inhibiting the response of PBL to PHA. Occasionally, the purified hormone showed up to 20–40% inhibition at very high concentrations of 5000–10,000 IU/ml, without significant inhibition at lower concentrations, and occasionally, the crude hormone preparations gave as much as 45% inhibition at concentrations as low as 100 IU/ml.

It should be noted that if the hCG concentration in these experiments was based upon bioassays of hormone activity, then all the results indicate that hormonal specific activity increased during purification while immunological suppressive activity decreased.

When both crude and purified hCG were added alone in the absence of mitogen, the incorporation of ^{3}H-thymidine by PBL was increased. Table 1 summarizes several experiments to illustrate this point. Using three different lots of crude and two different lots of purified hCG, the stimulatory effects of both types of preparations in fetal calf serum were equivalent. The extent of ^{3}H-thymidine incorporation was 2–3 times the control values at 100 IU/ml, 2–4 times at 1000 IU/ml and 3–6 times greater at 10,000 IU/ml. Increased ^{3}H-thymidine incorporation was not observed when lymphocytes were cultured with crude or purified hCG in autologous, AB or cord plasmas.

To demonstrate that the suppressive effect was not due to binding to the lectin by the crude hCG, which is a glycoprotein, the inhibitory effect was tested utilizing another nonspecific mitogen. Pokeweed (PWM) mitogen was chosen for study since it is a mitogen stimulatory for both T and B lymphocytes. At PWM concentrations of 1000, 2500 and 6000 μg/ml, crude hCG was again more inhibitory than the purified hormone. Figure 2 shows a representative experiment plotted in IU/ml. Crude hCG was usually inhibitory at 100 IU/ml and showed 80% or complete inhibition by 2000–4000 IU/ml, whereas purified hCG required concentrations of 6000 IU/ml or above for 50% inhibition.

TABLE 1

Effect of Crude and Purified hCG on Unstimulated Peripheral Blood Lymphocytes[a]

Type of hCG preparation	^{3}H-thymidine incorporation (cpm)			
	hCG concentration (IU/ml)			
	0	100	1,000	10,000
Crude				
#91685	4,186 ± 201	—	12,170 ± 1,800	16,776 ± 816
#91930	2,045 ± 140	7,053 ± 354	7,181 ± 144	11,828 ± 291
#91843	2,276 ± 212	—	7,798 ± 1,703	8,687 ± 1,630
Purified				
CR-118	5,440 ± 767	9,723 ± 307	—	15,523 ± 2,331
CR-118	2,101 ± 129	6,936 ± 342	7,726 ± 590	12,427 ± 1,471
CR-116	1,640 ± 99	2,903 ± 699	5,953 ± 1,000	7,594 ± 1,767

[a] Each culture contained 2 × 10^6 lymphocytes in 2 ml of culture medium with 15% fetal calf serum and was performed in triplicate according to the method of Valentine (25). Results are expressed as the arithmetic mean ± SE of counts per minute of ^{3}H-thymidine incorporation per culture.

Tuberculin positive individuals served as donors to compare the inhibitory capacity of the hCG preparations on PPD stimulated lymphocytes. A representative experiment is depicted in Fig. 3 comparing the inhibition in IU/ml. Although PPD stimulation was performed at both 1 and 10 μg/ml, the results were similar. Crude hCG showed inhibition at 100 IU/ml and 70% inhibition at 1000 IU/ml whereas equivalent inhibition (85%) required greater than 12,000 IU/ml of the purified hormone.

The last mode of lymphocyte stimulation to be evaluated was that initiated by allogeneic lymphocytes (MLR). In three separate experiments the crude preparation was more inhibitory than the purified. Figure 4 illustrates a representative experiment comparing the relative inhibition achieved by both preparations in IU/ml. Again, inhibition by crude hCG was noted with concentrations as low as 100 IU/ml and only at significantly higher concentrations, i.e., 14,000 IU/ml, did

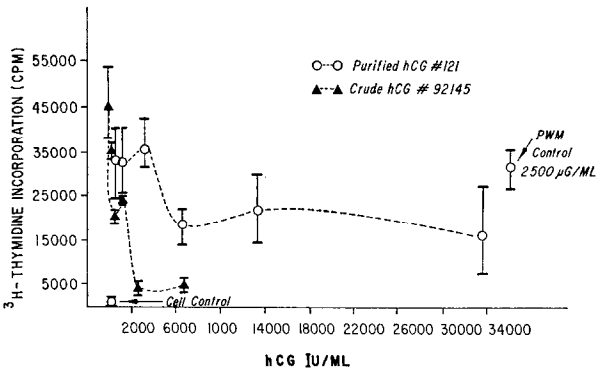

FIG. 2. Comparison of inhibitory effects of crude hCG #92145 and purified hCG #121 on PWM stimulated PBL expressed in IU/ml. The microtiter cultures contained 1 × 10^5 lymphocytes with 15% heated AB plasma and 2500 μg/ml of PWM.

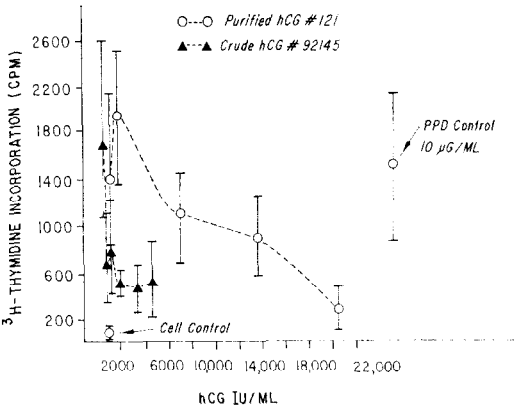

FIG. 3. Comparison of the inhibitory effects of crude hCG #92145 purified hCG #121 on PPD stimulated PBL expressed in IU/ml. The microtiter cultures contained 1×10^5 lymphocytes with 15% unheated AB plasma and 10 μg/ml of PPD.

the purified hormone produce inhibition. Despite the lower degree of stimulation achieved by mitomycin treated allogeneic lymphocytes, concentrations as high as 1500 μg/ml of both preparations (5000 IU/ml of crude and 20,000 IU/ml of purified hormone) did not completely inhibit the MLR response.

Although not depicted here, the inhibitory capacity of α and β subunits of hCG, and asialo-hCG were assayed in the PHA ad MLR systems. In the MLR the β subunit exhibited slight inhibition at concentrations of 10,000 IU/ml compared to the purified parent hormone or its α subunit. Both subunits showed 30% inhibition of PHA stimulated lymphocytes at concentrations of 10,000 IU/ml. The asialo-hCG produced no inhibition of stimulated lymphocytes at concentrations of 100–10,000 IU/ml.

In all of the results described above crude hCG preparations demonstrated significantly greater inhibitory activity in the mitogen, antigen and allogeneic lymphocyte stimulated cultures when compared with those containing comparable

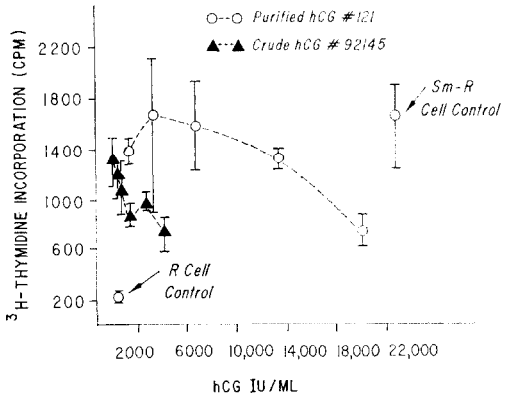

FIG. 4. Comparison of inhibition of crude hCG #92145 and purified hCG #121 on PBL stimulated by mitomycin C treated allogeneic lymphocytes. Each microtiter plate culture contained 1×10^5 stimulator (Sm) and 1×10^5 recipient (R) lymphocytes in 15% heated AB plasma.

biological activity of the purified hormone. On the basis of these results, it seemed likely that the principal immunosuppressive factors that were so active in crude hCG had been lost during purification of the hormone. In an effort to confirm that crude hCG contained immunosuppressive factors other than hCG, two separate batches of crude hCG were subjected to gel filtration through Sephadex G-100 i.e., an earlier SP-Sephadex adsorption step and subsequent dialysis was eliminated (*27*). Pooled fractions of the eluate were tested for immunosuppressive activity (Fig. 5). In both experiments the principal inhibitory activity of PHA stimulation was eluted later than the peak of hCG activity. Pools 1 through 3, shown in Fig. 5, contained the highest protein concentrations and over 90% of the hCG activity with bioassays yielding more than 10,000 IU/mg in each fraction. Since hCG is a glycoprotein, the hormone elutes with molecules in the range of 70,000 daltons rather than in the region of its actual molecular weight, which is 37,000 daltons (*22, 27*). Pools 4 through 6, which eluted later than hCG, appeared to contain the highest concentrations of inhibitory activity of PHA-stimulated lymphocytes, and the hCG biological activity was 1840, 25 and 9 IU/mg respectively. Thus the immunosuppressive activity is maximal in a portion of the eluate that exhibits rapidly declining hCG activity. Molecular weight markers were chromatographed on the same column indicating that this immunosuppressive activity elutes in the molecular weight range of 20,000–40,000 daltons. In another experiment, similar to that shown in Fig. 5 but employing batch #92145, a small molecular weight component of immunosuppressive activity also appeared in addition to that which is seen in Fig. 5. The presence of a small molecular weight component in some hCG crude material is consistent with the observation of Muchmore and Blaese

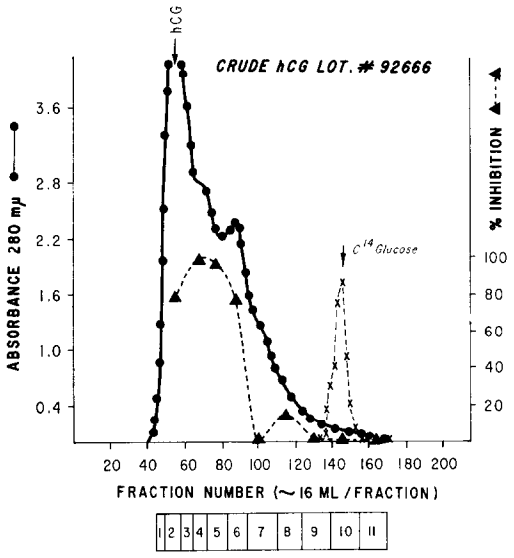

FIG. 5. Sephadex G-100 chromatography of crude hCG #92666 in 0.15 *M* NaCl, 0.02 *M* Tris buffer, pH 7.4. The fractions shown on the abscissa were combined to form pools 1 through 11 and equalized to the same optical density. The degree of inhibition of PHA stimulated lymphocytes by aliquots of these pools is plotted on the right ordinate. Since hCG is a glycoprotein, the hormone has a large Stoke's radius in relation to its molecular weight. Under the conditions employed for this column, the hormone elutes with a Ve/Vo of 1.22 (Ref. 27) and this position is shown by the arrow at the top.

that immunosuppressive activity can appear in the dialysate of crude hCG preparations (33).

DISCUSSION

The data indicate that purified hCG has little inhibitory activity on mitogen, PPD, and MLR induced lymphocyte transformation except at very high concentrations. The concentration of purified hCG required to demonstrate any significant inhibition, ranged from 2000 IU/ml to greater than 10,000 IU/ml, depending on the type of lymphocyte stimulation used. Multiple preparations of crude hCG exhibited much greater inhibitory capacity in these same systems. The inhibition by crude hCG usually appeared at concentrations of the equivalent of 100 IU/ml of hCG or less, but was always manifest at the higher concentrations of 1000 and 10,000 IU/ml. These differences were apparent regardless of the lot numbers of the hCG preparations, the sex and age of the donor cells, and irrespective of the protein in the culture medium, i.e., fetal calf, AB or autologous plasma.

The alpha and beta subunits as well as asialo-hCG were not inhibitory to the mitogen and allogeneic cell stimulated lymphocytes. Therefore, experiments which were initiated to determine the structural elements of the purified hormone that were immunosuppressive have led to a search for factors other than hCG in the crude preparations that exhibit inhibitory activity. In initial experiments to separate these factors by Sephadex G-100 column chromatography, the major inhibitory activity eluted in a region of lower Stokes radius than hCG. This confirms that the principal inhibitory activity is not hCG and can be distinguished from the hormone.

This increased inhibition by crude hCG, compared with the purified hormone, of PHA stimulated lymphocytes had been reported by us previously (22) and also recently by Gundert *et al.* (34) and Caldwell *et al.* (23). Because the observed inhibition of PHA stimulation by crude hCG could be largely an interaction between a lectin and glycoprotein (35) and unrelated to an effect of crude hCG on lymphocytes as suggested by Powell (36) it was considered important to extend the documentation of inhibitory activity to other stimulated lymphocyte systems such as PWM, PPD and the MLR reaction. The fact that the crude preparation inhibited PPD and allogeneic cell stimulation made a lectin-glycoprotein interaction unlikely as the explanation of the effect.

Considerable conflict exists regarding the inhibitory role of hCG in the MLR reaction. Beling and Weksler (21) reported that purified hCG with a potency of 13,700 IU/mg inhibited in a range of 40–200 IU/ml. Han (20), using crude commercial hCG, found inhibition of cultures stimulated by varidase and allogeneic cells with concentrations as low as 10 IU/ml. However, in agreement with data reported here, Caldwell *et al.* (23) recently concluded that purified hCG had no inhibitory activity on the MLR. The most likely explanation for the conflicting data regarding the role of the purified hormone is that the preparations studied were of varying purity and contained some of the same inhibitory material found in crude preparations. In fact, Gundert *et al.* (34) using PHA stimulated lymphocytes found decreased inhibition as their hCG preparation increased in purity, and noted that the immunosuppressive activity did not adsorb to SP-Sephadex while hCG did.

The significance of the inhibitory activity achieved by high concentrations of purified hCG noted here is unexplained. It could be the result of an impurity, a

specific effect dependent upon high concentrations, or due to a nonspecific adsorption effect at the surface of lymphocyte so that the stimulating substance whether mitogen, antigen or allogeneic cell, could not reach its appropriate receptor. It is also possible that the purified hormone is more readily inactivated than the crude material under the 5 day culture conditions utilized here.

The lack of significant inhibitory activity by purified hCG on stimulated lymphocytes noted here and by others (23, 34, 37) suggests that a direct role for immunosuppression by the pure hormone during pregnancy is unlikely. The possibility of hCG working through proteins or factors such as the stimulation of estrogen synthesis still exists and has not been evaluated in this study. Furthermore, the exact concentration of the hormone at the placental site is unknown although the synthetic rate generally rises to approximately 10^5 IU/day during the first trimester with blood levels reaching as high as 10 IU/ml (22). Since only *in vitro* lymphocyte transformation was studied, the role of purified hCG on other immune parameters such as antibody formation, delayed hypersensitivity, lymphokine production, etc. would have to be ascertained both *in vivo* and *in vitro* before one could definitively state that it does not exert an immunosuppressive role during pregnancy.

The cell inhibited by crude hCG was probably the lymphocyte, although the method of isolation did not exclude participation by the monocyte. In fact, insulin receptors have recently been shown to be associated with monocytes rather than lymphocytes (38). No attempt was made to ascertain whether crude hCG inhibited isolated B or T lymphocytes, although the inhibition of lymphocytes stimulated by PPD and allogeneic cells suggested that crude hCG inhibited T cell function. PWM, both a T and B cell mitogen, has found successful use as a stimulator of immunoglobulin production *in vitro* using human lymphocytes. It would be important to compare the inhibition of crude and purified hCG in this model before making any conclusions regarding inhibition of B cell proliferation.

Although the nature of the inhibitory material in the crude hCG preparations is unknown, several immunosuppressive substances found in the plasma and urine during pregnancy should be considered. These substances include the other gestational hormones: i.e., estrogens, progesterone, and placental lactogen (chorionic somatomammotrophin), which are all elevated during pregnancy. Diethylstilbestrol has been shown to inhibit the incorporation of ^{3}H-thymidine by PHA stimulated human PBL (39), whereas other investigators failed to find inhibition of the MLR and mitogen stimulated lymphocytes using physiologic concentrations of estrogen, progesterone and hCG (37). Even though estrogen, progesterone and ACTH have all been implicated as immunosuppressive substances with various degrees of certainty, their molecular weights are too low to be in the inhibitory peak on the Sephadex G-100 column studied here unless aggregated or bound to heavier substances. Placental lactogen, whose MW is approximately 20,000, has been shown by Contractor and Davies (18) to inhibit PHA-stimulated lymphocytes. Whether this hormone or a similar substance has been concentrated sufficiently in the commercial crude hCG preparations to account for the inhibitory activity is unknown and presently under investigation. Another possibility is that hCG acts synergistically with another hormone or substance in the crude preparation to provide immunosuppressive activity.

There are several plasma proteins which are elevated or unique to pregnancy, which could play a role in immunosuppression, or at least inhibit stimulated lymphocyte cultures. These proteins include: 1) immunoglobulin with antilympho-

cyte activity (40), 2) pregnancy B_1-glycoprotein described by Bohn (41), 3) pregnancy associated α_2-macroglobulin (42) originally described by MacLaren *et al.* (43), also identical to pregnacy associated globulin (PAG) (44), 4) PAPP-A, another recently described pregnancy associated α-globulin with MW 750,000 supposedly different from PAG (45); as well as α-fetoprotein. There is also a third α_2-globulin, called pregnacy zone protein (46) which appears to be immunologically distinct from the other two α_2-globulins of pregnancy just described, namely PAG and PAPP-A (47).

The presence of antilymphocyte antibodies in the maternal circulation has been documented in some patients and recently certain commercial hCG preparations have been shown to be contaminated with immunoglobulins, not necessarily anti-lymphocyte antibodies, in sufficient quantity to provide anticomplementary activity great enough to convert C3, the third component of complement (48). These immunoglobulins or complexes would be eluted from Sephadex G-100 in a region of higher molecular weight than the inhibitory action in Figure 5. As yet, pregnancy specific β_1-glycoprotein and PAPP-A have not been tested in cell mediated immune systems, and controversy exists regarding the ability of PAG or pregnancy associated α_2-macroglobulin to inhibit the mitogenic responses of PPD and PHA stimulated lymphocytes (44). Mouse α-fetoprotein with a known MW of 49,000 (79,000 on SDS gels) has definitely been shown to inhibit lymphocyte transformation (49) and antibody information (50). In summary, the possibility that the inhibitory activity found in crude hCG is related to these various pregnancy correlated factors which show an influence in different tests for cell mediated immunity has to be considered but whether these factors or their fragments exist in pregnancy urine in a biologically active state is unknown.

An unexpected finding was that both crude and purified hCG were stimulatory by themselves in the presence of fetal calf serum. The biological significance of this observation is completely unknown and it may be that the preparations merely removed substances inhibitory to lymphocyte transformation such as α-fetoprotein from the fetal calf serum.

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REFERENCES

1. D'Crux, I. A., Balani, S. G., and Iyer, L. S., *Obst. & Gynec.* **31**, 449, 1968.
2. Hardy, J. M. B., Azarowica, E. N., Mannini, A., Medearis, D. N., Jr., and Cooke, R. E., *Am. J. Pub. Health* **51**, 1182, 1961.
3. Anderson, G. W., Anderson, G., Skaar, A., and Sandler, F., *Am. J. Hyg.* **55**, 127, 1952.
4. Siegel, M., and Greenberg, M., *New Eng. J. Med.* **253**, 841, 1955.
5. Finn, R., St. Hill, C. A., Govan, A. J., Ralfs, I. G., Gurney, F. J., and Denye, V., *Brit. Med. J.* **3**, 150, 1972.
6. Andresen, R. H., and Monroe, C. W., *Am. J. Obs. & Gynec.* **84**, 1096, 1962.
7. Heslop, R. W., Krohn, P. L., and Sparrow, E. M., *J. of Endocrin.* **10**, 325, 1954.
8. Nelson, J. H., Jr., *Clin. Obst. & Gynec.* **8**, 263, 1965.
9. Pepper, F. J., *J. Endocrin.* **22**, 335, 1961.
10. Leiken, S., *In* "Proceedings Sixth Leucocyte Culture Conference" (M. R. Schwarz, Ed.) pp. 725–742. Academic Press, New York, 1972.
11. Leventhal, B. G., Buell, D. N., Yankee, R., and Rogentine, C. N., Jr., *In* "Proceedings Fifth Leucocyte Culture Conference" (J. E. Harris, Ed.), pp. 473–486. Academic Press, New York, 1970.

12. Kasakura, S., *J. Immunol.* **107**, 1296, 1971.
13. St. Hill, C. A., Finn, R., and Denye, V., *Brit. Med. J.* **3**, 513, 1973.
14. Smith, J. K., Caspary, E. A., and Field, E. J., *Lancet* **1**, 96, 1972.
15. Purtilo, D. T., Hallgren, H. M., and Yunis, E. J., *Lancet* **1**, 769, 1972.
16. Kaye, M. D., and Jones, W. R., *Amer. J. Obstet. Gynec.* **109**, 1029, 1971.
17. Adock, E. W., III, Teasdale, F., August, C. S., Cox, S., Meschia, G., Battaglia, F. C., and Naughton, M. A., *Science* **181**, 845, 1973.
18. Contractor, S. F., and Davies, H., *Nature* **243**, 284, 1973.
19. Muchmore, A. V., and Blaese, R. M., *Fed. Proc.* **33**, 750, 1974.
20. Han, T., *Clin. Exp. Immunol.* **18**, 529, 1974.
21. Beling, C. G., and Weksler, M. E., *Clin. Exp. Immunol.* **18**, 537, 1974.
22. Canfield, R. E., Birken, S., Morse, J. H., and Morgan, F. J., *In* "Proceedings of the Symposia on Drug Action-Peptide Hormones" (J. Parsons, Ed.), pp. 299–315. Macmillan and Co., London, 1976.
23. Caldwell, J. L., Stites, D. P., and Fudenberg, H. H., *J. Immunol.* **115**, 1249, 1975.
24. Boyum, A., *Scand. J. Clin. Invest.* **21**, (Suppl. 97) 77, 1968.
25. Valentine, F., *In "In Vitro* Methods in Cell Mediated Immunity" (B. R. Bloom and P. R. Glade, Eds.), pp. 443–454. Academic Press, New York, 1971.
26. Harrison, M. R., Thurman, G. B., and Thomas, G. M., *J. Immunol. Methods* **4**, 11, 1974.
27. Canfield, R. E., Morgan, F. J., Kammerman, S., Bell, J. J., and Agosto, G. M., *Rec. Prog. Horm. Res.* **27**, 121, 1971.
28. Canfield, R. E., and Morgan, F. J., *In* "Methods of Investigative and Diagnostic Endocrinology" (S. A. Berson and Yalow, R. S., Eds.), pp. 727–733. North-Holland Pub. Co., Amsterdam, 1973.
29. McArthur, J. W., *Endrocrinology* **50**, 304, 1952.
30. Van Hell, H., Goverde, B. C., Schuurs, A. H. W. M., DeJager, E., Matthijsen, R., and Homan, J. D. H., *Nature* **212**, 261, 1966.
31. Morgan, F. J., Canfield, R. E., Vaitukaitis, J. L., and Ross, G. T., *In* "Methods in Investigative and Diagnostic Endocrinology" (S. A. Berson and Yalow, R. S., Eds.), pp. 733–742. North-Holland Pub. Co., Amsterdam, 1973.
32. Morgan, F. J., Canfield, R. E., Vaitukaitis, J. L., and Ross, G. T., *Endocrinology* **94**, 1601, 1974.
33. Personal communication.
34. Gundert, D., Merz, W. E., Hilgenfeldt, U., and Brossmer, R., *FEBS Letters* **53**, 309, 1975.
35. Morse, J. H., *Immunology* **14**, 713, 1968.
36. Powell, A. E., *Science* **184**, 913, 1974.
37. Schiff, R. I., Mercier, D., and Buckley, R. H., *Fed. Proc.* **33**, 749, 1974.
38. Schwartz, R. H., Bianco, A. R., Handwerger, B. S., and Kahn, C. R., *Proc. Nat. Acad. Sci.* **72**, 474, 1975.
39. Ablin, R. U., Bruns, G. R., Guinan, P., and Bush, I. M., *J. of Immunol.* **113**, 705, 1974.
40. Revillard, J. P., Robert, M., Betnel, H, Lajour, M., Bonneau, M., Brochier, J., and Traeger, J., *Trans. Proc.* **4**, 173, 1972.
41. Bohn, H., *Arch. Gynäk.* **210**, 440, 1972.
42. Stimson, W. H., and Eubank-Scott, L., *FEBS Letters* **23**, 298, 1972.
43. MacLaren, J. A., Reid, D. E., Konugres, A. A., and Allen, F. H., Jr., *Vox Sang.* **11**, 553, 1966.
44. Horne, C. H. W., McLay, A. L. C., Tavadia, H. B., Carmichael, I., Mallinson, A. C., Yeung Laiwah, A. A. C., Thomas, M. A., and MacSween, R. N. M., *Clin. Exp. Immunol.* **13**, 603, 1973.
45. Lin, T. M., Halbert, S. P., Kiefer, D., and Spellacy, W. N., *Int. Arch. Allergy & Appl. Immunol.* **47**, 35, 1974.
46. Schlontz, B., von Stigbrand, T., and Tärnvik, A., *FEBS Letters* **38**, 23, 1973.
47. Lin, T. M., and Halbert, S. P., *Inter. Arch. Allergy & Appl. Immunol.* **48**, 101, 1975.
48. Loke, Y. W., and Pepys, M. R., *Am. J. Obs. & Gynec.* **121**, 37, 1975.
49. Murgita, R. A., and Thomasi, T. B., *J Exp. Med.* **141**, 440, 1975.
50. Murgita, R. A., and Tomasi, T. B., Jr., *J. Exp Med.* **141**, 269, 1975.