J.Biosci., Vol. 4, Number l, March 1982, pp. 105-114. © Printed in India.

# propertie s and characteristics of an anti-human Chorionic gonadotropin monoclonal antibody

S. K. GUPTA, S. RAMAKRISHNAN and G. P. TALWAR

Department of Biochemistry, All India Institute of Medical Sciences, New Delhi 110 029

MS received 17 August 1981

**Abstract.** The product of a hybrid cell clone, $P_3W_{80}$, obtained as ascites fluid from mouse peritoneal cavity had high titres of anti-human chorionic gonadotropin antibodies e.g. 30 to 40% binding of $^{125}$I-human chorionic gonadotropin at $10^7$ dilution in a radioimmunoassay. The antiserum $SB_6$ (raised against ß-human chorionic gonadotropin distributed by National Institutes of Health, USA gave similar binding at 5000 dilution in parallel runs. The monoclonal antibody recognized best human chorionic gonadotropin (0.3 mIU of hormone/tube with $B/B_0 < 75\%$), but also bound ß and α subunits of human chorionic gonadotropin, 12 and 800 folds lower than human chorionic gonadotropin respectively. No binding was observed with carboxy terminal peptides of β-human chorionic gonadotropin ranging from 93 to 145 amino acid residues, indicating the lack of recognition of the C-terminal region. No cross-reaction with human leutinizing hormone was obtained at the physiological surge levels, a significant competition ($B/B_0 < 75\%$, obtainable only at 60 mIU of LER 960 human leutinizing hormone/tube. The antibody had heavy chain of $IgG_1$ and light chain of kappa type. It neutralized the bio-activity of human chorionic gonadotropin both *in vitro* and *in vivo*.

**Keywords.** Human chorionic gonadotropin; monoclonal anti-hCG antibody; radioimmuno-assay; biological neutralization.

## Inroduction

itukaitis *et al.* (1972) obtained an antiserum by immunization of rabbits with beta unit of human chorionic gonadotropin (*ß*-hCG) which had a high specificity for man chorionic gonadotropin (hCG) and permitted the estimation of this mone in the presence of levels of hLH encountered in the serum. This anti-um, $SB_6$ has served as a valuable reagent for the estimation of hormone levels 1 for research on the hormone function (Goldstein *et al.,* 1974; Braunstein *et al.,* 6). Subsequently anti-sera were raised against synthetic carboxy-terminal tides of ß-hCG which were totally devoid of cross-reaction with human inizing hormone (hLH) (Chen *et al.,* 1976; Ramakrishnan *et al.,* 1979). vever, due to low association constant of the antibodies for hCG, the sensitivity

Abbreviations used: hCG; human chorionic gonadotropin; *ß* hCG, β subunit of hCG; hLH, human nizing hormone; hPRL, human prolactin; RIA, radioimmunoassay.

of their assay was one order lower for radioimmunoassay of HCG than $SB_6$ (Chen *et al.,* 1980). The anti-carboxy terminal peptides of ßhCG sera also failed to neutralize the bio-activity of hCG *in vivo* (Louvet *et al.,* 1974; Matsuura *et al.,* 1976).

We describe here the properties of a monoclonal antibody derived by hybridization of mouse myeloma cells with splenocytes obtained from mice immunized with highly purified hCG. This antibody resembles $SB_6$ in several of its characteristics and is obtainable in very high titres and unlimited amount. In contrast to the antibodies raised against carboxy terminal peptides of ßhCG, it neutralizes the bio-activity of hCG *in vivo.* This antibody, though highly specific for hCG does not bind with carboxy terminal peptides of ßhCG, which is suggestive of the presence within the core of hCG of epitopes unique to the hormone in immune-reactivity.

## Materials and methods

### *Hormones and peptides*

Human chorionic gonadotropin (hCG; 10,000 IU/mg) was made available by Dr. Tsong of the Population Council, New York, USA. Human luteinizing hormone (hLH; LER 960) was a generous gift from National Institutes of Health, Bethesda, Maryland, USA. Human prolactin (hPRL) was supplied by WHO under Quality Control Programme, β-subunit of hCG (CR-119) and α-subunit (CR-119) were made available by Drs S. Birken and R. E. Canfield of Columbia University, New York, USA. Carboxy-terminal peptides of ß-hCG; 93-145; 111-145 and 115-145 conforming to the sequence proposed by Morgan *et al.* (1975), were prepared by Dr. Karl Folkers of Texas University and made available through the International Committee for Contraception Research (ICCR) of the Population Council, New York, USA. Alkaline phosphatase-tagged goat anti-mouse $\mu$, $G_1$, $G_{2a}$, $G_{2b}$ $G_3$, K and λ chain-specific antibodies were obtained from Dr John Kearney, University of Alabama in Birmingham, Birmingham, Alabama, USA.

*Borate buffered saline* This was prepared by dissolving 6.185 g of boric acid, 9.54 g of sodium borate and 4.385 g of sodium chloride in 1 litre of double distilled water.

*Alkaline phosphatase substrate buffer* It was prepared by dissolving 2.45 mg $MgCl_2$ in 40 ml of distilled water containing 4.8 ml of diethanolamine (Sigma Chemicals, St. Louis, Missouri, USA). The pH of the solution was adjusted to 9.8 with 5N HCl and the volume made up to 50 ml with distilled water, *p* Aminophenyl-phosphate (Biochemical Unit, V. P. Chest Institute, Delhi, India) was dissolved in the ethanolamine buffer (1 mg/ml) fresh before use.

### *Monoclonal antibodies*

Hybridomas were prepared by fusion of spleen cells obtained from hCG immunized mice with P3-NSI/1-Ag4-1 (NSI) a non-secreting variant of P3-X63-Ag8 (cell line of Balb/c origin derived from MOPC-21) as described elsewhere (Gupta and Talwar, 1980); Hybrids positive for anti-hCG antibodies were cloned by limiting dilution technique. Out of 110 clones thus developed, 10 were studied for their binding to iodinated gonadotropins as described elsewhere (Gupta and

Talwar, 1980). One of the positive clones, namely, $P_3W_{80}$ was grown in the intraperitoneal cavity of the Pristane (Aldrich Chemical Co., Milwaukee, Wisconsin, USP) primed Balb/c mouse as ascites (Gupta and Talwar, 1980). Ascites fluid taped from intraperitoneal cavity was made cell-free by centrifugation at 800g for 15 min at 4°C Subsequently, it was heat-inactivated at 56°C for 30 min, centrifuged at 15,000 *g* to remove debris, diluted with an equal volume of 10 mM phosphate buffer, pH 7.4 and lyophilized in aliquots of 0.5 ml. It was reconstituted in distilled water and subsequent dilutions were made in 10 mM phosphate buffer pH 7.4 containing 0.1% sodium azide and 0.1% bovine serum albumin for radioimmunoassay. For biological neutralization studies, the dilutions were made in isotonic saline containing 0.1% bovine serum albumin.

*Specificity Studies:* The reactivity of the ascites fluid obtained from $P_3W_{80}$ clone of hybrid cells with various hormones was determined by competitive immunoassay. Iodination of hCG with carrier-free $Na^{125}I$ (Radio chemical Centre, Amersham, UK) was carried out by the method of Greenwood *et al.* (1963) as adopted by Vaitukaitis *et al.* (1972). The assay system contained 20 µl normal horse serum, 50 µl $^{125}I$-hCG (100 to 150 pg), 50 µI solution of different hormones at varying concentrations and 50 µl of $SB_6$ or $P_3W_{80}$ ascites fluid at the appropriate dilution to give 30 to 40% binding in absence of competition. Incubation of the assay mixture was carried out directly at 4°C for 18 to 20 h. The bound and free labelled hormone was separated by the addition of 1.0 ml of ammonium acetate-alcohol mixture as described by Salahuddin *et al.,* 1976.

*Neutralization of biological activity of hCG*

The mouse Lyedig cell bioassay system was used to investigate the ability of ascites fluid to neutralize the biological activity of hCG *in vitro.* To precipitate immunoglobulins, 0.2 ml of 30% polyethylene glycol solution was added to an equal volume of ascites fluid, vortexed and incubated overnight at 4°C. It was centrifuged at 1500 *g* for 15 min, supernatant discarded and the pellet was dissolved in 0.2 ml of phosphate buffer (pH 7.4). hCG (384 µIU) in 0.1 ml of phosphate buffer (pH 7.4) was preincubated with increasing dilutions of the precipitated immunoglobulins for 2 h at 37°C and subsequently at 4°C for 18 h. Leydig cell suspension was added to the preincubated hormone and the inhibition in the production of testosterone in presence of antibodies was estimated (Das *et al.,* 1978). The monoclonal antibodies were also tested for neutralization of the bioactivity of hCG *in vivo* by the mouse uterine weight gain assay. Prepubertal female mice of Balb/c strain, 20-21 days were given subcutaneously a total dose of 0.5 IU of hCG dissolved in 0.3 ml of isotonic saline with 0.1 % bovine serum albumin in three equally divided daily doses. Ascites fluid (0.1 ml) at the indicated dilutions was also given subcutaneously every day in test mice at a site different from hCG.

*Characterization of heavy and light chains of antibody secreted by $P_3W_{80}$ clone*

Alkaline phosphatase tagged goat anti-mouse µ, $G_1$, $G_{2a}$, $G_{2b}$, $G_3$, k and λ chain-specific antibodies were used in solid phase immunoassay to detect the chain specificity of the monoclonal antibodies. Essentially, the methodology described by Kerney *et al.* (1979) was followed except that polyvinyl microelisa plate (96 wells)

were coated with 100 μl of hCG solution (100 μg/ml in distilled water). At the end of the assay the reaction product of the wells were diluted with 0.3 ml of borate buffered saline and absorption of the reaction product was measured at 440nm using a Pye-Unicam Spectrophotometer SP8-100.

## Results

*Reactivity of monoclonal antibodies with hCG and its comparision with SB*

The mouse ascites fluid containing the product of $P_3W_{80}$ clone had a binding capacity of 30 to 40% of $^{125}$I-hCG at a dilution of $10^7$ in RIA. Using the same assay system, $SB_6$ gave 30-40% binding at 5000 dilution. hCG competed well with labelled hCG for binding to monoclonal antibody tested at $10^7$ dilution (figure 1).

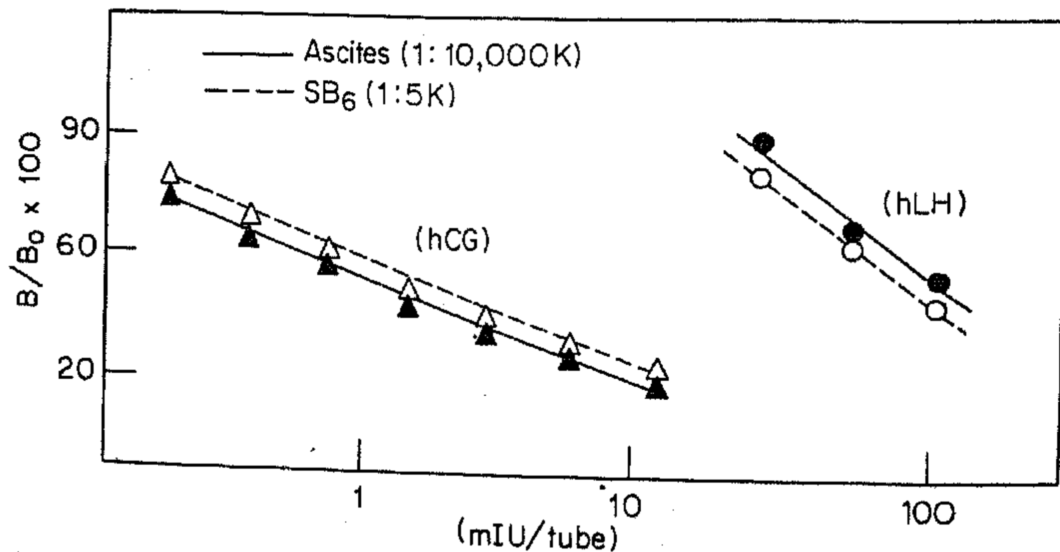


**Figure 1.** Comparative competitive inhibition profile of hCG and hLH.

The assay was done in a radioimmunoassay system using $^{125}$I-hCG as tracer with monoclonal anti-hCG antibodies (—; $P_3W_{80}$) or $SB_6$ serum (- - - -). $SB_6$ was used at $5\times10^3$ dilution in the assay. hCG ($P_3W_{80}$), (▲); hCG ($SB_6$), (Δ); hLH ($P_3W_{80}$), (●); hLH ($SB_6$), (O). B=radioactivity bound to the antibody in presence of labelled hCG and unlabelled hormone. $B_0$=radioactivity bound with labelled hCG alone.

As low as 0.3 mIU of hCG per tube gave a significant inhibition of $B/B_0$ (i.e. <75%). With $SB_6$ 0.45 mIU of hCG produced a similar inhibition. The slopes of $SB_6$ and monoclonal were by and large parallel. This monoclonal antibody was able to recognise hCG standards prepared either in male undiluted urine or serum (diluted 1:10 in 0.05 M phosphate buffer, pH 7.4 containing 0.1% bovine serum albumin and 0.1% sodium azide). The slopes and the amount of hCG required for $ED_{50}$ (i.e. $B/B_0$=0.5; B=radioactivity bound to the antibody in presence of labelled hCG and unlabelled hormone; $B_0$=radioactivity bound with labelled hCG alone.) for this antibody as compared to the standard NIH antiserum $SB_6$ are given in table 1. The amount of hCG required for $ED_{50}$ in urine (1.97 mIU/tube) was higher as compared to buffer (0.78 mIU/tube) and serum (0.93 mIU/tube). In all the three systems e.g. phosphate buffer, urine and serum, monoclonals were more efficient in assay of hCG as compared to $SB_6$. On a weight basis, the recognition capability of monoclonals was less (12 fold lower) for β-hCG as compared to the intact hCG and was still lower for α-hCG (800 fold) as compared to hCG (figure 2). $SB_6$

**Table 1.** Comparison of radioimmunoassay characteristics of $SB_6$ and the product of the clone $P_3W_{80}$

| Antiserum | RIA characteristics | | | | | |
|---|---|---|---|---|---|---|
| | Buffer | | Urine | | Serum | |
| | Slope (±S.D.) | $ED^*_{50}$ | Slope (±S.D.) | $ED^*_{50}$ | Slope (±S.D.) | $ED^*_{50}$ |
| $SB_6$ | −2.42±0.13 | 1.18 | −2.65±0.11 | 2.09 | −2.34±0.12 | 1.57 |
| $P_3W_{80}$ | −2.60±0.19 | 0.78 | −3.05±0.10 | 1.97 | −2.40±0.20 | 0.93 |

Competitive binding displacement by hCG of $^{125}$I-hCG binding to $SB_6$ and $P_3W_{80}$ ascites fluid was studied. Slope was calculated by the Linear Regression formula Y=mx+C where $Y=LogB/B_0$(B:cpm bound in the presence of $^{125}$I-hCG and unlabelled hCG; $B_0$: cpm bound in the presence of $^{125}$I-hCG alone) m=slope, x=Iog hormone cone, and C=intercept.

$ED^*_{50}$ is the amount of hCG (mlU/tube) required for $B/B_0=0.5$.

hCG standards were prepared in 0.05 M phosphate buffer, pH 7.4 containing 0.1% bovine serum albumin and 0.1% sodiumazide; undiluted urine and serum (diluted 1:10 in phosphate buffer) obtained from a healthy male.

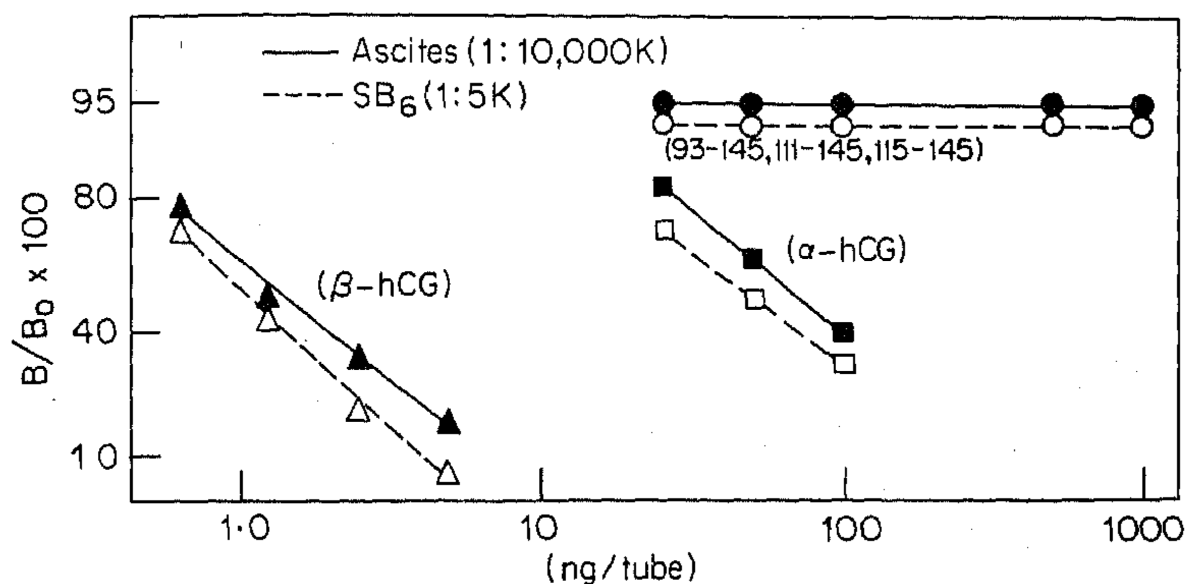


**Figure 2.** Radioactivity of monoclonal anti-hCG antibody ($P_3W_{80}$) ascites fluid and $SB_6$ with alpha and beta subunits of hCG and three carboxy terminal peptides of *β*-hCG of 93-145, 111-145 and 115-145 amino acid.

*β*-hCG($SB_6$), (Δ);α-hCG($SB_6$), (□);carboxy terminal peptides ($SB_6$), (O);ß-hCG ($P_3W_{80}$), (▲); α-hCG ($P_3W_{80}$); (■); carboxys terminal peptides ($P_3W_{80}$), (●)

showed a similar reactivity. None of the carboxy terminal peptides of *β*hCG (93-145,111-145,115-145) competed with $^{125}$I-hCG for binding sites to this monoclonal or $SB_6$ (figure 2). However, $SB_6$ at lower dilution (1:100) was shown to bind iodinated carboxy terminal peptides (93-145) which was not the case with monoclonale even when tested at 1:100 dilution (unpublished data). The slope of inhibition of $SB_6$ with *β*-hCG was also different from those of monoclonal (figure 2).

hLH at the maximum surge level (Shelly *et al,* 1973) concentration of 1.2 ng (5.04 mIU/tube) per assay tube did not compete for binding of labelled hCG with monoclonal. 60 mlU of hLH/tube was required to obtain a significant cross-reaction in this assay system ($B/B_o$ =70%) with monoclonals; the amount of hLH producing similar inhibition was 45 mlU with $SB_6$ (figure 1). The cross-reactivity of the antibody with hLH in the Leydig cell bioassay system is given in table 2. PRL was devoid of cross-reaction with monoclonal and $SB_6$ upto 10 mIU/ml tested. Due to non-availability of highly purified hFSH and hTSH (free of hLH), the cross-reaction of monoclonals with these hormones has not been tested.

**Table 2.** Effect of $P_3W_{80}$ antibody on hCG/hLH induced steroidogenesis by Leydig cell

| Dilution of $P_3W_{80}$ ascites fluid | Percent decrease in testosterone production | |
|---|---|---|
| | hCG | hLH |
| 1:100,000 | 83.6 | 5.7 |
| 1: 10,000 | 100 | 5.8 |
| 1: 1,000 | 100 | 7.3 |
| 1: 100 | 100 | 24.8 |

hCG (400 μIU), 2.5 ng of hLH (LER-960) dissolved in 100 μl of phosphate buffer (pH 7.4) were preincubated with 100 μl of the indicated dilution of the ascites fluid for 2 h at 37°C followed by 18 h at 4°C. Leydig cell suspension was added to the preincubated hormones and the testosterone produced was estimated by radioimmunoassay as described in Methods.

## *Biological neutralization studies*

The ability of monoclonal antibodies ($P_3W_{80}$) to block the biological activity of hCG was tested *in vitro* and *in vivo*. Figure 3 gives the amount of hCG neutralized

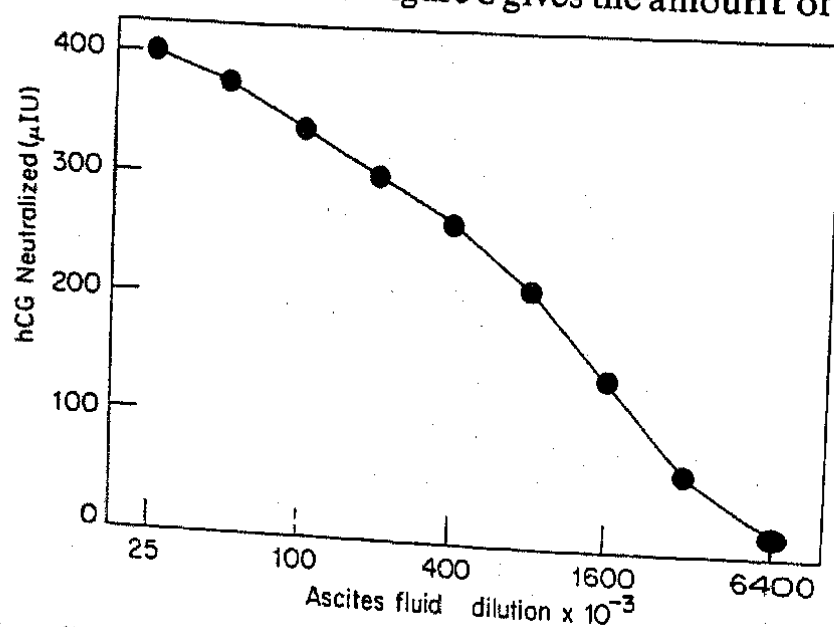


**Figure 3.** Neutralization of the biological activity of hCG as a function of dilution of monoclonal anti-hCG antibody ($P_3W_{80}$) ascites fluid in mouse Leydig cell bio-assay system. 384 μIU of hCG was used and each point represents a mean of quadruplicate determinations.

by various dilutions of ascites fluid in Leydig cell assay system. At 25,000 dilution, the ascites fluid neutralized the entire hCG (384 μIU) taken for the assay: 50% of the hormone was neutralizable at $8 \times 10^5$ dilution.

In the mouse uterine weight gain assay, immature mice injected with 0.5 IU of hCG alone showed about three fold increase (5.5 to 15.5 mg) in the wet weight of the uterus as compared to saline injected group. Ascites fluid (upto 1:1000 dilution) given with same quantity of hCG inhibited completely the hormone-induced increase of uterine weight (figure 4).

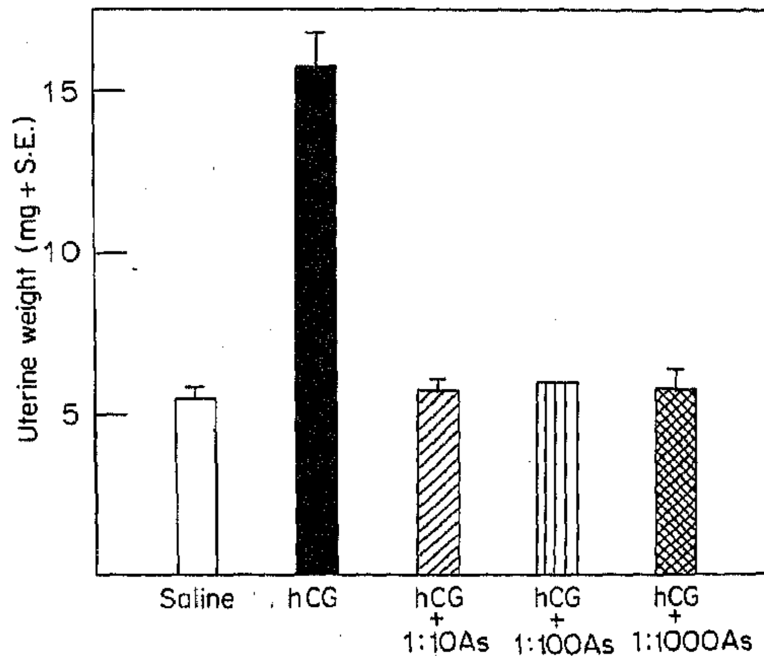


**Figure 4.** Effect of monoclonal anti-hCG antibody ($P_3W_{80}$) ascites fluid on hCG induced increase in mouse uterine weight. The bars represent mean values of 6 animals + S.E.

### *Characterization of heavy and light chains of the monoclonal antibody ($P_3W_{80}$)*

The culture fluid of the clone $P_3W_{80}$ as well as the ascites fluid developed in the mouse peritoneal cavity was tested for the light and heavy chain classes. Monospecific antisera against mouse μ, $G_1$ $G_{2a}$, $G_{2b}$, $G_3$, k and λ chains were utilized. The reactivity was assayed by enzyme linked immunoassay. The

**Table 3.** Enzyme linked assay for light and heavy chains in the product of the clone $P_3W_{80}$

| Test product | Test antiserum | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Blank | μ | $G_1$ | $G_{2a}$ | $G_{2b}$ | $G_3$ | K | λ |
| | Absorbance at 440 nM | | | | | | | |
| Culture fluid from NSI myeloma cells | — | 0.048 | 0.048 | 0.041 | 0.041 | 0.051 | 0.047 | 0.023 |
| $P_3W_{80}$ culture fluid | 0.11 | 0.18 | 2.64 | 0.14 | 0.08 | 0.095 | 1.395 | 0.055 |
| $P_3W_{80}$ mouse ascites fluid | 0.129 | 0.304 | 2.66 | 0.031 | 0.105 | 0.080 | 1.358 | 0.057 |

The assay was carried out as described in Methods.

product of the $P_3W_{80}$ clone in culture or from ascites fluid reacted only with anti-mouse $G_1$ and kappa serum as evidenced by absorbance at 440 nm which is shown in table 3. Other wells showed negligible absorbance. Culture fluid obtained from myeloma cells (NSI) gave negligible absorbance with all the tested antisera.

## Discussion

The antibody made by $P_3W_{80}$ clone is an $IgG_1$, k type; it can bind the complement and can bring about the antibody induced lysis of cells bearing hCG. This may be an interesting trait, as anti-hCG antibodies have been observed to exercise a complement-dependent cytotoxic action against choriocarcinoma cells *in vitro* (Currie, 1967; Talwar, 1980).

The antibodies are of neutralizing type. The antibodies abrogate the bio-activity of hCG both *in vitro* and *in vivo.* It may be recalled that antibodies generated against carboxy terminal peptides unique to ß-hCG fail to intercept the hCG action *in vivo* (Louvet *et al.,* 1974; Matsuura *et al.,* 1976). This may be partly due to the fact that antibodies generated by carboxy terminal peptides are comparatively of low affinity with $K_\alpha$ of $1.2\times10^9$ L/M (Chen *et al.,* 1980). It is, however, also possible that the epitopes against which the antibodies are directed has importance. The antibodies against carboxy terminal peptides read sequences located in a tetrapeptide and a dipeptide sequence, whereas the antibodies produced by this clone bind to other epitopes or conformation in the hCG/*β*-hCG molecules. These antibodies are devoid of recognition of carboxy terminal peptides of hCG. Leydig cell receptors do not respond to these peptides and the determinant(s) inducing biological effect of the hormone reside in core part of *β*-hCG and still better in the associated hCG molecule (Ramakrishnan *et al.,* 1978). Thus the binding characteristics of the monoclonal antibodies are of interest and amenable to applications. Preliminary studies in our laboratory demonstrate the ability of these antibodies to terminate pregnancy in mouse.

These antibodies just as other hybrid cell clone products can be obtained at extremely high titres. The present antibody binds 30 to 40% of $^{125}$I-hCG at $10^7$ dilution. The supply of these antibodies is abundant and theoretically unlimited. These can be used for radioimmunoassays and enzyme linked assays; the specificity is very similar to $SB_6$. These antibodies can also be used for neutralization of the bioactivity of hCG.

## Acknowledgements

This work was accomplished during the tenure of Jawaharlal Nehru Fellowship of G.P.T. We thank Lady Tata Memorial Trust for Fellowship support to S.K.G.

## References

Braunstein, G. D.,Rasor, J., Adler, D., Danzer, H. and Wade, M. E. (1976) *Am. J. Obstet. Gynecol.,* **126,** 678.

Chen H. C., Hodgen, G. D.,Matsuura, S.,Lin,L. J., Gross, E., Reichert (Jr.), L. E., Birken, S., Canfield, R. E., and Ross, G. T. (1976) *Proc. Natl. Acad. Sci. USA,* **73,** 2885

Chen H. C, Matsuura, S. and Ohashi, M. (1980) in *Chorionic gonadotropin* (ed. S.J. Segal) (New York: Plenum Press) p. 231.

Currie, G. A. (1967) *J. Obstet, Gynec,* **74,** 845.

as. C, Talwar, G. P., Ramakrishnan, S., Salahuddin, M., Kumar, S., Hingorani, V., Cutinho, E., Croxatto, H., Hemmingson, E., Johansson, E., Luukainean, T., Shahani, S., Sundaram, K., Nash, H. and Segal, S. J. (1978) *Contraception,* **18,** 35.

oldstein, D. P., Kosasa, T. S. and Skarim, A. T. (1974) *Surg. Gynecol. Obstet.,* **138,** 747.

reenwood, F. C, Hunter, W. M and Glover, J. S. (1963) *Biochem. J.,* **89,** 114.

upta, S. K. and Talwar, G. P. (1980) *Indian J. Exp. Biol.,* **18,** 1361.

earney, J. F., Radbruch, A., Liesegang, B. and Rajewsky, K. (1979) *J. Immunology,* **123,** 1548.

ouvet, J. P., Ross, G. T., Birken, S. and Canfield, R. E. (1974) *J. Clin. Endocrinol. Metab.,* **39,** 1155.

latsuura, S., Ohashi, M., Chen, H. C. and Hodgen, G. D. (1976) *Endocrinology,* **104,** 396.

lorgan, F. J., Birken, S. and Canfield, R. E. (1975) *J. Biol. Chem.,* **250,** 5247.

Ramakrishnan, S., Das, C. and Talwar, G. P. (1978) *Biochem. J.,* **176,** 599.

Ramakrishnan, S., Das, C, Dubey, S. K., Salahuddin, M. and Talwar, G. P. (1979) *J. Reprod. Immunol.,* **19,** 1.

alahuddin, M., Ramakrishnan, S., Dubey, S. K. and Talwar, G. P. (1976) *Contraception,* **13,** 163.

lelley, D. S., Brown, L. P. and Besch, P. K. (1973) *Clin. Chem.,* **19,** 146.

alwar, G. P. (1980) in *Immunology of contraception—A monograph in the series Topics in Immunology* (ed. J. Turk) (London: Edward Arnold), p. 114.

aitukaitis, J. L., Braunstein, G. D. and Ross, G. T. (1972). *Am. J. Obstet. Gynecol,* **113,** 751.