ASIAN PACIFIC JOURNAL OF ALLERGY AND IMMUNOLOGY (1998) 16: 127-135

# Characterization of Monoclonal Antibodies Recognizing α and β subunits of Human Chorionic Gonadotropin Hormone

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**SUMMARY** **Human chorionic gonadotropin (hCG) hormone is required for maintenance of early pregnancy and is a potential marker in the diagnosis and prognosis of both pregnancy and trophoblastic diseases. Murine hybridomas were generated against purified hCG. Seven hybrid clones secreting antibodies against hCG molecule with $IgG_1$/kappa subclass were established. The indirect ELISA result demonstrated that six MAbs (BEL-1 to BEL-6) recognized hCG in both holo and free β subunit form with negligible cross-reactivity against a closely related hormone, human luteinizing hormone (hLH). In this fusion, only one MAb (ALC-1) showed a cross-reaction with hLH, which designated an α subunit specific. The outcome of Western blot ascertained that ALC-1 recognized the conformational epitope on α subunit of hCG at *Mr* 23 kDa band in non-reducing condition (NR). In contrast, epitopes belonging to all MAbs recognized β subunit of hCG were in linear peptide structure at *Mr* 34 kDa band (NR) and *Mr* 26 kDa band (R). These six MAbs were further characterized by using two β subunit carboxy-terminal synthetic peptides (β109-119 and β109-145). Three of them (BEL-1, BEL-3, and BEL-4) recognized only epitope harboring in β109-145 fragment, the others recognized both types of the synthetic peptide. In order to select the most suitable MAbs specific to β subunit of hCG for exploiting with ALC-1 in the sandwich-type immunometric assay, competitive ELISA was employed. Six individual MAbs specific to β subunit of hCG were used to compete with biotinylated ALC-1 to evaluate the proximity of their epitopes on the holo form of hCG molecule. Of all six MAbs, BEL-5 depicted the lowest percent inhibition result, which indicated the bottom-most steric hindrance effect. Consequently, MAb BEL-5 will be the most appropriate antibody to utilize in concert with ALC-1 in place of devising a sandwich-type immunometric assay for measuring holo-hCG level.**

Human chorionic gonadotropin (hCG) is a hormone secreted from the placental syncytiotrophoblast of the embryo, and is required for maintenance of early pregnancy. The biochemically and immunologically closely-related hCG, luteinizing hormone (hLH), follicle stimulated hormone (FSH) and thyrotropin (TSH) are composed of two nonidentical glycosylated subunits linked by non-covalent bonds. Only the native intact (holo) hormone has biological activity.[1] Within a given species, the α subunit is common to all members, however, the β subunit is unique for each hormone. A high degree of sequence similarity of β subunit resides in the first 114 amino acids between hCG and the other hormones.[2] The hCG β subunit is distinguished among the β subunits due to the extra 31 amino acids of carboxy-terminal peptide (CTP). CTP is important for maintaining its longer *in vivo* half-life compared with other glycoprotein hormones.[3] The molecular basis for epitopes on both α and β subunits was elucidated by means of monoclonal antibodies (MAbs). Seven epitope and nine epitope clusters were represented on α and β sub-

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units accordingly.[4,5] The CTP of β subunit contributed to the formation of linear peptide epitopes $\beta_8$ (137-144) and $\beta_9$ (113-116). The others are conformationally determined epitopes localized on the core fragment of the β subunit. The hormone specific epitope, $\beta_1$ and $\beta_7$, also existed in this region. In 1994, Lapthorn[6] first demonstrated the three-dimensional crystal structure of hCG with the correct cystine knot and seat-belt characteristic of dimer. Success in identification of the three-dimensional structure initiated many robust efforts to identify gonadotropin residues that contact the receptor. Cosowsky *et al.*[7] designated the epitopes contacted with the LH receptor to be the proportion of β loops one and three near residue 74 on the outside hormone groove and parts of the CTP end of the seat-belt. The CTP region of the α subunit forms a part of the epitope indicating its proximity to the receptor-binding region.[8] Recently, Jackson *et al.*[9] identified the cross-reactive discontinuous residues presented on β subunit by random mutagenesis, which did not change the tertiary folding. The results suggested that residues 24, 25, 68 and 71 probably contribute to the $\beta_3$ cluster, residues 20, 21, 22, 75 and 77 to $\beta_6$ and residue 68 to clusters $\beta_2$, $\beta_4$ and $\beta_5$. The molecular topography of these epitopes was verified in term of synergistic antibody by Klonisch *et al.*[10,11] The combination of MAbs specific to hCG and MAb specific to CTP of β subunit showed the strongest enhancement in capturing hCG molecule. The flexibility of CTP region was contributed to explain this observation.

hCG is a potential marker in the diagnosis and prognosis of both pregnancy and trophoblastic diseases, such as choriocarcinoma, hydatidiform mole, and nonsemino matous testicular cancer. The MAbs, which recognized miscellaneous epitopes on hCG were utilized in developing various immunological assays.[12,13,14] Multiple forms of holo-hCG, free α, and β subunits occurs in biological fluid.[15,16] Total β hCG detected has proved useful in discriminating between normal pregnancies and pregnancies affected by Down's syndrome, which was first proposed by Bogart *et al.*[17] However, after an establishment of MAbs specific to $\beta_6$ and $\beta_7$ epitope clusters,[18] which are present only on the nonassociated subunit, the assay for the free β subunit level was developed. This diagnostic kit proved to have more potential value in screening for Down's syndrome than the detection of total β hCG.[19] The variants of hCG can also be detected in tumor patients as pI-isoforms of holo-hCG and their free subunits. The major difference of these variants is due to the abnormal highly negative carbohydrate-associated surface charges. The panel of MAbs evaluated could recognize all variants. According to this study, one could conclude that the carbohydrate moieties play no significant role in the immunological make-up of hCG.[20] In addition, the variation in expression degrees of hCG subunits and fragments on cultured human cancer cell membrane was investigated in order to establish a phenotypic marker.[21] Currently, hCG and its CTP are under investigation as an antigenic target in both anti-cancer[22] and anti-fertility vaccines.[23,24] In addition, Delves *et al.*[9] described an epitope-focused vaccine by using hCG as a model. The cross-reactive epitopes on β subunit of hCG, which elicited antibodies to LH, were modified and tested by panel of MAbs.

In this study, we produced and characterized seven MAbs against each subunit epitopes residing on holo-hCG using a solid phase immunoassay. Our approach has been to select the MAb specific to β subunit, which recognized the epitope localized in binding distant from the epitope recognized by the MAb specific to α subunit. The MAbs selected will be applied in a two-monoclonal antibody, sandwich-type of immunometric assay in the future.

## MATERIALS AND METHODS

### Monoclonal antibodies

Female BALB/c mice were immunized with the following schedule: 30 μg of human chorionic gonadotropin (holo-hCG, Profasi, Switzerland) emulsified with Freund's complete adjuvants, *ip* on days 0 and 14. One month later, mice were given an *iv* dose of 10 μg free β subunit hCG (Zymed, USA) in NSS. After 72 hours, 5 x $10^7$ splenocytes were isolated and fused with 1 x $10^7$ X63-Ag 8.653 myeloma cells using 50% polyethylene glycol 4000 (GIBCO BRL, USA) as the fusogen. The hybrid cells selected in RPMI (GIBCO BRL, USA) containing 10% FBS (Seromed, Germany), hypoxanthine, thymidine and aminopterin. To detect hybrids secreting antibodies against hCG, culture supernatants were tested by using indirect enzyme-linked immunosorbent assay.

### Ascites fluid

BALB/c mice were primed

with pristane *ip* 10 days prior to receiving injections of the selected hybridoma cells (2 x $10^6$ cells/animal). One week after injection, the ascites fluid was harvested, and the antibody was purified by protein-A affinity chromatography (Econo-Pac, BIO-RAD, USA).

**Biotinylation of monoclonal antibody**

The protein-A chromatographic purified MAb ALC-1 specific to α subunit of hCG was biotinylated using aminohexanoyl-biotin-N-hydroxysuccinimide ester (AH-BNHS, Zymed, USA). Ten mg/ml of purified MAb in bicarbonate pH 8.4 was mixed with 10 mg/ml of AH-BNHS in dimethylformamide at 10:1 (w/w) ratio. The mixture was incubated for 1 hour at room temperature with continuous shaking, dialysed extensively in 0.05% sodium azide in PBS pH 7.5. Biotinylated antibody was store at 4°C.

**Enzyme-linked immunosorbent assay**

Polystyrene flat bottom plates (MaxiSorp, Nunc, Denmark) were coated with holo-hCG in 0.1 M carbonate buffer pH 8.6 buffer at a concentration of 2.5 μg/ml at 4°C overnight (each well was incubated in 50 μl). Plates were then washed three times with washing buffer, 0.5% Tween 20 in PBS pH 7.5 and blocked with 200 μl in each well with 2% BSA in PBS pH 7.5 for 1 hour at 37°C. The hybrid cells were screened by adding 50 μl of culture supernatant to the coated wells and incubated for 1 hour at 37°C. Following the incubation, the plates were washed and the presence of anti-hCG antibodies was assessed with polyspecific rabbit anti-mouse immunoglobulin-peroxidase conjugate (Zymed, USA). The enzyme conjugate was diluted in 1% BSA in PBS pH 7.5 and was revealed with the TMB chromogenic substrate (Zymed, USA). After 30 minutes of room temperature incubation, enzymatic reaction was stopped with 5 N $H_2SO_4$. The optical densities detected were read on a microtiter plate reader (EL 340, BIO-TEK, USA) at 450 nm. The contents of wells showing an optical density of > 0.8 were cloned by limiting dilution culturing. The immunoglobulin subclass was determined using an isotyping kit (GIBCO BRL, USA).

The specificity of the MAbs produced was further characterized by using free β subunit hCG (Zymed, USA), synthetic carboxy-terminal peptide (CTP) of sequence β109-119 (TCDDPRFQDSS) and β 109-145 (TCDDPRFQDSSSSK-APPPSLPSPSRLPGPS DTPILPQ) purchased from Sigma, USA and human luteinizing hormone (hLH, Zymed, USA) as antigen with the same procedure described above.

**Competitive enzyme-linked immunosorbent assay**

The coating of holo-hCG and subsequent blocking steps were prepared as described above. Fifty microliters of the competitive MAbs diluted to 1.5 OD in reaction against holo-hCG using indirect enzyme-linked immunosorbent assay was incubated in each coated well for 1 hour at 37°C, followed by washing three times. Fifty microliters of biotin-conjugated ALC-1 at dilution of 1:2,500 was applied to the reaction plate and incubated for 1 hour at 37°C. After washing three times, wells were incubated for 1 hour at 37°C with 1:5,000 dilution of peroxidase-Z-avidin (Zymed, USA). TMB chromogenic substrate was added after washing another three times. Five normal $H_2SO_4$ was added after 30 minutes of incubation at room temperature and the absorbance of the reaction product measured with a microtiter plate reader at 450 nm.

Reduction in OD of each reaction well was used to compare with a no competitor control well. The positive inhibition control well in this experiment was non-labeled ALC-1 as self-competitor. The percent inhibition was calculated from these raw data.

**Polyacrylamide gel electrophoresis (PAGE)**

Both standard protein marker (SDS-6, Sigma, USA) and holo-hCG were boiled for 3 minutes in a sample buffer containing 1% SDS, 62.5 mM Tris-HCl pH 6.8, 10% glycerol, with or without 5% 2-mercaptoethanol (2-ME) and a trace amount of tracking dye (Bromophenol blue, Fisher, USA) before electrophoresis. Descending electrophoresis was performed by allowing a current of 5 mA/gel using 15% acrylamide separating gel as a bridge. The chamber was filled with electrophoresis buffer pH 8.3 and the current was turned on until the tracking dye reached the lower edge of the gel. Lane containing standard protein marker was cut off for staining with 2% Coomassie brilliant blue R-250 (Sigma, USA). Sample lanes were further processed for Western blot analysis.

**Western blot analysis**

Proteins resolved by PAGE

were electrophoretically transferred to nitrocellulose membrane (Nitro-Pure, MSI, USA) at 150 V for 60 minutes. Sample lanes were treated for 5 minutes with washing buffer (1% Tween20 in PBS pH 7.5) and incubated in blocking buffer (2% BSA in PBS pH 7.5) for 1 hour at 37°C. After washing three times, selected MAbs were applied to nitrocellulose strips for 1 hour at room temperature. Reaction bands were probed by a 1:1,000 dilution of horseradish peroxidase conjugated anti-mouse immunoglobulins antibody (Zymed, USA) after washing the membrane extensively three times. Following 1 hour of incubation at room temperature, the last washing step was performed. Immunoreactive bands on each strip were visualized by adding chromogenic substrate (diaminobenzidine, Sigma, USA). The enzymatic reaction was stopped by running tap water after 5-minute incubation.

## RESULTS

### Monoclonal antibodies to hCG selection

Splenocytes from a single animal possessing the highest serum antibody titer against holo-hCG (1:280,000) by indirect ELISA were harvested. After the fusion process, the mixture of cells was expanded to 960 wells of 96-well tissue culture plates. Ten days later hybridomas were colonized in 119 wells under selective conditions. Seven hybrid wells secreted antibodies against holo-hCG by screening indirect ELISA with OD > 0.8. All of them were cloned in a 96-well tissue culture plate. Culture supernate of wells harboring single hybrid colony were reaped to examine their specificity with holo-hCG, free β subunit hCG and hLH by indirect ELISA. The reactive activity can be demonstrated by the OD value (Table 1). Six hybrid clones secreting MAbs recognized epitopes presented on both holo-hCG and free β subunit hCG without cross-reactivity against hLH. These clones were named BEL-1 to BEL-6. Nevertheless, the only MAb, ALC-1, which was established in this study, recognized both holo-hCG and hLH equally. No significant reaction with free β subunit was observed. Moreover, all of these MAbs except for ALC-1 were examined with synthetic CTP of sequence β109-119 containing $\beta_9$ epitope cluster and β109-145 containing both $\beta_9$ and $\beta_8$ epitope clusters (Fig. 1). The results demonstrated that MAbs BEL-1, BEL-3, and BEL-4 recognized sequence β109-145, while, MAbs BEL-2, BEL-5, and BEL-6 recognized both β109-119 and β109-145 sequences equally (Table 2). Regarding the immunoglobulin subclass identification, all of MAbs selected were of $IgG_1$/kappa.

### Biotinylation of MAb ALC-1

In order to produce ascites fluid, 2 x $10^6$ cells of ALC-1 hybrid line were injected into mouse *ip*. The ascites fluid achieved was further purified by protein-A chromatography. Purified immunoglobulin was diluted to 10 mg/ml in bicarbonate buffer pH 8.4 and mix with 10 mg/ml of AH-BNHS in dimethylformamide at 10:1 (w/w) ratio. In addition, the biotinylated ALC-1 was examined for its antibody retaining activity toward hCG by avidin-biotin indirect ELISA. The highest titer obtained was 1:8,000. This single preparation of biotinylated ALC-1 was employed throughout the experiment.

**Table 1** Indirect ELISA reactivities of MAbs against holo-hCG, β subunit hCG, and hLH. The degree of each reaction well was represented in the mean of optical densities (OD) value at 450 nm. Each assay was done in triplicate.

| Antigens | Monoclonal antibodies | | | | | | |
|---|---|---|---|---|---|---|---|
| | BEL-1 | BEL-2 | BEL-3 | BEL-4 | BEL-5 | BEL-6 | ALC-1 |
| holo-hCG | >2.00 | >2.00 | >2.00 | >2.00 | >2.00 | >2.00 | >2.00 |
| β hCG | >2.00 | >2.00 | >2.00 | >2.00 | >2.00 | >2.00 | 0.07 |
| hLH | 0.06 | 0.07 | 0.07 | 0.12 | 0.07 | 0.08 | >2.00 |

**Table 2** Indirect ELISA for epitope localization of MAbs against synthetic carboxy terminal peptide sequence β109-119 and β109-145. The binding efficient of each MAb was represented in the mean of optical densities (OD) value at 450 nm. Each assay was done in triplicate.

| Antigens | Monoclonal antibodies | | | | | |
|---|---|---|---|---|---|---|
| | BEL-1 | BEL-2 | BEL-3 | BEL-4 | BEL-5 | BEL-6 |
| β109-119 | 0.05 | 1.85 | 0.15 | 0.07 | 1.98 | 1.90 |
| β109-145 | 1.70 | 1.89 | 1.77 | 1.80 | 1.91 | 1.75 |

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                    .          .          .          .          .
hCG:(1-50)      SKEPLRPRCRPINATLAVEKEGCPVCITVNTTICAGYCPTMTRVLQGVLP
ALIGN           S+EPLRP C PINA LAVEKEGCPVCITVNTTICAGYCPTM RVLQ VLP
hLH:(1-50)      SREPLRPWCHPINAILAVEKEGCPVCITVNTTICAGYCPTMMRVLQAVLP
                    .          .          .          .          .
hCG:(51-100)    ALCQVVCNYRDVRFESIRLPGCPRGVNPVVSYAVALSCQCALCRRSTTDC
ALIGN            LPQVVC YRDVRFESIRLPGCPRGV+PVVS+ VALSC+C  CRRST+DC
hLH:(51-100)    PLPQVVCTYRDVRFESIRLPGCPRGVDPVVSFPVALSCRCGPCRRSTSDC
                    .          .          .          .
hCG:(101-145)   GGPKDHPLTCDDPRFQDSSSSKAPPPSLPSPSRLPGPSDTPILPQ
ALIGN           GGPKDHPLTCD P+
hLH:(101-121)   GGPKDHPLTCDHPQLSGLLFL
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**Fig. 1** Comparison of the amino acid sequences of the β subunit hCG and β subunit hLH hormones, non homology was designated as gap, + symbol referred to similar amino acid. The $\beta_9$ and $\beta_8$ epitopes were accordingly represented as open and shaded box. Analyzed by BlastP Enhanced Alignment Utility.

**Polyacrylamide gel electrophoresis (PAGE)**

To determine the purity of urinary preparation of pregnancy holo-hCG purchased from Profasi, PAGE was performed and gel stained with 2% Coomassie brilliant blue R-250 (Fig. 2). Holo-hCG hormone was separated into two discrete bands at *Mr* 23 kDa (α subunit) and *Mr* 34 kDa (β subunit) in non-reducing condition. In contrast, bands at *Mr* 20 kDa (α subunit) and *Mr* 26 kDa (β subunit) were found in reducing condition (with 2-ME in sample buffer). These relative size differences on PAGE corresponding to the standard molecular weight, SDS-6 was determined by 1D image analysis program (ONE-Dscan version 1.0, Scanalytic, USA). The result obtained was similar to highly purified urinary NIH standard hCG molecular size described by Berger *et al.*[18]

**Western blot analysis**

According to the result shown in Fig. 3, the Western blot reaction of MAbs BEL-1 to BEL-6 against holo-hCG exhibited an homology binding pattern in both non-reducing (NR) and reducing conditions (R). Polypeptides bearing specific epitopes for these MAbs were localized at *Mr* 34 kDa band (NR) and *Mr* 26 kDa band (R). However, the reactive bands visualized in the R condition was more intense than in the NR condition. In comparison, MAb ALC-1 rendered a specific recognition epitope residing on polypeptide with *Mr* 23 kDa (NR) and no reactive band was visualized under R condition. There was absolutely no background band observed on the control strips in both R and NR condition.

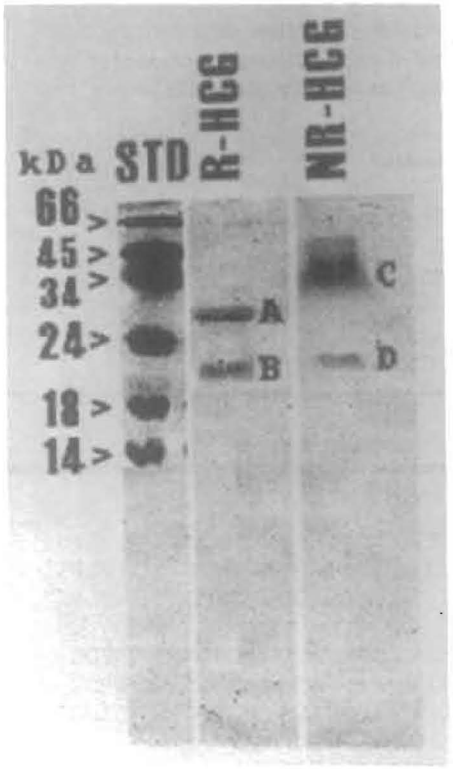


**Fig. 2** The PAGE results of reduced (R) and non-reduced (NR) holo-hCG protein. The STD lane contained standard protein molecular weight (SDS-6), specific *Mr* of each band was shown on the far-left side. A, C illustrated β subunit hCG and B, D depicted α subunit hCG.

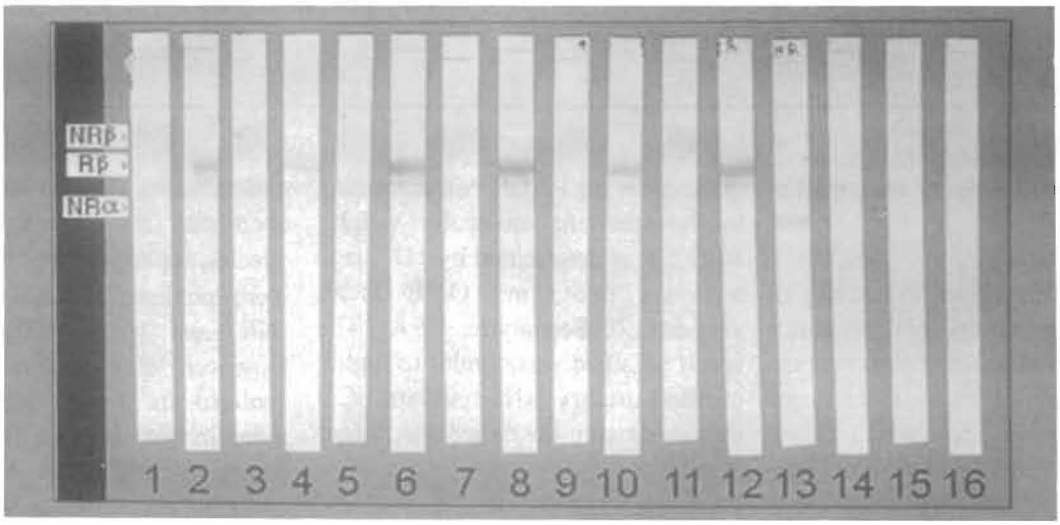


**Fig. 3** Immuno-enzyme reaction patterns of Western blot holo-hCG probed with MAbs BEL-1 (lanes 1 and 2), BEL-2 (lanes 3 and 4), BEL-3 (lanes 5 and 6), BEL-4 (lanes 7 and 8), BEL-5 (lanes 9 and 10), BEL-6 (lanes 11 and 12), and ALC-1 (lanes 13 and 14). The myeloma culture supernatant was used as a negative control, which exhibited in lanes 15 and 16. The holo-hCG protein separated in non-reducing and reducing conditions were represented in the odd and even lane numbers, accordingly. The labels designating the specific location of each visualized band was shown on the far-left side.

**Table 3** Effect of MAbs BEL-1 to BEL-6 in binding to holo-hCG target monitored with biotinylated MAb ALC-1 by competitive ELISA. The results were expressed in percent inhibition by comparing with no competitor reaction well. Each assay was done in triplicate.

| MAbs | Mean OD at 450 nm | % Inhibition |
|---|---|---|
| BEL-1 | 0.46 | 51.68 |
| BEL-2 | 0.71 | 20.22 |
| BEL-3 | 0.40 | 55.05 |
| BEL-4 | 0.45 | 49.43 |
| BEL-5 | 0.75 | 15.73 |
| BEL-6 | 0.70 | 21.34 |
| ALC-1* | 0.17 | 80.89 |
| No competitor | 0.89 | 0.00 |

ALC-1* was used as self-competitor in this system.

**Competitive enzyme-linked immunosorbent assay**

In accordance with the highly sensitive competitive ELISA using avidin-biotin system developed, six MAbs specific to β subunit hCG (BEL1 to BEL6) were supplied to compete with the MAb specific to α subunit hCG (ALC-1) conjugated biotin. The holo-hCG coated on solid phase was subjected to be a target molecule. As shown in Table 3, each competitor gave a distinct degree of percent inhibition. Regarding this result, the competitors could roughly be separated into two clusters: MAbs BEL-1, BEL-3, and BEL-4 with percent inhibition above 22, whereas, MAbs BEL-2, BEL-5, and BEL-6 with percent inhibition below 22. Among these competitors, MAb BEL-5 showed the lowest percent inhibition.

## DISCUSSION

The specific immunization protocol used successfully produced hybridomas secreting MAb specific to CTP of β subunit hCG, which was poorly immunogenic.[25] Only mouse B-lymphocytes which expressed specific antibodies against β subunit hCG were triggered by restricting the *iv* prefusion dose with the free β subunit hCG, therefore, removing the strong immunogenicity of α subunit hCG.[26] This protocol was applied from the previous report which found that immunization with free β subunit hCG yielded polyclonal antibodies with much lower cross-reaction to hLH.[27] Evidence presented herein showed that the MAbs BEL-1 to BEL-6 specifically reacted with β subunit hCG because they did not cross-react with hLH molecule in indirect ELISA. The identical binding patterns of these MAbs were observed both in the R and NR conditions by Western blot analysis. This group of MAbs could retain its activity when reacted with the epitopes on β subunit hCG treated with 2-ME. Hence, these MAbs recognized the epitopes, which did not rely on the conformational structure. Nevertheless, the intensity of the band viewed on the strip of non-reduced antigen was duller and more disperse than the strip of reduced antigen. This could apparently be explained in that the ratio of SDS occupied on the heavily glycosylated incomplete unfolding of β subunit hCG was not correct. Thus the similar protein had a different moving rate in PAGE. As previously precluded, so far there were only two linear peptide epitope clusters, which discriminated β subunit hCG from other closely related hormones. These epitopes lined in the CTP region of β subunit hCG, $\beta_8$ (137-144) and $\beta_9$ (113-116).[28] For that reason we chose two synthetic peptides of sequence β109-119 and β109-145 to further characterize these MAbs. The result differentiated these MAbs into two groups. First group was BEL-1, BEL-3, and BEL-4 recognized only β109-145. The other was that BEL-2, BEL-5, and BEL-6 recognized both types of synthetic peptide. These outcomes indicated that the set of BEL-1, BEL-3, and BEL-4 restricted to the $\beta_8$ epitope cluster. In contrast, BEL-2, BEL-5, and BEL-6 directed towards the $\beta_9$ epitope cluster.

Another MAb harvested in this study, MAb ALC-1, demonstrated an absolutely different immunological reaction. It bound only to epitopes localizing on holo-hCG and hLH, but not on free β subunit hCG in indirect ELISA. This result implied that MAb ALC-1 had a specific reaction to α subunit of holo-hCG, which shared among the closely related hormones. This consequence correlated with the Western blot result, since MAb

ALC-1 reacted with the α subunit hCG at *Mr* 23 kDa of NR condition. Disrupting disulfide loops in the holo-hCG molecule absented the recognition of MAb ALC-1 to α subunit hCG. Thus the epitope recognized by this MAb confined the native structure of α subunit hCG. As we did not further characterize the epitope recognized by this MAb, the cluster of epitope could not be specified. However, $\alpha_6$ and $\alpha_7$ could be excluded because $\alpha_6$ restricted to the unbound form of α subunit hCG and $\alpha_7$ was a member of linear peptide epitope.[4]

To determine the distant topography of epitopes recognized by the set of MAb specific to β subunit hCG and one MAb specific to α subunit hCG, competitive ELISA was exploited. Each MAb of BEL-1 to BEL-6 inhibited the specific binding of biotinylated ALC-1 to α subunit hCG epitope in the different degree. Since all MAbs belonged to $IgG_1/k$ subclass, they could practically be compared in this experiment. The smallest percent inhibition bestowed the most distant of that β subunit epitope and ALC-1 epitope. The group of MAbs specific to the $\beta_8$ epitope cluster contended with MAb ALC-1 in binding to its epitope more effectively than the $\beta_9$ epitope cluster specific group. With respect to the result, MAb BEL-5 had the bottom-most interfering effect in binding of MAb ALC-1 to its epitope. This percent inhibition had validity for selecting a MAb BEL-5, specific to β subunit hCG to utilize with MAb ALC-1 in developing a two-monoclonal antibody, sandwich-type of immunometric assay. The highest sensitivity in the detection of holo-hCG will be achieved from this criterion.

At present, at least 50 distinct commercial kits are available for measuring hCG levels. Different combinations of MAbs in the sandwich-type assay are used in each kit. The interpretation of these results is cumbersome because the hCG detected by unlike kits is in a different form.[29] In any event, the correlation of these consequences may play a high potential role in classifying patients with trophoblastic disease. The kit, which will be prepared for detecting serum holo-hCG level is possibly helpful in the prognosis of a patient's condition in combination with the serum level of total β subunit hCG. In addition, MAbs specific to β subunit hCG can be exploited in preparation of an affinity purified hCG. This extremely purified hCG is essential for the remedy of Kaposi's sarcoma[30,31] and for antifertility vaccination.[32]

While the three-dimensional structure of β subunit hCG CTP part had not yet been elucidated, this study also contributed an insight into how CTP interacted with α subunit hCG in native structure of holo-hCG. Since the $\beta_8$ epitope cluster resides in the extreme end of the β hCG molecule. Hence, it is more flexible compared to the $\beta_9$ epitope cluster,[10] which is adjacent to the core fragment of the molecule. Therefore, when MAbs reacted with $\beta_8$ epitope, CTP might fold back and hinder the epitope specific for MAb ALC-1.

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