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# INDUCTION OF IMMUNE RESPONSE IN *Camelus bactrianus* AND *Camelus dromedarius* AGAINST MUC1 - PEPTIDE PRODUCED HEAVY-CHAIN ANTIBODIES WITH EFFICIENT COMBINING PROPERTIES

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## ABSTRACT

Camelidae are known to possess antibodies devoid of light chains and $C_H1$ domains. Antigen-specific fragments of these heavy-chain IgGs (VHH) are of great interest in biotechnology applications. We report here the first example of a successfully raised heavy-chains antibodies in *Camelus dromedarius* and *Camelus bactrianus* against the MUC1 peptide. Camels were immunised against cancerous tissue and peptide conjugated to bovine serum albumin. Both conventional and heavy-chain IgG antibodies were produced in response to MUC1- peptide. Enzyme-linked immunosorbent assays (ELISAs) and Western blotting for MUC1 peptide conjugated to BSA, deglycosylated human milk fat globule membrane (HMFG), and cancerous breast tissues were established to investigate the titre development. Three subclasses of IgG of both camels were separated chromatographically. All three subclasses of IgG in both of camels were bound to MUC1 peptide. This study demonstrates specific *in vitro* targeting of MUC1 peptide by camels heavy-chain antibodies. It may open prospective for their future and practical application as tumor-targeting tools, due to their small size and soluble behaviour.

**Key words:** *Camelus bactrianus, Camelus dromedarius*, heavy-chain antibody, immune response, MUC1

Hamers-Casterman *et al* (1993) reported a novel class of two-heavy chain IgG antibodies, in addition to conventional four-chain IgG in Camelidae. However, these antibodies have never been documented in species of closely related taxonomic suborders such as sheep, bovine and swine. Therefore, these heavy-chain antibodies are considered as a unique event arising in the Tylopoda (Arbabi Ghahroudi *et al*, 1997; Nguyen *et al*, 2001 and Nguyen *et al*, 2002). In *Camelus dromedarius*, three subtypes of IgGs have been identified on the basis of their binding pattern to staphylococcal protein A and G. $IgG_2$ and $IgG_3$ ("heavy-chain antibodies") lack light chains and the $C_H1$ domain. $IgG_2$, a homodimer of 46 KDa chains, is characterised by an extended hinge region of Pro-X repeats (X = glutamic acid, glutamine or lysine). $IgG_2$ binds only to protein A whereas $IgG_3$, which consists of two chains of 43 KDa after reduction, binds to protein A and G (Hamers-Casterman *et al*, 1993 and Lange *et al*, 2001). A comparative study of old world and new world camelids showed that heavy-chain antibodies are abundant in the sera of all species examined and total up to 75% of the molecules binding to protein A.

The heavy-chain IgG antigen-binding domain of these antibodies consists of the variable domain of the heavy chain, referred to as VHH. VHH have been proposed as valuable potential tool for biotechnological applications (Arbabi Ghahroudi *et al*, 1997). The size of these VHH fragments is reduced to a bare minimum (a single immunoglobulin domain) and their levels of expression and solubility are significantly higher than those of classical Fab or Fvs (Variable fragments of immunoglobulins). Several VHHs have been raised against different protein antigens and a few haptens. VHH from llama or camel have affinity constants for their

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protein antigens are compatible to those of Fab or Fvs (van der Linden *et al*, 2000, Spinelli *et al*, 2000 and Vranken *et al*, 2002). However, there is no report of raising antibody response of camels against peptide antigens.

In this study, we characterised the immune response of *C. dromedarius* and *C. bactrianus* and evaluated the binding capacity of the different IgG subclasses by ELISAs for the MUC1 peptide as well as for the large protein molecule BSA. We used the MUC1 peptide in order to evaluate the possible use of camelid antibodies for targeting cancerous tissues bearing MUC1 proteins antigen. MUC1 is a transmembrane molecule of which the major extracellular domain is composed of tandem repeat units of 20 amino acids (PDTRPAPGSTAP-PAHGVTSA). The repeated units contain potential O-glycosylation sites represented by serine and threonine residues, which act as a scaffold for the attachment of O-glycans, resulting in the formation of a highly glycosylated extended repetitive structure (Qi *et al*, 2001). In carcinomas, not only is MUC1 overexpressed, but the core protein is also aberrantly glycosylated, making the tumor-associated mucin antigenically distinct from the normal mucin (Taylor-Papadimitriou *et al*, 1999). Epitope mapping tests have been performed for antibody reactivity against overlapping synthetic peptides and results were largely consistent between different groups. The dominant feature of epitopes within the MUC1 protein core is the presence, in full or part, of the hydrophilic sequence of PDTRPAP (Price *et al*, 1998). Interestingly MUC1 is a tumor-associated antigen which is over-expressed on various adenocarcinoma including breast adenocarcinoma, pancreatic cancer, ovarian cancer, prostate cancer, colon cancers and multiple myeloma (Xing *et al*, 1992). Therefore, rapid and sensitive screening methods for the detection of this molecule and their efficient targetting for immunotherapy of cancer is highly desirable. Referring to these possible applications, low molecular weight (MW) antigen binders, like camels' heavy-chain antibodies, may open new prospective of accelerated, sensitive and very stable test systems and targeting vehicles.

## Materials and Methods

***Materials and reagents :*** Two synthetic mucin peptides ($P_{27}$ = TSAPDTRPAPGSTAPPAHGVT SAPDTR and $P_{16}$ = APDTRPAPGSTAPPAH), corresponding to the mucin core protein, which were chemically conjugated to BSA by reaction with glutaraldehyde, were purchased from Q. Biogene, Co., France. Sheep anti rabbit conjugated to HRP was purchased from Serotec Co. All other reagents used in this study were of analytical grade and purchased from Sigma Chemical Co., St. Louis, MO.

***Tissue samples preparation:*** Tissue samples of breast and pancreatic cancer were kindly provided along with pathological reports by Cancer Institute, Imam Hospital, Tehran, Iran, immediately after the surgery was performed. Tissues were transferred to the lab in ice cold containers, treated with liquid nitrogen and homogenised mechanically in 20 mM PBS, pH 7 containing PMSF. The resulting suspension was centrifuged (40,000 xg), supernatant was collected and used as a source for immunisation, as well as antigen to recognise the antibody binding activity.

***Antigen preparation: Purification, deglycosylation and characterisation of HMFG*:** Fresh mature milk and colostrum samples from healthy mothers were collected. Fat globules were recovered by centrifuging milk at 40,000 xg for 1 h at 5°C, washed three times with 0.9% saline and subjected to one cycle of freezing (overnight at -70°C) and thawing. Membranes were collected by suspending the ruptures globules in warm (35°C) 0.9% saline and centrifuged at 40,000 xg for 1.5 h at 5°C. The pellet was recovered and subjected to two resuspension-centrifugation cycles (Keenan *et al*, 1970). For the preparation of defatted HMFG, the washed cream fraction of human milk was extracted twice with 2 volumes of chloroform and twice with 1 volume of ether and lyophilised (Ceriani *et al*, 1977).

Sodium cholate (2% w/w; 0.2 g/ml), urea (8 M; 0.48 g/ml), and 2-mercaptoethanol (2-ME) (1% v/v; 10 micro litre/ml ) were added to HMFG suspension (approximately 10 mg/ml) for solubilisation. The mixture was then incubated at 37°C for 30 min. The solubilised sample was centrifuged at 10,000 xg for 30 min at 15°C and a clear aqueous portion was recovered (Shimizu and Yamauchi, 1982).

Chemical deglycosylation of MUC1 (dried HMFG) was performed by incubation of the extensively dried sample (10 mg) in trifluromethane-sulfonic acid (400 µl) for 2 h at 0°C, followed by neutralisation with 1.5 ml of pyridine/water (3:2)

at -20°C and dialysis against phosphate buffer (20 mM, pH 7) for 24 h (Hanisch *et al*, 1996). SDS-PAGE analysis was carried out according to Hames and Rickwood (1990) using 5% gel. Proteins were stained with coomassie blue R-250 and silver nitrate.

### 2.4. Immunisation and production of antibodies

Two different MUC1 antigens cancerous tissue samples, prepared as explained under materials and methods section, and a synthetic mucin peptide conjugated to BSA were used for immunisation of two young adult male camels (*C.dromedarius* and *C. bactrianus*) purchased from local cattleman. Camels were immunised with nearly 12 mg homogenised tissues in Freund's complete adjuvant and boosted for 4 times every 2-3 weeks with nearly 7 mg homogenised tissues in a Freund's incomplete adjuvant and then boosted for 5 times every 2-3 weeks with 250 µg of $P_{27}$–BSA in a water-in-oil emulsion (Van der Linden *et al*, 2000). Antiserum titration was started 1 week after the $7^{th}$ immunisation. Blood collection was performed by puncturing the vena jugularis. The blood samples were centrifuged in 4°C, serum was collected and stored at -70°C.

### 2.5 Serum fractionation

Sera from camels were fractionated using protein G and protein A affinity chromatography (Hitrap, Pharmacia, Upsala, Sweden) according to the procedure of Hamers-Casterman *et al* (1993). Sera fractions were immediately dialysed against PBS (100 mM, pH 7) and the protein contents of the affinity chromatography fractions were quantified by means of Bradford assay.

### 2.6 Electrophoretical characterisation and comparison of the antibody subclasses

$IgG_1$, $IgG_2$, $IgG_3$ fractions from *C. bactrianus* were subjected to 12% SDS-PAGE in absence or presence of 2-ME (Hamers-Casterman *et al*, 1993). Bands were scanned and analysed after staining of the gel. The MW of the IgG peaks was approximated with the help of a calibration curve (Hames and Rickwood, 1990).

### 2.7. ELISAs

#### *2.7.1. Preparation of purified rabbit anti-camel IgGs*

Anti-camel IgG fractions were prepared in four rabbits and purified following the procedure described by Levy and Sober (1960). The immunoglobulin fractions of these antisera were precipitated by 33% ammonium sulphate (w/v) and purified using protein A affinity chromatography following the standard procedure (Tijssen, 1992).

#### *2.7.2. Preparation of rabbit anti-camel immunoglobulin conjugated to Horse radish peroxidase (HRP) (rabbit anti-camel–HRP)*

Purified rabbit anti-camel immunoglobulins exhibiting lower cross-reaction with either BSA, HMFG or normal and cancerous tissue preparations were selected by ELISA (Rassaie *et al*, 1992). Good titre antibodies were conjugated to HRP following a simplified $NaIO_4$ method, with small reduction in activation of peroxidase and improved yield of conjugate (Ausubel *et al*, 1999). Finally a titration assay was performed in order to assign the best dilution of conjugate to be used in the assay procedure.

#### *2.7.3. Purified camel immunoglobulins*

The globulin fraction of camel antiserum was precipitated by 26% ammonium sulphate (w/v) and dialysed against PBS (100 mM, pH 7). Alternatively camel antiserum was precipitated by polyethylene glycol 6000 according to Polson *et al* (1985) with minor modifications.

#### *2.7.4. ELISA using rabbit anti-camel IgGs conjugated to HRP*

To detect antisera and IgG fractions in camels sera, two synthetic mucin peptides ($P_{16}$, $P_{27}$) conjugated to BSA were coated onto the wells of microtitre plates at 37°C overnight (same concentration of BSA was used as NSB). Then the wells were emptied, washed and blocked with 1% solution of BSA supplemented PBS (10 mM, pH 7.2) for 1 h at 37°C. At the end of incubation time the wells were washed and added with dilutions of sera, purified immunoglobulins and IgGs fractions. In each experiment normal camel sera with a dilution of 1:600 or PBS were used for non-specific binding (NSB) for dilutions of serum and IgGs fractions, respectively. The contents were incubated at 37°C for 3 h, washed, added with rabbit anti-camel–HRP, and incubated at 37°C for 75 min. At the end of incubation time, wells were washed and added with 100 µl of substrate, 3,3',5,5'-tetramethylbenzidine and incubated for 10 min. The enzyme reaction was terminated using

50 µl of a 1 N sulfuric acid solution and the colour development was measured at 450 nm (Rahbarizadeh *et al*, 2000).

### *2.7.5. ELISA using sheep anti-rabbit IgGs conjugated to HRP*

In another set of experiments in order to detect antiserums and IgGs fractions in camels sera, a two-step immunostaining using rabbit anti-camel and a sheep anti-rabbit–HRP labeled was carried out. In this way very low non-specific binding and cross-reaction were observed.

### *2.7.6. ELISA for HMFG and deglycosylated HMFG*

Delipidated HMFG and deglycosylated and delipidated HMFG in PBS (0.17 M NaCl, 3.3 mM KCl, $Na_2HPO_4$ 10 mM, $KH_2PO_4$ 1.84 mM) containing 0.03% Triton X100 to give a concentration of 1 µg/well were added to each well of a 96-well Nunc plate. The plates were allowed to dry in a warm chamber overnight. Immediately before use, they were blocked with PBS containing 1.0% BSA and 0.01% sodium azide for 1 h. The rest of experiment was performed as explained in previous sections.

### *2.8. Western blotting*

HMFG, deglycosylated HMFG, cancerous breast tissues, normal breast tissues and $P_{27}$–BSA were subjected to 5% SDS-PAGE and blotted on nitro- cellulose paper. Blots were blocked with 5.5% skimmed milk in PBS containing 0.02% Tween 20 for 60 min at room temperature. Subsequently, the blots were incubated for 120 min with different purified sera. Bound antibodies were detected by using polyclonal rabbit anti-camel IgG and sheep anti-rabbit IgG–HRP. Blots were developed with 3,3′-diaminobenzidine as a substrate of HRP.

## 3. Results

### *3.1. Preparation of antigen*

The results of antigen preparation are shown in Fig 1 SDS-PAGE. Delipidated and solubilised HMFG are shown in lanes 2, 5 and 6 with different dilutions where by a > 400 KDa band is clearly seen, secretory mucins in native state. However, after deglycosylating HMFG (lane 4), this band disappears and instead a broad band at MW of 200-400 KDa can be observed, which is probably the MUC1-specific peptide motif. Sample of cancerous tissue is shown in lane 1. On comparison the results showed that in all cases of non-synthetic natural antigens used for immunisation and immunoassays a glycoprotein of 200-400 KDa was found in samples (same samples with equal concentration were used in immunoblotting, Fig 6).

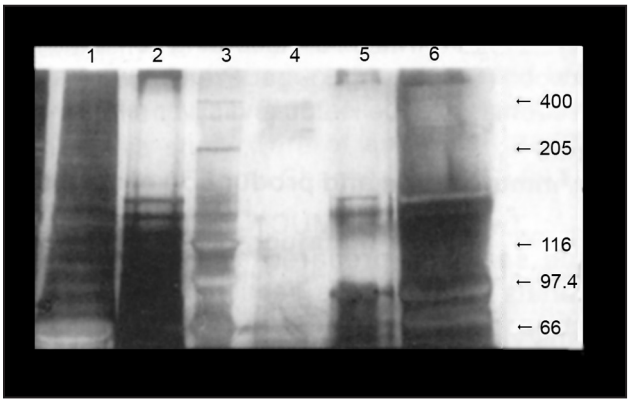


**Fig 1.** 5% SDS-PAGE, silver-stain. Lane 1: cancerous breast tissue, lanes 2, 5, 6: solubilised delipidated HMFG (with different dilutions), lane 3: MW marker, lane 4: deglycosylated HMFG.

### *3.2. Fractionation of camel serum*

Serum from two non-immunised, *C.bactrianus* and three non-immunised, *C.dromedarius* individuals were fractionated into heavy-chain and conventional IgG antibodies (Fig 2, 10% SDS-PAGE of whole and purified serum (Fig 3, 12% SDS-PAGE of IgG subclasses: A, in the absence of 2-ME; B, in the presence of 2-ME).

Two purification methods were performed using ammonium sulphate and polyethylene glycol 6000 and the results were compared and the results showed no difference between the electrophoretic pattern of sera from *C.bactrianus* and *C. dromedarius*. Higher yields were obtained with ammonium sulphate but better purification was obtained with polyethylene glycol 6000 method. The conventional *C.bactrianus* IgG antibodies were found in the $G_1$ fraction (identified as "$IgG_1$" according to Hamers-Casterman *et al*, 1993), identical to that of *C. dromedarius*. In both of camels this fraction ($IgG_1$) contains molecules of approximately 160-170 KDa (Fig 3 (A), lanes 1 and 2) which upon reduction yield 50 KDa heavy chains and 30 KDa light chains ($IgG_1$

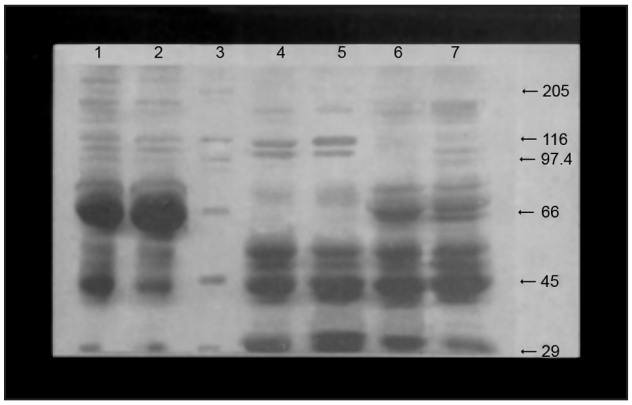


**Fig 2.** 10% SDS-PAGE, coomassie stain. Lane 1: whole serum of *Camelus bactrianus*, lane 2: whole serum of *Camelus dromedarius*, lane 3: MW marker, lane 4: purified serum by polyethylene glycol of *Camelus bactrianus* lane 5: purified serum by polyethylene glycol of *Camelus dromedarius*, lane 6: purified serum by ammonium sulphate of *Camelus bactrianus*, lane 7: purified serum by ammonium sulphate of *Camelus dromedarius*.

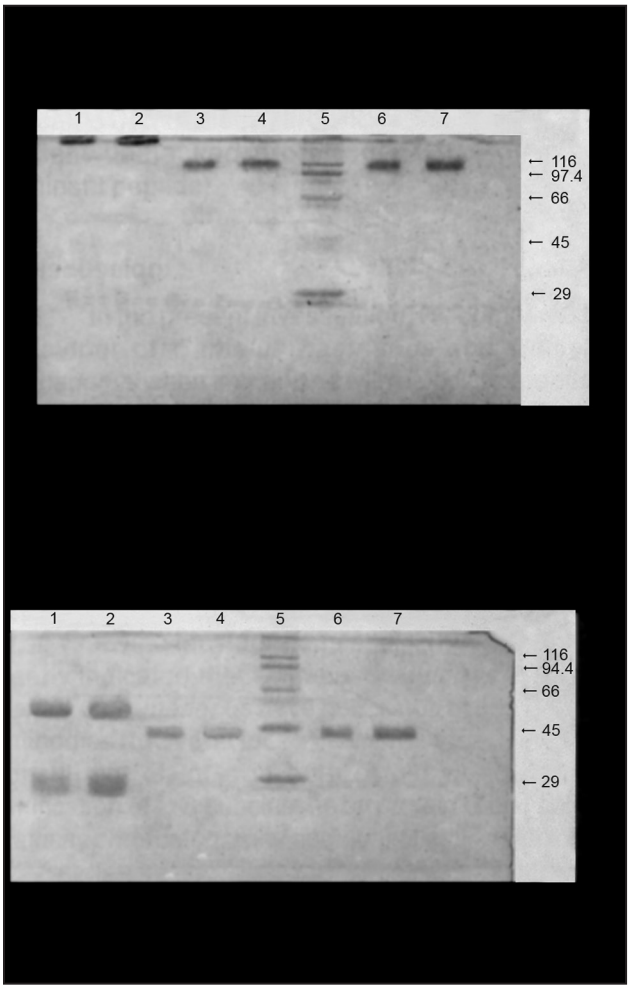


**Fig 3.** 12% SDS-PAGE coomassie stain. (A) In the absence of 2-ME. Lane 1: $IgG_1$ of *C. dromedarius*, lane 2: $IgG_1$ of *C. bactrianus*, lane 3: $IgG_2$ of *C. dromedarius*, lane 4: $IgG_2$ of *C. bactrianus*, lane 5: MW marker, lane 6: $IgG_3$ of *C. dromedarius*, lane 7: $IgG_3$ of *C. bactrianus*. (B) In the presence of 2-ME. Lane 1: heavy chains and light chains of $IgG_1$ of *C. dromedarius*, lane 2: heavy chains and light chains of $IgG_1$ of *C. bactrianus*, lane 3: heavy chains of $IgG_2$ of *C. dromedarius*, lane 4: heavy chains of $IgG_2$ of *C. bactrianus*, lane 5: MW marker, lane 6: heavy chains of $IgG_3$ of *C. dromedarius*, lane 7: heavy chains of $IgG_3$ of *C. bactrianus*.

fraction binding to protein A and protein G, eluted in pH 2.7) [Fig 3 (B), lanes 1 and 2]. The two other immunoglobulin fractions contained molecules of approximately 100 KDa [$IgG_2$ in Fig 3 (A), lanes 3 and 4 and $IgG_3$ in Fig 3 (A), lanes 6 and 7] which upon reduction yield only heavy chains of, respectively, approximately 46 KDa ($IgG_2$ fraction binding only to protein A, eluted at pH = 3) [Fig 3 (B), lanes 3 and 4] and approximately 43 KDa ($IgG_3$ fraction binding to protein A and protein G, eluted in pH = 3.5) [Fig 3 (B), lanes 6 and 7]. These two IgG subclasses appeared to lack the light chain completely. Fractionation results showed that although IgGs of llama were reported to be different from that of *C. dromedarius* from MW point of view, there was no difference between the immunoglobulins of the old world camels. The concentration of $IgG_1$ in *C. bactrianus* was lesser than *C. dromedarius* and the total of $IgG_2$ and $IgG_3$ that in *C. bactrianus* contributed higher. On the other hand the $IgG_2$ in *C. dromedarius* was much lower than $IgG_3$ concentration in *C. bactrianus*. The relative amounts of the isolated camels IgG subclasses were determined (Table 1). The total amount of IgG in serum of both the species ranged between 8-12 mg/ml.

### *3.3. Camel immune response*

Two camels (*Camelus bactrianus* and *Camelus dromedarius*) were immunised with cancerous tissues and P27-BSA. The immune responses were followed by analysis of whole serum IgG and purified serum (precipitation methods) with antigen-specific ELISAs (Fig 4). However, since we found good results for purified IgG, only those results are reported here. In both of camels, a significant antibody response was detected.

Sera obtained on day 270 post immunisation were fractionated by protein G and A affinity chromatography to detect antigen-specific antibodies of each IgG subclasses rabbit anti-camel-HRP as

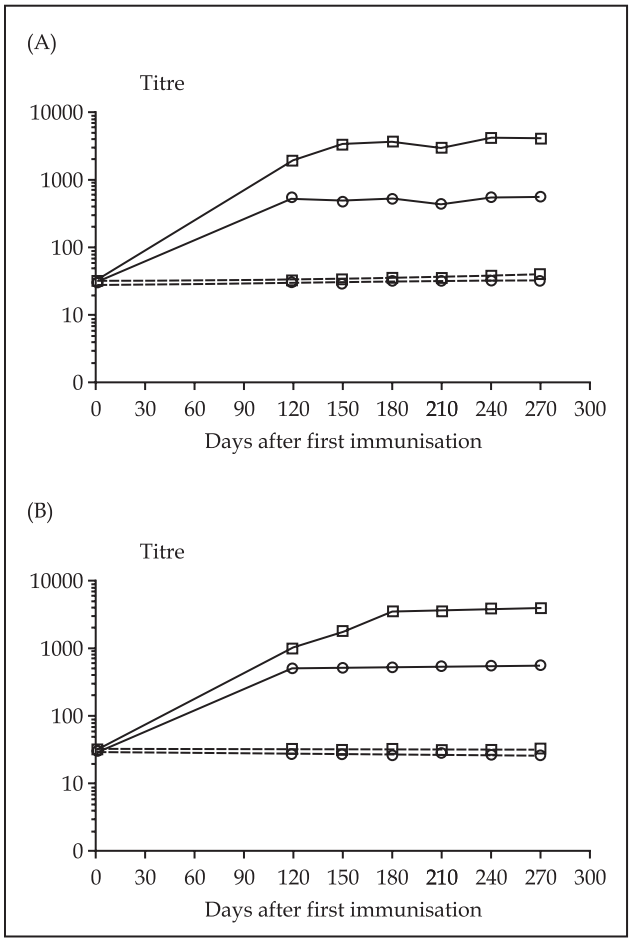


**Fig 4.** Titres of camels purified serum (purification with ammonium sulphate precipitation). (A) *Camelus dromedarius*, (B) *Camelus bactrianus*. Solid line: serum of immune camel, dashed line: serum of normal camel, square: P27-BSA, circle: BSA.

tracer (Table 2). These results were comparable when sheep anti-rabbit-HRP was used as label (the results not shown here). High difference between the optical densities of BSA and peptide-BSA coated wells indicated that camels were successfully immunised and the sera contained antibodies for immuno-dominant antigen of MUC1. These results also indicated that a good titre of antibody for heavy-chain $IgG_3$ was generated. Antibodies of different classes also reacted in much higher affinities with deglycosylated HMFG (contain the MUC1-specific peptide motif). However, there was no significant difference in ODs of IgG fractions reacted with normal and cancerous breast tissues.

### *3.4. Antigen specificity of the different camel antibodies*

High difference between the OD 450 of IgG fractions of wells containing BSA and peptide-BSA

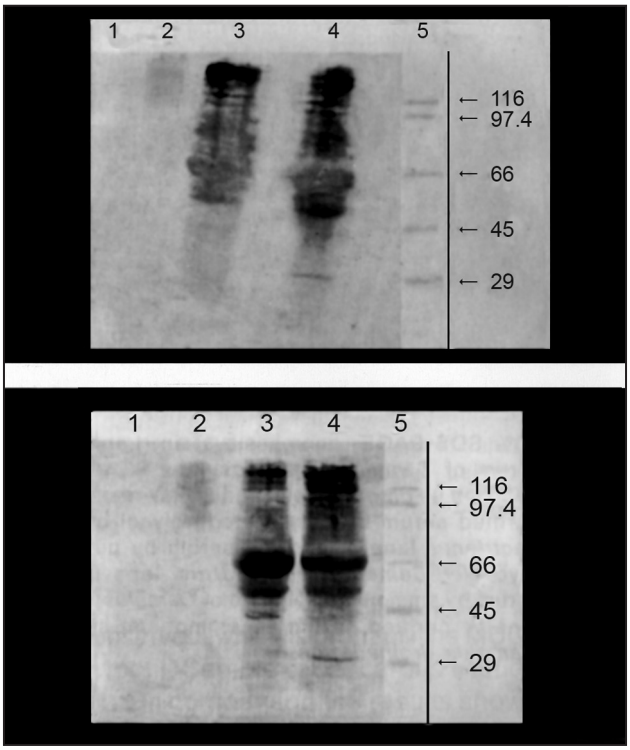


**Fig 5.** Immunoblotting of immunoglobulin fraction of (A) *Camelus bactrianus*. Lane 1: BSA, lane 2: $P_{27}$-BSA, lanes 3: normal tissue, lane 4: breast cancerous tissues and lane 5: MW marker. (B) *Camelus dromedarius*. Lane 1: BSA, lanes 2: $P_{27}$-BSA, lane 3: normal breast tissue, lane 4: breast cancerous tissues and lane 5: MW marker.

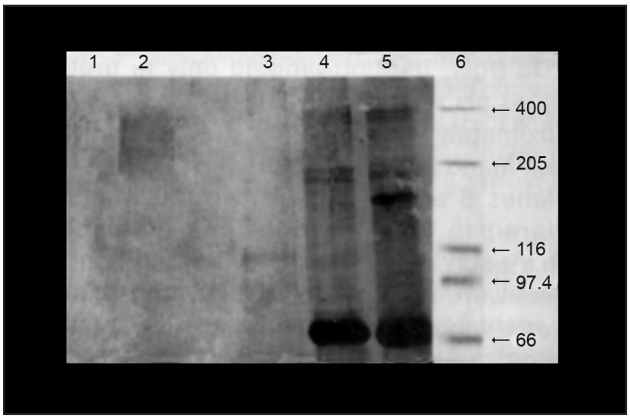


**Fig 6.** Immunoblotting of purified serum of *Camelus bactrianus*. Lanes 1 and 3 (with different concentrations): HMFG, lane 2: deglycosylated HMFG, lane 4: cancerous breast tissue, lane 5: normal breast tissue, lane 6: MW marker.

prompted a further investigation of purified sera of camels. Using purified serum (precipitation method using polyethylene glycol 6000) antigen specificity in different camel immunoglobulins was analysed by immunoblotting. Homogenised normal and cancerous breast tissue, BSA and synthetic peptide conjugated to BSA were separated by 10% SDS-

**Table 1.** Percentages of different IgG fractions found in *Camelus bactrianus* and *Camelus dromedarius*.

| Camel species | Fractions (%) | | | Total IgG (mg/ml) |
|---|---|---|---|---|
| | $IgG_1$ | $IgG_2$ | $IgG_3$ | |
| *C. bactrianus* | >25 | 25-35 | 40-45 | 8-12 |
| *C. dromedarius* | >25 | 25-30 | 35-45 | 8-12 |

**Table 2.** ELISAs results at $OD_{450}$ nm for various antigens using rabbit anti-camel-HRP as tracer.

| Antigens (1 µg/well) | *C. bactrianus* | | | *C. dromedarius* | | | PBS* |
|---|---|---|---|---|---|---|---|
| | $IgG_1$ | $IgG_2$ | $IgG_3$ | $IgG_1$ | $IgG_2$ | $IgG_3$ | |
| BSA | 0.8 | 0.75 | 0.8 | 0.66 | 0.54 | 0.8 | 0.56 |
| $P_{27}$–BSA | 1.80 | 1.20 | 1.86 | 1.86 | 1.48 | 1.98 | 0.61 |
| $P_{16}$–BSA | 2.20 | 1.25 | 2.30 | 2.27 | 1.48 | 2.61 | 0.60 |
| HMFG | 1.68 | 1.40 | 1.39 | 1.96 | 1.62 | 1.90 | 0.7 |
| D-HMFG | 2.49 | 2.22 | 2.52 | 2.36 | 2.31 | 2.10 | 0.7 |
| N-T | 1.30 | 1.10 | 1.70 | 1.70 | 0.90 | 1.90 | 0.90 |
| C-T | 1.50 | 1.30 | 1.90 | 1.80 | 1.20 | 2.20 | 0.80 |

PAGE under reducing conditions and membranes were developed with purified sera and by using rabbit anti-camel (Fig 5A) and then sheep anti-camel-HRP as label (Fig 5 B). The results confirmed that antibodies only react with peptide part of peptide-BSA conjugation.

### *3.5. Binding of camels serum immunoglobulin to HMFG and deglycosylated HMFG*

The results of these experiments using *C. bactrianus* antibodies are shown in Fig 6 of only one broad band around the MW of 200 KDa up to 400 KDa indicated the presence of the MUC1 specific peptide motif (lane 2). However, no band was observed in lanes 1 and 3 where intact HMFG in higher concentrations were used. These results clearly indicated that camels were successfully immunised against unmasked protein of MUC1 (immuno-dominant peptide).

## 4. Discussion

In the present investigation, we compared the behaviour of *Camelus bactrianus* and *Camelus dromedarius* when immunised with a peptide of definite size and antigenic determinant, whether the two species of camel would exhibit different immune response with different fractions of immunoglobulin binding the peptide and BSA part of the molecule. In *Camelus dromedarius* two immunoglobulin fractions contain molecules of 100 KDa. These two IgG classes appear to lack the light chain completely (Lauwereys *et al*, 1998 and Muyldermans *et al*, 2001) Our results clearly indicated that *Camelus bactrianus* as like as *Camelus dromedarius* have both kinds of heavy-chain antibodies ($IgG_2$ and $IgG_3$). However, $IgG_3$ is the main constituent of IgGs in both of old world camels. Purification of IgG subclasses by protein G and protein A affinity chromatography and quantification of the total IgG fraction showed that in general there exist about 8-12 mg/ml of IgG in serum of *Camelus bactrianus* out of which $IgG_3$ is up to 45%. Thus $IgG_3$ titre is higher in *Camelus bactrianus* compared to *Camelus dromedarius*. In addition, a different profile of binding activity of immunoglobulin fractions was observed in which $IgG_3$ exhibited over all better reactivity with either of $P_{27}$-BSA, $P_{16}$-BSA, deglycosylated HMFG, and cancerous tissue preparations. This phenomenon was observed in both species of camels. We conclude that using the techniques described, antigen-specific heavy-chain antibodies are readily accessible from *Camelus bactrianus*, thus providing highly valuable binding molecules for variety of applications.

To assess the application and behaviour of heavy-chain antibodies, we selected a model peptide MUC1, tumor-associated epitopes on a high-molecular-weight glycoprotein molecule found in human milk (Girling *et al*, 1989). The expression of MUC1 is dramatically increased and pattern of glycosylation is altered when the cells became malignant. Thus in the breast, ovarian, lung, prostate, colon and pancreatic cancer mucin, glycosylation changes result in certain epitopes in the core protein being exposed. These epitopes (MUC1) are masked in the mucin produced by normal cells, and the epitope in the 20-amino acid tandem repeat sequence (Prince *et al*, 1998) was found to be accessible in these cancers (Taylor-Papadimitriou *et al*, 1999). We also prepared a 27 mer peptide-BSA and immunised camels with the cancerous tissues and peptide-BSA. ELISAs and immunoblotting assays of purified HMFG whole molecule, deglycosylated HMFG and normal and cancerous breast tissues indicated that good antibodies were obtained which reacts with deglycosylated MUC1 in better proportion.

Camels' heavy-chain antibodies have been found to be difficult to raise against peptides as antigen.

Recently, However, several antibodies have been raised against BSA-hapten or even RR6, copper containing dye (Spinelli *et al*, 2000).

Increasing affinity of antibodies with subsequent immunisation and individual factors like age has been described by Lange *et al* (2001). They described an ELISA with polyclonal anti-clenbuterol antiserum from llama. In our study, we produced good titre anti-peptide IgGs in young camels (after 150 days in *Camelus dromedarius* and after 180 days in *Camelus bactrianus*).

To the best of our knowledge, this is the first report on the use of a limited peptide (the epitope in tandem repeat of core protein of the MUC1 mucin) as a immunogen for production of heavy-chain antibodies in camels and the first report on the induction of immune response in two-humped camel (*Camelus bactrianus*) and also the comparative study of IgG subclasses antigen binding and specificity of antibodies of *Camelus bactrianus* and *Camelus dromedarius*.

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