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Production and Characterization of a Recombinant Camel Full Heavy Chain Antibody against Human IgE

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Abstract

Camel heavy chain antibodies (HCAbs) have novel properties that render them useful in diagnosis and immunotherapy of various diseases. The purpose of this study was to produce recombinant camel HCAbs directed against a synthetic loop polypeptide that mimics the FcεRI binding site on human IgE. A recombinant camel HCAb was purified and characterized after being cloned using One Shot TOP10 *Escherichia coli*, expressed in BL21 Star (DE3) *E. coli*. Out of nineteen successful clones only one named IgG211 was found to contain the IgG2 HCAb coding sequence in the correct orientation with 85% homology to camel IgG2. A 62kDa fusion protein was expressed in an insoluble form under Isopropyl β-D-1-Thiogalactopyranoside (IPTG) induction. Probond purified fusion protein was localized by immunoblot using mouse anti camel antibody. The reactivity of recombinant camel IgG211to its corresponding antigen using ELISA was 72.9% of the camel polyclonal IgG2. Thus, a successful production of a recombinant camel HCAb of the IgG2 isotype was achieved. Such achievement may contribute towards the application of the unique properties of camel HCAbs in the field of antibody-based therapy for the treatment of asthma and allergy.

Keywords: Camels, Antibodies, Immunoglobulin E, *Escherichia coli*, DNA, Recombinant protein, Antibody-based therapy.

1. Introduction

Among mammals, the Arabian camel (Camelus dromedarius) and other Camelidae produce special kinds of antibodies in their sera, known as heavy chain antibodies (HCAbs) (Hamers-Casterman et al., 1993, Arbabi Ghahroudi et al., 1997). The HCAb molecule lacks both light chains and consists of only two heavy chains that lack the CH1 domain. The small size, high affinity, solubility, close homology of the variable domain to that of human IgG, and stability at high temperature or in the presence of denaturing agents are remarkable characteristic features for the HCAbs variable heavy chain domains (VHH). Such novel properties of HCAbs has led to intensive research towards the production of HCAbs and VHH nanobodies against various viral, bacterial, protozoal and helminthic parasites, toxins and tumors as well as other immunologic and functional protein targets. In this regard, recombinant HCAbs and VHH were successfully prepared in bacteria and yeast through cloning, expression and selections of antigen-specific HCAbs and VHHs (Van de Laar et al., 2007; Muyldermans et al., 2009; Franco et al., 2010).

IgE antibodies are reaginic antibodies involved in atopic diseases such as allergic asthmas (Beeh et al., 2000; MacLean and Eidelman, 2001; Novak and Bieber, 2003). IgE binds to its specific FcεR1 receptor mast cells as well as blood basophils. Such interaction triggers a series of cascade reactions in these cells that results in various symptoms characterizing asthma and other IgE mediated allergic diseases (Gould and Sutton, 2008).

Allergic asthma is one of the most common and highly variable chronic disorders of respiratory airways with many symptoms and complications. Prevalence of allergic asthma with severe manifestations is increasing worldwide, particularly in industrial countries (Beasley et al., 2000). The currently available treatment strategies are inadequate to control the symptoms of severe asthma (Bukstein et al., 2005). Alternative drugs, based on the production of anti-human IgE monoclonal antibodies (anti huIgE MCA), have been attempted. Such anti huIgE MCA efficiently bind IgE and do not induce histamine release in vitro (Davis et al., 1993, Presta et al., 1994, Rabe et al., 1998). Also, camel polyclonal HCAbs that

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* **List of non-standard abbreviations:** huIgE: human IgE, MCAs: monoclonal antibodies, SLP: synthetic loop peptide, IB: Immunoblot.

efficiently block IgE binding to its FcεRI on human basophils in vitro and resulted in histamine release inhibition were efficiently developed (Al-Qaoud et al., 2015). Moreover, a novel strategy based on the generation of humanized MCAs against huIgE-FcεRI binding site have been engineered and approved by the Food and Drug Administration (FDA) in the USA as early as 2003 to prevent subsequent allergic symptoms (Doding et al., 2005).

As a further approach towards the search for therapeutic strategies in asthmatic patients, an attempt to develop and purify recombinant camel HCAbs against a synthetic polypeptide that mimic FcεRI binding site on huIgE was made in the present investigation.

2. Materials and Methods

2.1. Immunogen Preparation, Peptide Synthesis and Camel Immunization

Immunogen used in camel immunization was modified Synthetic Loop Peptide (SLP) with the basic sequence CGETYQSRVTHPHLPRALMRSTTKC (Wang et al., 2003). The SLP was prepared according to multiple antigenic peptides system (MAPS) (Tam 1988) forming SLP-MAPS immunogen (Alpha Diagnostic International Co., San Antonio, USA). A Local male camel (Camelusdromedarius) was immunized with an initial 0.5 mg of SLP-MAPS mixed with Stimune adjuvant (Prionics, Schlieren-Zurich, Switzerland) at a 1:1 ratio. The camel was immunized 5 times at 2 week intervals as in our earlier work (Al-Qaoud et al., 2015). At the end of the immunization protocol, anticoagulated blood was collected for lymphocytes isolation.

2.2. Lymphocytes Isolation, RNA Extraction and cDNA Synthesis

Lymphocytes were obtained from heparinized peripheral blood samples of SLP-MAPS immunized camel using Ficoll-Paque (PAA laboratories, Linz, Austria). The cell number was adjusted to (5×106) for RNA isolation. RNA was isolated using a Nucleospin RNA II Total RNA isolation kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions. Fiveμgof RNA was converted to cDNA by Reverse Transcriptase kit (Promega, Wisconsin, USA). The cDNA was used as a template to amplify the coding sequence for camel HCAbs.

2.3. Design of PCR Primers and pET102/D-TOPO Expression Vector Cloning Site

Two separate PCRs were done to clone the HCAb coding sequence in the pET102/D-TOPO expression vector using a shared reverse primer (Cam2) and one of two different forward primers. The forward primers that were used in the first and second PCRs were called CamV1 and CamV-Topo, respectively. The sequences of the primers were as follows:
CamV1: (5′ ACACACCATGGAGCTGGGGC 3'),
CamV-Topo:(5′ CACCGATGTGCAGCTGCAGGAGTC 3′), and
Cam2:(5'CTAGTGGGTCAGAAGCCCATTT3')
(Saerens et al., 2004).

The pET102/D-TOPO expression vector was designed to directionally clone the gene between N-terminal HP-thioredoxin and C-terminal V5 epitope and 6xHis tags and allows the inducible expression of proteins in *E. coli* strains under the control of T7 promoter (Figure 1).

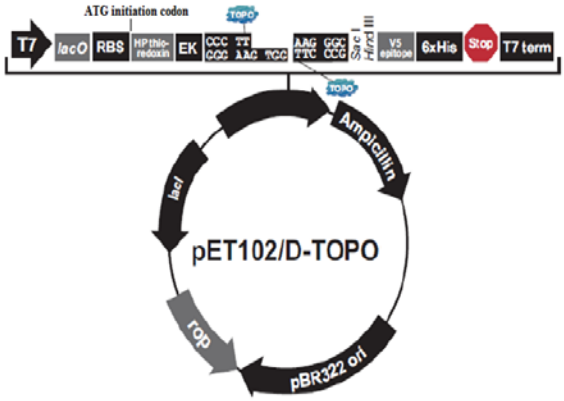

Figure 1: The pET102/D-TOPO expression vector cloning site and characteristics. The vector contains T7 promoter, lac operator (lacO), Ribosome binding site (RBS), ATG initiation codon, His-patch (HP) thioredoxin open reading frame (ORF), TrxFus forward priming site, EK recognition site, directional TOPO Cloning site, V5 epitope, Polyhistidine (6xHis), T7 reverse priming site, T7 transcription termination region, Ampicillin resistance gene, and pBR322 origin of replication.

2.4. Production of Blunt-End PCR Products

The HCAbcoding sequence was amplified by nested PCR using Phusion HF DNA polymerase(New England BioLabs, Massachusetts, USA) and ESCO Swift Max Pro Thermal Cycler (Esco Technologies, Changi, Singapore).The first PCR was done to separate HCAbs from the conventional IgG1. The PCR was done with 35 cycles at 60C annealing temperature. Separation of PCR product was done using 1% agarose gel electrophoresis after staining with ethidium bromide. The two bands near 1200 and 1300 base pairs, representing the two HCAbs isotypes coding sequences, were used as templates for the secondary PCR. The HCAbs were amplified in the second PCR reaction and the bands representing IgG2 and IgG3 coding sequences from the two second PCR reactions were extracted using Wizard SV Gel and PCR Clean-up system kit (Promega, Wisconsin, USA).

2.5. Assembly of the HCAb Expression Construct and Transformation of One Shot TOP10 E. coli Competent Cells

The Champion pET Directional TOPO Expression Kit (Invitrogen, California, USA) was used for cloning. The purified IgG3 and IgG2 PCR products were cloned into pET102/D-TOPO vector as per manufacturer instructions. The ligation mixture was transformed into One Shot TOP10 *E. coli* using the heat shock method.

2.6. Analysis of Positive Transformants by Colony PCR and DNA Sequencing

All the colonies that appeared on the LB-Amp (100μg/ml) plates were prepared for analysis by colony PCR using the TrxFus forward and T7 reverse sequencing primers supplied with the Topo cloning kit. The positive construct was sequenced to check the identity and orientation of the HCAb coding sequence using the

TrxFus forward sequencing primer (5'-TTCCTCGACGCTAACCTG-3´) with the aid of Eaton Corporation (Ohio, USA).The pET102/D-TOPO-HCAb construct was extracted using GenElute plasmid miniprep kit (Sigma, Missouri, USA) following specific manufacturer instructions.

2.7. Expression of the Recombinant HCAb Fusion Protein

Seven ng of the pET102/D-TOPO containing the expected anti-SLP-MAPS HCAb sequence was transformed into BL21 Star (DE3) *E. coli* using heat shock method following themanufacturer instructions. The culture containing the transformation reaction was grown overnight at 37ºC with shaking at 200 rpm and induced with 1mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) (Promega, Wisconsin, USA). The cells were harvested and resuspended in lysis buffer (50 mM potassium phosphate, 400 mM NaCl, 100 mM KCl, 10% glycerol, 0.5% Triton X-100, 10 mM imidazole, pH 7.8). The cells were lysed by freezing/thawing cycles (five times) using liquid nitrogen and 42°C water bath. After centrifugation, the supernatants containing the soluble fraction and pellets which contained the insoluble fraction were both used for analysis on 12% SDS-PAGE.

2.8. Purification of the Recombinant HCAb Fusion Protein and Analysis by SDS-PAGE

A fifty ml-sample of IPTG induced culture was used for the purification of the recombinant camel HCAb fusion protein using ProBond Purification System (Invitrogen, California, USA). Probond column was washed seven times and the unbound fractions were removed as per manufacturer instructions. Fractions were collected and analyzed by 12% SDS-PAGE.

2.9. Assessment of Recombinant Fusion HCAb by ELISA and Immunoblot (IB)

ELISA was used for the detection of the binding activity of the recombinant HCAb fusion protein with the SLP-MAPS synthetic peptide. Captured camel anti SLP-MAPS HCAbs were detected by HRP-labeled mouse anti camel antibodies prepared according to MonoJo's Standard Operating Procedures (SOP). Furthermore, the purified recombinant HCAb fusion protein, the induced bacterial lysate and uninduced bacterial culture were electrophoresed by SDS-PAGE and blotted on a nitrocellulose membrane using semidry immunoblotter purchased from Wealtec (Taipei, Taiwan). The presence of camel HCAbs was detected by the incubation with mouse anti camel-HRP as tracer. A relative reactivity index for recombinant IgG211 to anti-huIgE polyclonal IgG2 was calculated as per the formula: the reactivity of recombinant IgG211 divided by the reactivity of polyclonal IgG2 multiplied by 100.

3. Results

3.1. Camel HCAb Gene Amplification and Transformation

Nested PCR was performed to amplify the HCAbs coding sequence from lymphocytes of SLP-MAPS immunized camel (Figure 2). The resulting PCR products from the first reaction were three bands near 1500, 1300, and 1200bp. These bands represent camel IgG1, IgG2 and IgG3 coding sequences, respectively (Figure 2a). The second PCR re-amplified the gel purified IgG2 and IgG3 coding sequences (Figure 2b).The constructs containing the gel purified IgG2 and IgG3 coding sequences were transformed into TOP10 *E. coli* competent cells. The ligation efficiency was very low where only 15 and 4 bacterial colonies that resultedfrom the transformation of constructs contained IgG2 and IgG3 coding sequence, respectively.

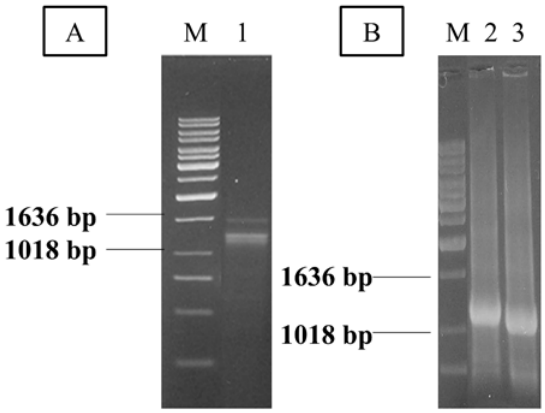

Figure 2: Nested PCR amplification products using 1% agarose gel. CamV-1 and Cam2 primers were used on camel lymphocyte cDNA.In A, IgG1 appears as a discrete band of ~ 1500bp while the two bands near 1018bp represent the HCAbs IgG2 and IgG3 complete coding sequence (Lane1). In B,CamVTopo and Cam2 primers were used on the gel purified IgG2 coding sequence (Lane 2), and on the gel purified IgG3 coding sequence (Lane 3), M: 1Kb DNA ladder.

3.2. Colony PCR and DNA Sequencing of Positive Transformants

All the 4 and 15 colonies were analyzed by colony PCR using the TrxFus forward and T7 reverse primers that annealed on the pET102 vector around the cloning site. Accordingly, the expected size of the clone containing the plasmid with an insert was around 1500bp. None of the 4 colonies yielded positive result, whereas only colony number 11 from the 15 colonies that resulted from transforming construct containing IgG2 coding sequence yielded positive result (Figure 3). This clone was named IgG211. Alignment of the sequence result of IgG211 on the NCBI database revealed homology with *Camelus dromedarius* heavy chain region with 84% identity. The sequence alignment showed a plus/plus result which indicates that the gene is in correct orientation.

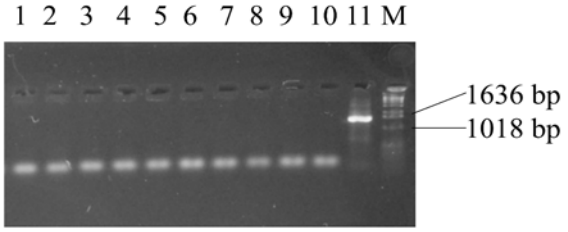

Figure 3: Colony PCR analysis of bacterial transformants harboring the pET102/D-TOPO-IgG2 construct using the TrxFus Forward and T7 Reverse primers. (Lanes 1-11) are bacterial colonies. Only colony number 11 contained the construct with the IgG2 coding sequence that was named $IgG_2$11. M: 1Kb DNA marker.

3.3. Expression, Analysis and Purification of Recombinant Fusion IgG211

Both the IPTG induced and uninducedIgG211 in BL21 (DE3) *E. coli* were both analyzed using SDS-PAGE to test for the expression of IgG2. Evidently, an over-expressed band near 65 kDawas revealed in the induced but not in the un-induced culture (Figure 4). Meanwhile, the expected band size was approximately 62 kDa including the expression of the N-terminal HP-thioredoxin and C-terminal V5 and 6His epitopes. The IPTG induced bacterial culture was analyzed for soluble/insoluble protein. A fusion form of IgG211 protein of approximately 65 kDa was completely found in the insoluble fraction (Fig 4). The recombinant IgG211 fusion protein was eluted and most of it was eluted in the first two fractions that yielded approximately 70% of the protein (Figure5).

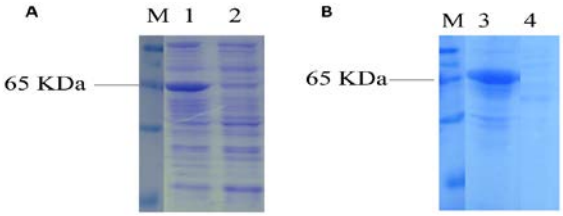

Figure 4: SDS-PAGE of IPTG induced and un-induced BL21 (DE3) *E coli* cell lysate harboring the $IgG_2$11 construct (A). Lane 1 for induced and lane 2 for un-induced. In B induced lysate was analyzed for insoluble (lane 3) and soluble protein (Lane 4). IgG211 appeared completely in the insoluble fraction (B lane 3). M: prestained molecular weight marker.

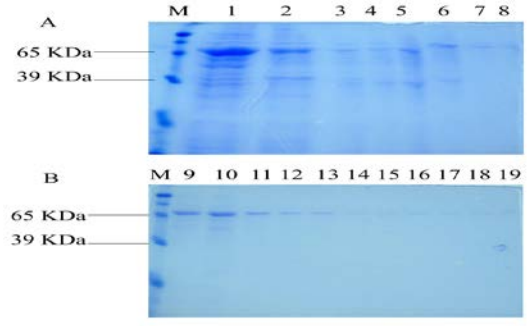

Figure 5. Purification of IgG211 from a 50 mL of induced BL21 bacterial culture using ProBond column. A 12% SDS-PAGE was done on washing (A) and elution (B) fractions. (Lane 1): induced BL21 bacterial cell lysate with abundant IgG211 fusion protein; (Lanes 2-8): wash fractions, (Lanes 9-19) IgG211 elution fractions with native elution buffer pH 7.8.

3.4. Activity Assessment of Purified Recombinant IgG211 Fusion Protein

The purified recombinant IgG211 fusion protein was analyzed by IB using mouse anti camel-HRP to confirm its identity. Purified IgG211 was captured by the mouse anti camel-HRP and appeared as discrete faint band. In addition, many nonspecific bands appeared in the un-induced bacterial lysate (Figure 6). Furthermore, the specificity of the purified recombinant IgG211 fusion protein was analyzed by ELISA. Low binding specificity was observed using SLP-MAPS as coating antigen. However, the relative reactivity index of recombinant IgG211 compared to polyclonal IgG2 was65.9% (Figure 7).

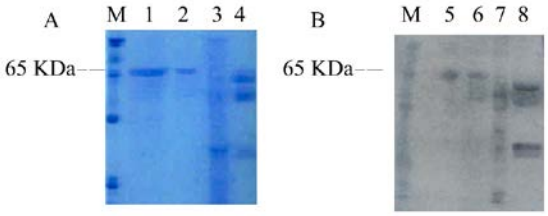

Figure 6. SDS-PAGE (A) and IB with mouse anti camel-HRP as a tracer (B) of the purified $IgG_2$11. (Lanes 1 and 5): induced BL21 bacterial lysate; (Lanes 2 and 6): purified $IgG_2$11; (Lanes 3 and 7): un-induced BL21 bacterial lysate; (Lanes 4 and 8): a mix of purified camel IgG1 and IgG3 as positive control, respectively.

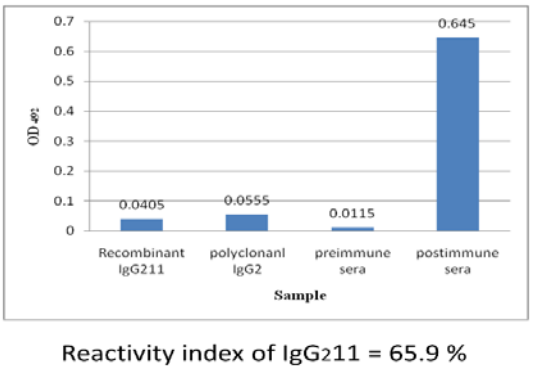

Figure 7. ELISA results of purified recombinant $IgG_2$11 to SLP-MAPS. Both preimmunized and postimmunized camel sera were used as negative and positive controls, respectively.

4. Discussion

As research on the dissection of camel immune response and antibody production is still in early stages, the lack of fusion myeloma partner for antibody production hindered the production of camel monoclonal antibodies (MCAbs). Nevertheless, recombinant HCAbs and VHH were successfully prepared through cloning, expression and selections of antigen-specific HCAbs and VHHs in bacteria and yeast (Arbabi Ghahroudi et al., 1997; Van der Vaart et al., 2006; Muyldermans et al., 2009; Franco et al., 2010). In this study, we produced a recombinant full camel HCAb against human IgE in *E coli* for the first time. IgG2 HCAb with coding sequence

in the correct orientation was confirmed upon the cloning and transformation in one *E. coli* colony named IgG211.

The IPTG-inducible expression of the recombinant IgG211 fusion protein in BL21 (DE3) *E coli* revealed that the IgG211 fusion protein was completely found in the insoluble fraction. This was opposite to what was expected, due to the presence of HP-thioredoxin at the N-terminal end which enhances solubility. However, the possible formation of insoluble recombinant proteins in form of inclusion bodies using *E coli* as n expression host was previously described (Ventura and Villaverde, 2006). Those workers indicated that aggregation of protein in the inclusion bodies renders it as unfolded or improperly folded protein.

The 46kDa size IgG211 was eluted as part of the 62kDa fusion protein which contained a 16 kDa HP-thioredoxin at the N-terminal end and a 6His-tag and V5 epitope at the C-terminal end. Due to the presence of the 6His-tag at the C-terminal of the IgG211 fusion protein, it was easily purified using ProBond nickel-chelating resin. Over 70% IgG211 was recovered in the first two elution fractions with relatively high purity. This high purity can be related to its presence in the inclusion bodies aggregates that is common to occur in *E coli* expression host (Ventura and Villaverde, 2006).

The other observation pertaining to this purified recombinant IgG211 fusion protein was its low reactivity against SLP-MAPS immunogen using the ELISA (Fig. 7). In our earlier work, (Al-Qaoud et al., 2015), we showed that the reactivity polyclonal IgG2 against SLP-MAPS was low using the ELISA, but was highly reactive using the flow cytometry technique. Thus, further analysis of the IgG211 recombinant protein by flow cytometry is warranted.

The production and use of orally administered anti huIgE HCAbs as immunotherapeutic agents is of great importance. This is due to several reasons which prefers the use of the aforementioned HCAbs compared to conventional antibodies. The high stability, solubility, affinity and most importantly low immunogenicity of HCAbs has been repeatedly documented. In addition, no cytotoxicity or anti-nanobody antibodies were detected in serum of mice injected with nanobodies for therapeutic purposes (Dumoulin et al., 2002; Cortez-Retamozo et al., 2004; Revets et al., 2005; Coppieters et al., 2006) that favors camel nanobodiesto be used in the treatment of various diseases. Moreover, high sequence homology between camel VH and human VH was confirmed by amino acid sequence analysis that revealed a difference of only 14 amino acid which in turns potentiate humanization process and rendering the humanized camel HCAb less immunogenic than humanized conventional antibody (Vincke et al., 2009; Deschacht et al., 2010). Furthermore, one key advantage that favor the use of camel HCAbs as blocking non-anaphylactogenic anti huIgE antibodies is their monovalent nature in contrast to the bivalent nature of mouse conventional IgG allowing HCAbs capable of targeting free IgE but not crosslinking bound IgE (Harmsen and De Haard, 2007). With these novel properties it is expected that the use of camel HCAbs as anti huIgE blocking antibodies will overcome the side effect that accompanied the use of the commercially available humanized mouse anti huIgE known as Omalizumab (Davydov, 2005; Doding et al., 2005; Slavin et al., 2009).

5. Conclusions

In conclusion, the superiority of camel HCAb (IgG2 and IgG3) against huIgE Cε3 over conventional camel IgG1 as revealed in earlier work (Al-Qaoud et al., 2015) paved the way toward successful production of recombinant camel HCAbs against SLP-MAPS. IgG211 was produced and expressed in E. coliand initial experiments to characterize it were done here. Evidently, the selection of higher affinity HCAb may be achieved by other techniques such as phage display technology which offers larger repertoire of antigen specific antibodies (Carmen and Jermutus, 2002; Arbabi-Ghahroudi et al., 2009). Presently, we are trying to produce camel nanobodies to be used as orally administered therapeutic drug against huIgE using the recombinant phage display technology.

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References

Al-Qaoud K. Yousef S. Rawashdeh A. Khalil R and Abdel-Hafez S. 2015. Blocking of histamine release and IgE binding to FcεRI on human basophils by antibodies produced in camels. *Allergy Asthma Immunol Res*, **7**: 583-589.

Arbabi-Ghahroudi M. Tanha J and MacKenzie R 2009. Isolation of monoclonal antibody fragments from phage display libraries. Bacteriophages. Springer, 502**:** 341-364.

Arbabi-Ghahroudi M. Desmyter A. Wyns L. Hamers R and Muyldermans S. 1997. Selection and identification of single domain antibody fragments from camel heavy-chain antibodies. *FEBS Lett*, **414**: 521-526.

Beasley R. Crane J. Lai CK and Pearce N. 2000. Prevalence and etiology of asthma. *J Allergy Clin Immunol*, **105**: S466-S472.

Beeh K. Ksoll M and Buhl R. 2000. Elevation of total serum immunoglobulin E is associated with asthma in nonallergic individuals. *Eur Respir J*, **16**: 609-614.

Bukstein DA. Jones CA. Ledford DK. Smith P. Wechsler ME and Urbano FL. 2005. Discussing the COSTS of asthma: controlling outcomes, symptoms, and treatment strategies. *Am J Manag Care*, **11**: S318-S336.

Carmen S and Jermutus L. 2002. Concepts in antibody phage display. *Brief Funct Genomic Proteomic*, **1**: 189-203.

Coppieters K. Dreier T. Silence K. Haard HD. Lauwereys M. Casteels P. Beirnaert E. Jonckheere H. Wiele CV and Staelens L. 2006. Formatted anti–tumor necrosis factor α VHH proteins derived from camelids show superior potency and targeting to inflamed joints in a murine model of collagen-induced arthritis. *Arthritis Rheum*, **54**: 1856-1866.

Cortez-Retamozo V. Backmann N. Senter PD. Wernery U. De Baetselier P. Muyldermans S and Revets H. 2004. Efficient cancer therapy with a nanobody-based conjugate. *Cancer Res*, **64**: 2853-2857.

Davis F. Gossett L. Pinkston K. Liou R. Sun L. Kim Y. Chang N. Chang T. Wagner K and Bews J 1993. Can anti-IgE be used to treat allergy?. Springer semin immunopathol, **15**: 51-73

Davydov L. 2005. Omalizumab (Xolair) for treatment of asthma. *Am Fam Physician*,**71**: 341-342.

Deschacht N. De Groeve K. Vincke C. Raes G. De Baetselier P and Muyldermans S. 2010. A novel promiscuous class of camelid single-domain antibody contributes to the antigen-binding repertoire. *J Immunol*, **184**: 5696-5704.

Doding S. Richter D. Cepelak I and Benko B. 2005. Anti-IgE therapy with omalizumab in asthma and allergic rhinitis. *Acta Pharm*, **55**: 123-138.

Dumoulin M. Conrath K. Van Meirhaeghe A. Meersman F. Heremans K. Frenken LG. Muyldermans S. Wyns L and Matagne A. 2002. Single-domain antibody fragments with high conformational stability. *Protein Sci*, **11**: 500-515.

Franco EJ. Sonneson GJ. DeLegge TJ. Hofstetter H. Horn JR and Hofstetter O. 2010. Production and characterization of a genetically engineered anti-caffeine camelid antibody and its use in immunoaffinity chromatography. *J Chromatogr B*, **878**: 177-186.

Gould HJ and Sutton BJ. 2008. IgE in allergy and asthma today. *Nat Rev Immunol*, **8**: 205-217.

Hamers-Casterman C. Atarhouch T. Muyldermans S. Robinson G. Hammers C. Songa EB. Bendahman N and Hammers R. 1993. Naturally occurring antibodies devoid of light chains. *Nature*, **363**: 446-448.

Harmsen M and De Haard H. 2007. Properties, production, and applications of camelid single-domain antibody fragments. *Appl Microbiol Biotechnol*, **77**: 13-22.

MacLean JA and Eidelman FJ. 2001. The genetics of atopy and atopic eczema. *Arch Dermatol*, **137**: 1474-1476.

Muyldermans S. Baral T. Retamozzo VC. De Baetselier P. De Genst E. Kinne J. Leonhardt H. Magez S. Nguyen V and Revets H. 2009. Camelid immunoglobulins and nanobody technology. *Vet Immunol Immunopathol*, **128**: 178-183.

Novak N and Bieber T. 2003. Allergic and nonallergic forms of atopic diseases. *J Allergy Clin Immunol*, **112**: 252-262.

Presta L. Shields R. O'Connell L. Lahr S. Porter J. Gorman C and Jardieu P. 1994. The binding site on human immunoglobulin E for its high affinity receptor. *J Biol Chem*, **269**: 26368-26373.

Rabe KF. Watson N. DENT G. Morton BE. Wagner K. Magnussen H and Heusser CH. 1998. Inhibition of human airway sensitization by a novel monoclonal anti-IgE antibody, 17-9. *Am. J. Respir Crit Care Med*, **157**: 1429-1435.

Revets H. De Baetselier P and Muyldermans S. 2005. Nanobodies as novel agents for cancer therapy. *Expert Opin Biol Ther*, **5**: 111-124.

Saerens D. Kinne J. Bosmans E. Wernery U. Muyldermans S and Conrath K. 2004. Single domain antibodies derived from dromedary lymph node and peripheral blood lymphocytes sensing conformational variants of prostate-specific antigen. *J Biol Chem*, **279**: 51965-51972.

Slavin RG. Ferioli C. Tannenbaum SJ. Martin C. Blogg M and Lowe PJ. 2009. Asthma symptom re-emergence after omalizumab withdrawal correlates well with increasing IgE and decreasing pharmacokinetic concentrations. *J Allergy Clin Immunol*, **123**: 107-113.

Tam JP. 1988. Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. *Proc Natl Acad*, **85**: 5409-5413.

Van de Laar T. Visser C. Holster M. Lopez CG. Kreuning D. Sierkstra L. Lindner N and Verrips T. 2007. Increased heterologous protein production by Saccharomyces cerevisiae growing on ethanol as sole carbon source. *Biotechnol Bioeng*, **96**: 483-494.

Van der Vaart J. Pant N. Wolvers D. Bezemer S. Hermans P. Bellamy K. Sarker S. van der Logt C. Svensson L and Verrips C. 2006. Reduction in morbidity of rotavirus induced diarrhoea in mice by yeast produced monovalent llama-derived antibody fragments. *Vaccine*, **24**: 4130-4137.

Ventura S and Villaverde A. 2006. Protein quality in bacterial inclusion bodies. *Trends Biotechnol*, **24**: 179-185.

Vincke C. Loris R. Saerens D. Martinez-Rodriguez S. Muyldermans S and Conrath K. 2009. General strategy to humanize a camelid single-domain antibody and identification of a universal humanized nanobody scaffold. *J Biol Chem*, **284**: 3273-3284.

Wang CY. Walfield AM. Fang X. Hammerberg B. Ye J. Li ML. Shen F. Shen M. Alexander V and MacGlashan DW. 2003. Synthetic IgE peptide vaccine for immunotherapy of allergy. *Vaccine*, **21**: 1580-1590.