DEVELOPMENT OF A NOVEL FORMAT OF STABLE SINGLE CHAIN ANTIBODIES AGAINST *Staphylococcus aureus* AND ALLERGEN-SPECIFIC IgE IN ALLERGIC ASTHMA

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Antibiotics, while having the power to control infection, neither have the capacity to counter effects of toxins produced by the microbes while in the host nor control inflammatory responses. Anti-inflammatory drugs address specific receptors, molecules or second messengers of the inflammatory cascade but have no effect on the pathogen. Immunosuppression to control infection-induced or allergic inflammation by steroids leave the host vulnerable to further secondary pathogen attack. This system of therapy not only create a negative spiral of more and more drugs and therefore greater and greater drug-related toxicity or worse, multi-drug resistance, this ultimately also leads to rampant abuse of the ecosystem that exists both inside and outside a living organism.

We propose to develop technology that is entirely biological and most importantly eco-compatible. This study aims to develop a library of novel format stable single chain nanobody library without the disadvantages of conventional antibodies that can have therapeutic, diagnostic and cosmetics use as well as food supplement as nutraceuticals. This grant proposal summarizes plans to develop such a library of **E**-compatible (eco-compatible) Targeted Biobodies or nanobodies (**E**TB**)** using the novel short chain variable fragments (scFv) technology or isolating camelid antibodies (VHH or Variable Heavy Chain antibodies) and generating an antibody repertoire for further downstream screening for specific uses.

The aim of the project will be to generate a naïve and a biased library of nanobodies (**E**TB) in the camelid format and screen against one of several different antigens including infectious agents, allergens and biomolecules. A combination of more than one fused dimeric antibody or a mixture of the two upto five, in appropriate formulation to curb specific needs and associated complications will be devised. The funded project involves generating proof-of-concept for the technology and raising VHH antibodies using phage display, as well as bacterial and yeast expression systems.

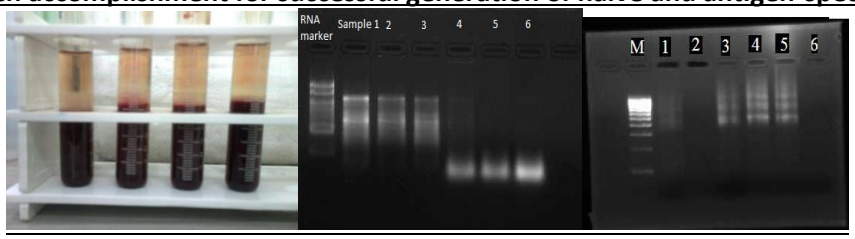
Goals achieved till date (Jan 2012-Aug 2013)

- Establishment of a molecular biology facility, procurement of important instruments and scientific workers a fellow and a technician were hired for three years
- Collaborative formalities established with Professor Serge Myuldermans, Flanders University, Belgium, Professor Dirk Saerens, Flemish Institute for Biotechnology, Brussels
- MTA signed for (phage display) expression system from above collaborators and materials received
- MoU signed with NRCC, Bikaner for work on camels

Research accomplishment till date (Jan 2012-Aug 2013)

- Optimization of PBL separation and total RNA preparation
- Successful synthesis of cDNA library from camelid VHH RNA
- Successful cloning and expression of VHH clonal proteins by proprietorial phage display system
- High throughput screening by ELISA and column chromatography for isolation of best binding clones
- Validated P-O-C of platform technology

Research accomplishment for successful generation of naïve and antigen-specific camelid antibodies



A. B. C.

Fig.1 RNA from fresh camel blood (A. PBL isolation from whole blood) collected from camels (B & C. Lane 1marker, Lanes 2-6, samples from various camel breeds)

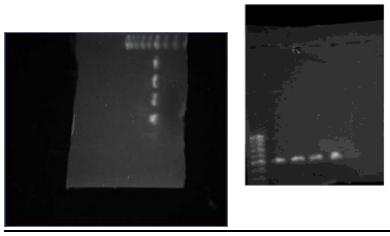
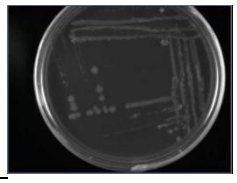


Fig. 2A & B. cDNA amplification and agarose gel electrophoresis



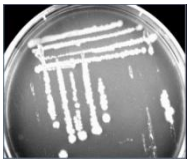
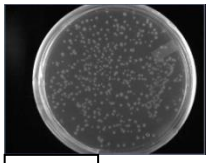
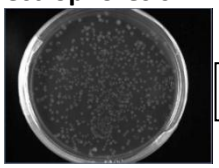


Fig.3 A & B. *E.coli* TG1 bacterial culture WK6 bacterial culture



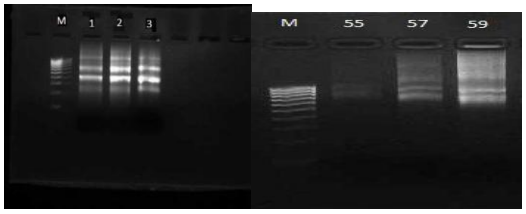
4A



4B

Fig. 4A&B. Colonies of transformed *E.coli* DH5α with pET16b (A) and transformed *E.coli* BL21 with pET16b (B) in Ampicillin + plates

5A



5B

Fig.5A&B. showing the PCR product of cDNA, at 57°C is selected (A) and **2nd PCR using notI & pstI Lane 1 is Marker and 2, 3,4 are sample**

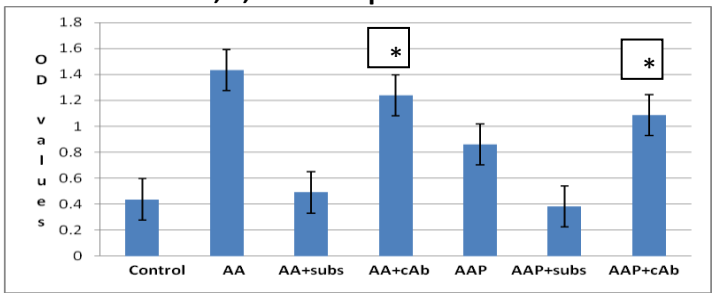


Fig.6. ELISA screening of antibody clones

alpha amylase (AA)	AA+cAb	anti AA camelid	AAP+subs	AAP+substrate
		anti AAP	Control (negative	Non-specific
alkaline phosphatase (AAP)	AAP+cAb	camelid	control)	Ag

*denotes p value <0.001 compared to OD taken with only enzyme (AA or AAP) or positive control