produced in bacteria, is very stable and highly soluble and highly yield and it binds the antigen with high affinity and specificity. Very often the Nanobody recognizes an epitope that is difficult to target with human or mouse antibodies. the largest advantage of Nanobodies comes from their strict monomeric behaviour and the ease to tailor them into larger pluripotent constructs. Now, nanobody applied for many application for example in tumor targeting for Search maximal tumor load and fastest blood clearance (invivo imaging) and in therapy in Ab dependent enzyme prodrug therapy (Nbs against African trypanosomes). There are more than 25 programmes in research and development on nanobody production, and there were five Nanobodies in clinical development. Two Nanobodies are on track to reach potential clinical proof-of-concept during 2011(TNFα, vWF, RANKL, IL-6R ,CXCR4, RSV,...).

Keywords: Nanobody, Nanotechnology, Nature

290. Production of Nanobodies from Camel-derived Heavy Chain Antibodies against Vascular Endothelial Growth Factor (VEGF)

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Background: Vascular Endothelial Growth Factor (VEGF) is a molecule which has important roles in blood vessels in tumors and therefore is an attractive target for anti-cancer therapy. Heavy chain antibodies (HcAbs) are a special kind of antibodies produced in the Camelidae family and have unique features in medical applications. In this research Camelid heavy chain antibodies have been produced against recombinant VEGF and a phage library was constructed. Materials and Methods: A 110 bp sequence of the VEGF protein was synthesized and subcloned into the pet32 vector. The recombinant protein was expressed in *E. coli* and purified by Ni-NTA column and was then injected to a camel. Lymphocytes were extracted from the blood and RNA purification was performed. cDNAs were constructed from RNAs by RT-PCR. Amplification of the cDNA is performed using Nested PCR. and VHH fragments were constructed from the cDNA. The VHH fragments were cloned into phagemid PComb3x and a library was constructed via phage display technique. Results and Conclusion: The 35 kDa VEGF peptide was expressed successfully. After injection, immunization of the camel was confirmed by ELISA. Lymphocytes were extracted successfully from the blood. Total RNA extraction was confirmed with the presence of 28s and 18s RNAs in the Agar gel and cDNA construction was accomplished successfully. The 600 and 700 bp bands were observed in the gel from the first PCR, and 400 bp from the second PCR. Cloning of the VHH domain was done in PComb3x and the titer of the library was high.

Keywords: VEGF, Heavy chain antibody, VHH domain , phage display

291. Tumour Inhibitory Effects of EGFR-Mimotope Bacteriophage Based Vaccine

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Background: Epidermal growth factor receptor is one of many tyrosine kinase receptors with oncogenic potential. Its over-expression is usually associated with cancer progression, invasion and metastasis. It is over-expressed in a wide variety of human malignancies. Several small molecule inhibitors and monoclonal-antibodies have been used to treat EGFR over-expressing tumours. One example of such antibodies is ICR-62 produced in rat which showed promise in preclinical studies. Previously in our lab, a peptide mimotope of EGFR corresponding to the binding region of ICR62 was isolated from a phage display library and was displayed on P-VIII of M-13 phage and used to immunize mice, the antibody response was evaluated. In this study we assessed the tumour inhibitory potential of the EGFR-mimotope-bacteriophage based vaccine *in vivo*. Both prophylactic and therapeutic effects of EGFR vaccine were evaluated. Materials and Methods: In prophylactic group of animals, the EGFR-momotopre-phage vaccine was used to immunize mice. Helper-phage and PBS injected groups of mice were used as negative control. Two weeks after the last immunization, mice were challenged sc in the right flank by LL/2 cells and tumour volumes were measured for four weeks. In therapeutic group the animals were injected sc in the right flank with LL/2 cells. After tumour formation, the mice were immunized by EGFR-mimotope-phage vaccine, helper phage and PBS as negative control. Tumour volumes were measured for four weeks. Results and Conclusion: In prophylactic group of animals there was no significant difference in tumour growth rate of animals immunized by EGFR-mimotope phage vaccine in comparison with helper phage immunized and non-immune mice.In therapeutic group of animals, both EGFR-mimotope vaccine and helper phage significantly reduced tumour growth in comparison with non-immune mice. However there were no significant difference between EGFR mimotope phage vaccine immunized and helper phage immunized mice. Thus we are yet to decide whether this vaccination strategy is successful.

Keywords: EGFR-Mimotope-bacteriophage, Vaccine, *in vivo*

292. *Erwinia Chrysanthemi* L-Asparaginase: Epitope Mapping and Production ofAntigenically Modified Enzymes

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This study shows that the antigenicity of *Erwinia chrysanthemi* Lasparaginase can be reduced by site-directed mutagenesis. Interest in L-asparaginase (EC 3.5.1.1) has grown considerably since this enzyme was found to have anti-tumour activity. The bacterial L-asparaginases from *Erwinia chrysanthemi* and *Escherichia coli* are effective in treating acute lymphoblastic leukaemia and lymphosarcoma, but their use against other forms of leukaemia or solid tumours is limited, since remissions are invariably of short duration .L-Asparaginase catalyses the hydrolysis of Lasparagine. Some tumour cells are deficient in L-asparagine synthetase and cannot synthesize sufficient L-asparagine. These cells are dependent on extracellular sources of the amino acid in order to complete protein synthesis. They can therefore be destroyed by starving them of L-asparagine by the administration of L-asparaginase.

Ten B cell epitopes of the enzyme were identified using synthetic hexapeptides and polyclonal antisera from rabbits and mice. The region 282GIVPPDEELPG292 near the C-terminus was an immunodominant epitope. Binding of two hexapeptides (2831VPPDE288 and 287DEELPG292) to the antibodies was dependent on Pro285, and Pro286, since their replacement by almost any other amino acid resulted in reduced binding. The other residues were less important for binding the antibodies, as binding was relatively unaffected by amino acid substitutions. Three site-directed mutant enzymes, P285T (proline-285 -. Threonine etc.), P286Q and E288A, were expressed in Escherichia coli. The pI values of P285T, P286Q and the wild-type enzymes were 8.6, and that for the mutant E288A was 9.2. The kcat and Km values for the mutants P286Q and E288A with L-asparagine and L-glutamine were comparable with those of the wild-type enzyme. The Km values for the mutant P285T with both substrates was similar to that of the wild-type enzyme, whereas the kcat was reduced by 2-fold with L-asparagine and by 4-fold with L-glutamine. The change proline -+ threonine reduced the antigenicity of the enzyme by 8-fold, as shown in sandwich using monoclonal antibodies raised against the wild-type enzyme.

Keywords: Epitope Mapping, Antigenically , Enzymes

293. Construction of an Efficient Expression Vector Encoding Soluble Form of Human Hyaluronidase Type PH20 for Purpose MS CompoundTherapy

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Background: The hyaluronidases are the enzymes hydrolyze β-1, 4 glycosidic linkage of hyaluronan. Hyaluronan is a polymer consisting of a repeating disaccharide unit found in cumulus ovuforus complex, semen liquid and other tissue. Addition to hydrolyzing the hyaluronan , hyaluronidase can penetrate through the cumulus cells layer that surrounds the oocyte, thus it terms spreading factor. Moreover, it is used to