

## **9. Production of Single Chain Antibodies against a Variety of Carbohydrate Epitopes**

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Phage-display technology has been applied to obtain human single chain antibodies (scFvs) against carbohydrate antigens. To establish methodologies as a first step, phage-displayed antibodies (phage Abs) that recognized mannose residues were isolated. Phage Ab library representing a large repertoire was prepared by CDR and VL/VH shuffling methods with unique vector constructs. The library was subjected to four rounds of panning against neoglycolipid synthesized from mannotriose (M3) and dipalmitoylphosphatidylethanolamine (DPPE) by reductive amination. Of 672 clones screened by ELISA using M3-DPPE as an antigen, 25 positive clones with different amino acid sequences were isolated as candidates for phage Abs against M3 residues. TLC-overlay assays and surface plasmon resonance analyses revealed that phage Abs thus far analyzed bound to neoglycolipids bearing mannose residues at non-reducing termini. In addition, selective binding of the phage Abs to RNase B carrying high mannose type oligosaccharides but not to fetuin carrying complex type and *O*-linked oligosaccharides was confirmed. Characterization of scFvs expressed from respective phages demonstrated affinity and specificity for non-reducing terminal mannose residues. These results demonstrate the usefulness of this strategy in constructing human scFvs against various carbohydrate antigens. Isolation and characterization of clones against Lewis-related carbohydrates, T antigen, and gangliosides are now in progress.