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Selection and Characterization of Genetically Engineered Lectins through a Phage Display System

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Cell surface carbohydrates are known to be potentially involved in cell recognition and trafficking. Structural variations of cell surface carbohydrates are believed to be as diverse as the recognition molecules in the immune system, yet the range of specificity of endogeneous carbohydrate recognition molecules is not diverse enough to correspond to the variations of carbohydrate chains.

Maackia amurenis hemagglutinin (MAH), a leguminous lectin, is known to recognize carbohydrate chains containing sialic acid residues. To generate novel lectins having a wide variety of specificities, the 24 nucleotides comprising the carbohydrate-recognition domain of MAH cDNA were randomly mutated based on the knowledge of structure-function relationships. The mutant lectins were expressed as glutathione-S-transferase fusion proteins in *Escherichia coli* and 16 clones were randomly chosen. Although all of 16 recombinant lectins reacted strongly with anti-MAH polyclonal antibody, the carbohydrate-recognition domain of each was unique. As shown by agglutination studies, each mutant MAH lectin was able to bind to erythrocytes from one or more of five animal species in very distinct patterns. Thus, novel plant lectin libraries can be used to discriminate in a highly specific manner among a variety of cell types. This technology may prove to be very useful in a number of different application requiring a high level of specificity in cell identification.

Two phage display systems, pComb3 and pComb8, were adapted to select high affinity mutant lectins from a library of virtually unlimited numbers of novel lectin with diverse specificities. First, carbohydrate binding capacity of phages displaying wild-type MAH lectin was assessed. Specific bindings of pComb3 and pComb8 phages expressing MAH to affinity purified polyclonal anti-MAH antibody and to glycophorin was demonstrated. Both phages also showed strong hemagglutinating activity to intact but not sialidase-treated human erythrocytes, which is consistent to the specificity of naive MAH. A library of mutant MAH containing randomized carbohydrate-recognition domains was constructed into pComb3 phagemid. The generated library of ~10^8 components was panned to human erythrocytes. Most of the selected phages contained the amino acid sequence of native MAH, although it is highly unlikely that

MAH is naturally selected for its binding to human erythrocytes during the evolution. Ten MAH mutant lectins having distinct amino acid seqences of carbohydrate-recognition domain were obtained. These mutant lectins were tested for their carbohydrate specificities by the use of glycophorin and carbohydrate-conjugated soluble polyacrylamides with Neu5Acα2-3Galβ1-3GalNac, Galβ1-3GalNac, Neu5Acα2-6GalNac, or 3' sialyllactosamine. Ten mutant lectins showed three different patterns of binding activities. One group bound only glycophorin A. The second group bound both glycophorin A and Neu5Acα2-3Galβ1-3GalNac-conjugated polyacrylamide with the intensities of this order of magnitude. The third group also bound both with a preference to Neu5Acα2-7Galβ1-3GalNac-conjugated polyacrylamide. Thus phage libraries with mutated MAH are useful source to prepare unique plant lectins with high affinity toward a variety of carbohydrate chains. This technology should be effective in a number of different applications, for instance, cancer therapy, tissue engineering, and the production of useful biological compounds.