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IMMUNOREACTIVE HCV NS3/4A PROTEIN WITHOUT NS3 SERINE PROTEASE ACTIVITY

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Background:

Hepatitis C virus nonstructural protein 3 (NS3) is 630 amino acid protein containing three functional domains. The serine protease domain is located in the amino terminus, whereas the helicase and NTPase are in the carboxy terminus. The serine protease of NS3 is responsible for the cleavage at the junction of NS3/4a, NS4a/b, NS4b/5a and NS5a/b. Previously we have expressed NS3/4a in yeast and purified the protein in non-denaturing conditions. The purified NS3/4a is a conformational protein, and is more sensitive than c200 or c33c antigen in early seroconversion antibody detection. In antibody assays using NS3/4a and MEFA 7.1 (Multiple Epitope Fusion Antigen) as antigens, we found the new antigens achieved 2-14 days of earlier detection of seroconversion antibodies in comparison with currently marketed assays. However, the NS3/4 protein did undergo self-hydrolysis and cleave MEFA 7.1 due to the protease activity. This study investigated if the immunoreactivity of NS3/4a might be preserved while the protease activity is eliminated.

Methods:

The mutant proteins, NS3(S1165A) and NS3/4a(S1165A), were generated where Ser1165, one of the catalytic triad amino acids in the protease domain of NS3, is mutated to Ala. The protease activity and immunoreactivity of the mutant proteins were studied and compared with normal NS3/4a.

Results:

The mutants, NS3(S1165A) and NS3/4a(S1165A), had expression levels in yeast similar to that of NS3/4a, and was purified to similar purity as in NS3/4a (>90% purity). In antibody assays, NS3/4a(S1165A) showed 90% immunoreactivity as compared with NS3/4a, whereas NS3(S1165A) had 80% immunoreactivity. When co-coated with MEFA 7.1 on an ELISA plate, both mutant proteins achieved immunoreactivity very similar to that of NS3/4a in early seroconversion antibody detection. It has been confirmed on SDS-

PAGE and Western blot, that the mutant NS3/4a(S 1165A) showed the full length of the protein without cleavage of NS4a, and neither mutant protein cleaved MEF A 7.1.

Conclusions:

This is the first evidence demonstrating that the enhanced immunoreactivity of the NS3/4a protein is not affected by elimination of the serine protease activity of the protein. To further improve assay sensitivity and ease of assay development, we hope to adopt this conformational sensitive protein to well characterized streptavidin plate. To achieve that, studies are underway to derivatize the mutant with a short amino acid tag that can be recognized by a high affinity biotinylated antibody.