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Diagnostic reagents for hepatitis C virus

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Summary. The development of diagnostic methods for hepatitis C virus is presented. Special attention is paid to the selection of antigenic markers, the type of assay selected and the interpretation of results. A few of the pitfalls and ambiguities of various assays are discussed and possible future methods are described.

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Parenterally-transmitted non-A, non-B hepatitis (PT-NANBH) is an important public health problem. With effective screening for HBV, PT-NANB accounts for more than 90% of post-transfusional hepatitis with perhaps 10% of transfusions resulting in disease. Additional routes of transmission include intravenous drug abuse, contaminated blood products and other parenteral transmissions. The virus cannot be cultured and there are only low levels of virus in infectious material (e.g. blood or liver).

For many years there have been false alarms concerning the identification of the infectious agent. Recently PT-NANB-specific DNA was isolated from cDNA libraries prepared from infectious chimpanzee plasma [1]. The initial clone (5-1-1) was used to identify overlapping clones and the sequence of the non-structural gene region of the virus was published. The virus has been designated hepatitis C virus (HCV) and is described as having a single-stranded, positive sense RNA genome which expresses its gene products as a single polyprotein.

The putative NS4 region of HCV has been expressed as a fusion protein with human superoxide dismutase in yeast and this recombinant protein (C-100-3) has been used in immunoassays to screen for the presence of HCV-specific antibodies [2]. Previously surrogate markers (anti-HBc and

ALT) have been used to screen blood donations for potential NANB-infected units in some countries. A confirmatory assay (RIBA) using printed strips of test and control antigens is now available to supplement the EIA. A second EIA, using the same antigen but on beads, plus a neutralisation-based confirmatory assay are now available from a different supplier. These assays represent a step forward in PT-NANBH research but in some ways have raised as many questions as they have answered.

Very little is known about the immune response to HCV antigens; indeed little is known about the HCV antigens themselves. It would be remarkable if C-100-3 were the most effective antigen to use in screening blood donations. In addition, what are the implications for an individual found to seropositive for anti-C100-3 antibodies? Are RIBA and the neutralisation test the best ways to confirm HCV? We have attempted to answer some of these questions using HCV-specific reagents produced from our own independent clones.

The published HCV sequence has been used by a number of groups who have presented partial sequences from different isolates [3, 4] including the structural protein region from Japanese patients [5, 6]. Independently of the published sequences, we have isolated cDNA clones from known human carriers of PT-NANBH. In particular, we have identified two overlapping clones, JG2 and JG3, which come from the putative NS5 region as well as a single clone, BR11, which contains structural sequences. These clones were recombined with a baculovirus based expression vector to produce the encoded antigens in insect cells. The non-structural (NS) recombinant was designated BHC-7 and the structural (S) recombinant BHC-9.

The two antigens were first used independently in an anti-human immunoglobulin format EIA to determine the antibody status of various sera from individuals at high-risk of HCV-infection e.g. haemophiliacs. We found that BHC-9 detected antibody in a higher proportion of the samples than did BHC-7 (25/32 vs 17/32 haemophiliacs); however using both antigens together 26/32 were positive. As might be expected, the structural antigen was more effective than the non-structural but there are some sera which appear to have antibodies only to non-structural regions. These observations were borne out by other risk groups and all future work has used both antigens together.

Initially the combined antigens were compared with the commercially-available, C-100-3-based EIA. In general the two antigens together detect antibody in a higher proportion of any patient group than C-100-3 alone even though there are some samples which are C-100-3 positive but negative with BHC7+9. These discrepancies must be resolved. The first point to note is that we are not comparing like with like and, given our current state of knowledge, it is possible that sera which react with one HCV antigen might not react with all other HCV antigens. Those samples which are BHC7+9 positive and C-100-3 negative have been assayed with the two antigens separately. We find that the majority of these samples react with only the

structural antigen BHC-9. Whilst the majority of reactive sera contain antibodies to all three antigens, significant numbers of sera have antibodies to only one or two of the antigens. As yet the prognostic value of these different patterns of antibody response is unknown.

Another related approach to analysing these discrepant samples is to use a confirmatory assay such as an immunoblot or a neutralisation test. We have our own in-house western blot using various purified HCV recombinant proteins to confirm BHC7+9 reactive sera. In general, many of the C-100-3-positive, BHC7+9-negative samples are RIBA negative or indeterminant whereas we find that only a few BHC7+9 reactive samples are falsely positive due to reaction with non-HCV-protein contaminants.

PCR amplification can be used to determine the presence of viral RNA in the sample. Care must be exercised when using PCR, not only technically to avoid contamination but also in interpreting the results. A positive signal under properly-controlled conditions will indicate current infection; but a negative result may mean that an infection has resolved or that the level of virus is below the limit of the method or that the sequence of the virus is sufficiently distinct that the amplification primers do not function efficiently. We do not know enough about the biology of HCV yet to address these points. Nevertheless we currently find that about 45% of the samples which are reactive with both C-100-3 and BHC7+9 are PCR-positive. Of those which react with only BHC7+9, about 30% are PCR-positive; whereas only 6% of those reactive only with C-100-3 are PCR-positive. Expressed another way, of 25 PCR-positive samples, 24 react with BHC7+9 and only 18 react with C-100-3.

In conclusion, we are at an exciting stage of the study of PT-NANBH. The most important agent of this disease has been identified and reagents are becoming available to study the response to infection. As more and more different antigens of HCV are used, it is clear that the antibody profile can differ from individual to individual and we may be able to identify those who will progress to chronic disease. Additionally we should be able to identify prognostic markers which will provide an early indicator of changes in the disease state.

References

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