Low Prevalence of Anti-E1 Antibodies Reactive to Recombinant Type 1b E1 Envelope Protein in Type 2, 3, and 4 HCV Sera, but High Prevalence in Subtypes 1a and 1b

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In order to measure the prevalence or the degree of cross-reactivity of hepatitis C virus (HCV) anti-E1 antibodies in a panel of 100 HCV sera that were previously classified into 4 genotypes and their subtypes by means of a new Line Probe Assay (LiPA), recombinant E1 protein was expressed in mammalian cells from a recombinant vaccinia virus containing a cDNA clone derived from the type 1b isolate, HCV-B, and used to coat microtiter plates. Sera were tested both on E1 and wild-type (wt) vaccinia virus-derived material. The following number of sera with signal to noise ratio (S/N) > 1 and E1/wt > 2 were found: 50% (7/14) for type 1a, 68% (23/34) for type 1b, 29% (6/21) for type 2, 20% (4/20) for type 3, and none of the 11 type 4 sera was positive. Surprisingly, although one-fifth of the E1 protein differs between the type 1 subtypes 1a and 1b, type 1a sera reacted nearly to the same degree with the type 1b E1 protein as did the much more homologous type 1b sera. As expected, only a few non-type 1 sera from Brazil were reactive with the type 1 E1 protein, whereas all European and African non-type 1 sera failed to react.

Key words: Anti-E1—HCV—Envelope—Cross-reactivity—Genotyping—LiPA

Introduction

After the discovery of several new genotypes of hepatitis C virus (HCV) [1–5] and the development of the HCV Line Probe Assay (LiPA) typing assay [5], it recently became possible to analyze the prevalence of anti-E1 antibodies against the homologous (derived from the same subtype) E1 antigen and to measure the degree of cross-reactivity between subtypes and types of HCV.

The E1 antigen, used to determine the presence of anti-E1 antibodies in the present study, can be classified as type 1b based on the sequence homologies with other isolates. The classification system of this study has been described in detail elsewhere [5]. About 1 in 5 amino acids in this recombinant protein differ from the type 1a sequence, and about 1 out of 3 amino acids differ from E1 proteins of other HCV types [6]. A panel of 100 sera that had been genotyped by means of a new LiPA [5] was used to determine whether the type 1 subtypes 1a and 1b, or the different HCV genotypes 1, 2, 3, and 4, exhibited cross-reactivity at the E1 envelope level.

Methods

Serum Samples

Serum samples were collected from blood transfusion centers, hemodialysis units, and university hospitals in Europe (mainly Belgium and the Netherlands), Brazil, and Gabon. All sera were positive by Innotest HCV Ab II (Innogenetics, Ghent, Belgium), confirmed by INNO-LIA HCV Ab II or III (Innogenetics, Ghent, Belgium), and by polymerase chain reaction (PCR) in the 5’ non-translated region. These PCR fragments were subjected to genotyping by means of the prototype HCV Line Probe Assay (LiPA) [5]. The classification system used throughout this work has been explained in Stuyver et al. [5]. The samples could be classified into 14 type 1a, 34 type 1b, 19 type 2a, 2 type 2b, 20 type 3, and 11 type 4 sera.
cDNA fragments covering the complete structural region of the Belgian type 1b isolate HCV-B were cloned (G. Maertens et al., unpublished data) and expressed by means of recombinant vaccinia viruses (G. de Martynoff et al., unpublished data). Cell lysates of E1 and wild type (wt) vaccinia virus-infected cells were partially purified.

Anti-E1 ELISA

Semi-purified material obtained from E1 or wt vaccinia virus-infected cell lysates was diluted 20 times in 50 mM sodium carbonate buffer pH 9.6 and coated onto Nunc maxisorb microtiter plates (Nunc, Denmark) for 16 h at 4°C. The plates were washed twice with PBS/1% Tween 20, blocked with PBS/0.5% casein for 2 h at 24°C, and incubated with a 1/20 dilution of the sera in PBS/0.5% casein/0.2% Triton/5% wt/vol cell lysate, for 1 h at 37°C. Plates were washed 5 times as indicated above, and antibodies were detected by means of a goat anti-human IgG peroxidase conjugate (Dako, Denmark) for 1 h at 37°C. Plates were washed 5 times, after which bound conjugate was revealed by incubation with 87 μg/ml tetramethylbenzidine in substrate buffer for 30 min at 24°C, and optical density (OD) values were read at 450 nm. Each sample was analyzed twice for reactivity with E1 and twice for reactivity with wt proteins, and for most samples this procedure was repeated several times. The cutoff was calculated as the average of OD values obtained with 10 blood donor sera that frequently showed aspecific reactivity (to cellular or vaccinia proteins), elevated with 3SD. Signal to noise ratios (S/N) were calculated as the E1 OD/cutoff. E1 to wt type ratios (E1/wt) were calculated as E1 OD/wt OD. Samples were considered positive when S/N > 1 and E1/wt > 2. All samples with S/N > 1 had E1/wt ratios of > 2.

Results and Discussion

Anti-E1 Cross-reactivity Between HCV Type 1 Subtypes

At first, the cross-reactivity between the ‘American’ subtype 1a and the ‘Japanese’ subtype 1b was investigated using the type 1b E1 antigen. In fact, these subtypes are distributed throughout the world, and the study was not performed with American or with Japanese sera, but mainly with Brazilian and Belgian sera. Figure 1 shows the S/N ratios obtained with the 14 type 1a sera and the 34 type 1b sera in the anti-E1 ELISA, and Fig. 2 shows the average S/N ratios of positive versus negative sera. Surprisingly, although the prevalence of anti-E1 antibodies has been reported to range from 7% to 23% [7] or from 36% to 42% [8], 68% of type 1b and 50% of type 1a sera were positive. When sera with E1/wt > 2, but S/N < 1 are included, 9 more samples become positive, 5 of which are type 1a, and 3 of which are type 1b; only 1 of the 52 non-type 1 sera (a type 3) becomes positive, suggesting the presence of a low-titer of anti-E1 antibodies in some of the sera. Applying these less stringent criteria, as much as 86% (12/14) of type 1a and 76% (26/34) of type 1b sera are positive (79% of all type 1 sera tested). This high degree of positivity could partly originate from using genotyped samples, but presentation of the E1 protein in a more native way as in our ELISA system compared to the detection of fixed antigen in an immunofluorescence assay [7], or the use of the S2 protein (a part of the HCV polyprotein derived from the E1 region expressed in S. cerevisiae [8]) in a radioimmune assay could have influenced the detection of anti-E1 antibodies. Higher S/N ratios were obtained in the type 1b group compared to type 1a samples, which correlates with the homology of both groups with the E1 antigen used in the ELISA (93% and 79%, respectively). Although each type 1b epitope can potentially be mutated compared to type 1a (21% difference), the mutations between type 1 subtypes are usually more conserved in nature than those observed between types (see Fig. 1B in [6]). It can be concluded, supposing that the prevalence of anti-type 1a E1 antibodies and of anti-type 1b E1 antibodies is similar, that divergence between type 1 subtypes 1a and 1b affects the inter-subtype cross-reactivity of anti-E1 antibodies to some degree, but most antibodies still show cross-reactivity.

Anti-E1 Cross-reactivity Between HCV Types

Reactivities of the 48 type 1 sera were compared with reactivities of 52 non-type-1 sera. Figure 3 shows the S/N ratios for all 100 sera. Sixty-eight percent of the type 1 sera were positive, while 29% of the type 2, 20% of the type 3, and none of the type 4 sera were positive. Type 1 (negative or positive) sera had a positive S/N of 1.62, while type 2, 3, or 4 average S/N were always negative (0.10 to 0.82), despite the positivity of some of the sera in the latter groups. While most non-type 1 sera were negative, six type 2 and