Establishment of a Competitive Binding Assay Identifying the Different Characteristics of Neutralizing Epitopes of Hepatitis E Virus

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Keywords

Hepatitis E virus · Competitive binding assay · Monoclonal antibody · Neutralizing epitope

Abstract

Aims: To confirm the different characteristics of genotype-specific and common neutralizing epitopes of hepatitis E virus (HEV). **Methods:** A competitive binding assay was established with known genotype-common neutralizing monoclonal antibodies (mAbs) 3G1 and 5G5 as well as genotype-specific neutralizing mAbs 2B1 and 4C5. HEV ORF₂ recombinant p166W01 derived from genotype 1 and p166Chn derived from genotype 4 were used as coated antigens, to determine whether the mAbs recognize independent, similar, or overlapping epitopes. mAbs were produced, purified, and conjugated with horseradish peroxidase (HRP). HRP-conjugated 2B1 could react only with p166W01 but not p166Chn, HRP-conjugated 4C5 could react only with p166Chn but not p166W01, while HRP-conjugated 3G1 and 5G5 could react both with p166W01 and p166Chn. Thus, competitive binding assays were performed successively using p166W01 and p166Chn antigen. **Results and Conclusion:** The results of competitive binding assays revealed that the binding of HRP-conjugated 2B1 to p166W01 could not be inhibited by 5G5 or 3G1. Similarly, the binding of HRP-

conjugated 4C5 to p166Chn could not be inhibited by 5G5 or 3G1. However, the mAbs 5G5 and 3G1 blocked each other's binding to p166W01 and p166Chn, suggesting that common and genotype-specific neutralizing mAbs recognize independent epitopes.

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Introduction

Hepatitis E virus (HEV) is a nonenveloped, positive-strand RNA virus that is transmitted mainly through the oral route and causes human acute hepatitis E [1, 2]. HEV infection can lead to detrimental prognostics, for example life failure, severe acute hepatitis, chronic infection in the organ transplanted and in immunosuppressed patients, and high mortality in pregnant women [3–5]. In addition to confirmed infection in humans, HEVs also infect animals and can be transmitted from animals to human beings; the incidence of hepatitis E infection in the developed countries has also increased significantly [6]. These findings indicate that HEV poses a public health threat across the globe [7].

Although one serotype has been recognized, HEV isolates, derived from different areas all over the world, could be classified into 4 major genotypes [8]. HEVs of

genotypes 1 and 2 differ from genotypes 3 and 4 in their epidemiological, antigenic, and immunogenic characteristics [9].

To date, research on antigenic epitope characterization mainly based on the HEV structural protein for an in vitro culture system is unavailable [10]. pORF₂ is the major HEV structural protein encoded by ORF₂, which is 1 of 3 overlapping open reading frames (ORFs) of the viral genome [5]. Predominant immunogenic regions of pORF₂ have been localized at the C-terminal 2/3 region, which were the main targets of vaccine development [11].

Our previous study has revealed the existence of an immunogenicity difference between the hepatitis E vaccine p179 (439–617 amino acids of the ORF₂ protein) derived from genotype 4 and p239 (Hecolin) derived from genotype 1, the difference may be attributed to the presence of HEV genotype-specific epitope(s) [9]. Monoclonal antibodies (mAbs) produced in mice immunized respectively with p166Sar (452–617 amino acids of the ORF₂ protein) derived from genotype 1 and p166Chn derived from genotype 4 have been used to confirm the presence of genotype-specific epitope(s). Besides 2 genotype-common neutralizing mAbs, 3G1 and 5G5, 2 genotype-specific neutralizing mAbs, 2B1 and 4C5, were also obtained. 2B1 against p166Sar could specifically bind to genotypes 1 and 2 recombinant proteins and neutralized only genotypes 1 and 2 strains in vitro; 4C5 against p166Chn immunoreacted specifically against genotypes 3 and 4 recombinant proteins and neutralized only genotype 3 and 4 strains, while 3G1 against p166Sar and 5G5 against p166Chn could bind to genotypes 1–4 recombinant proteins and neutralized 4 genotype strains [9]. For further study of the different characteristics of genotype-common and genotype-specific neutralizing epitopes of HEV, a competitive binding assay was developed using mAbs 2B1, 3G1, 4C5, and 5G5, to confirm whether epitopes recognized by mAbs are independent, similar, or overlapping.

Materials and Methods

Recombinant Proteins

Recombinant p166 was a truncated protein of pORF₂, the nucleotide sequences were derived from the following HEV strains: W01 (genotype 1, JX857689) and China-9829 (genotype 4, AY789225). Recombinant plasmids were constructed and expressed in *Escherichia coli* [10, 12, 13]. The 2 His-tagged p166 proteins were designated as p166W01 and p166Chn, respectively.

mAb Production

A total of 4 neutralizing mAbs, 2B1, 3G1, 4C5, and 5G5 described previously, were used to develop a competitive enzyme-

linked immunosorbent assay (ELISA). 2B1 and 3G1 were raised against p166Sar; 4C5 and 5G5 were raised against p166Chn [9]. The mAbs were produced by injecting 10⁶ hybridoma cells into the peritoneal cavity of a BALB/c mouse; ascites fluid containing mAbs was harvested after 7–10 days, filtered, centrifuged, and stored at –80 °C [14].

Purification of mAbs

The mAb ascites fluid was purified using protein G-sepharose column affinity chromatography (Sigma, Germany). Fractions containing antibody were collected using the acidic elution buffer (0.1 M glycine-HCl, pH 2.8) from the immobilized protein G and then determined at 280 nm; absorbance at 280 nm (A₂₈₀) can be used to roughly quantify the mAbs. Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to confirm and quantify the purified fractions using bovine serum albumin [15, 16].

Coupling of Horseradish Peroxidase to mAbs

Purified mAbs were dialyzed against 100 mM carbonate/bicarbonate buffer (pH 9.3) at 4 °C overnight [17]. Antibodies were coupled to horseradish peroxidase (HRP) by the manufacturer's instructions (KPL). About 1.5 mg of HRP was mixed with 0.5 mg of mAb in 0.5 mL total volume of HRP conjugation buffer, by gently stirring at room temperature for 1 h. The mixture was left at room temperature for 15 min after having added 10 µl of the reducing agent, and then, 1 volume of distilled glycerol was added for storage and further use.

Cross-Reactivity of HRP-Conjugated mAbs to Heterologous p166 Antigen

The ELISA method was applied to confirm the cross-reactivity of HRP-conjugated mAbs with p166W01 and p166Chn antigen [9]. Briefly, wells of microplates were coated with 100 ng of the p166W01 and p166Chn antigen, respectively, incubated for 2 h at 37 °C. This was followed by removal of coated antigens; 200 µL of phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin were added in wells, and then microplates were incubated at room temperature with gentle shaking. Two hours later, the blocking buffer was discarded, and the wells were washed twice with PBS containing 0.05% Tween-20 (PBST). Serial 2-fold dilutions of HRP-conjugated mAbs ranging from 1:400 to 1:25,600 in 1% casein PBS were added in corresponding wells, followed by 45 min of incubation at 37 °C; casein PBS was removed by washing 5 times with PBST. Tetramethylbenzidine liquid substrate was added for color development for incubation at 37 °C for 10 min. Finally, the optical density (OD) of each well was read at 450 nm, with a 630-nm reference wavelength.

Determination of Antigen and mAb Titers

The titers of antigen and mAbs were determined using an ELISA. Initially, serial 2-fold dilutions of HRP-conjugated mAbs ranging from 1:400 to 1:25,600 in 1% casein PBS were prepared, then added to wells precoated with serial dilutions of p166W01 or p166Chn antigen. Followed by a 45-min incubation at 37 °C, unbound mAbs were removed by washing 5 times with PBST. Tetramethylbenzidine liquid substrate was added for color development for a 10-min incubation at 37 °C. Finally, the OD of each well was read at 450 nm, with a 630-nm reference wavelength, to determine the dilution that was subsaturating and gave an OD reading of approximately 1.0 at 450 nm, with a 630-nm reference wavelength.

Fig. 1. Reactions with the 2 p166 proteins p166W01 (a) and p166Chn (b) at different mAb dilutions.

Competitive Binding Assay

The different characteristics of common and genotype-specific neutralizing epitopes of HEV were investigated using a pairwise competitive binding assay established with common and genotype-specific neutralizing mAbs [18]. The methods were similar to the procedures described above, except that p166W01 and p166Chn were used as the coating antigen, respectively, and mixtures of HRP-conjugated mAb at twice the concentration and a saturated concentration of unconjugated mAb were added to the coated wells. The OD was measured at 450 nm, with a 630-nm reference wavelength. The percentage inhibition was calculated using the formula: $100 \times [1 - (A_{450, 630} \text{ of HRP-mAb and mAb}) / (A_{450, 630} \text{ of HRP-mAb})]$. Competition was rated as positive if the inhibition percentage was more than 50%.

Results

Cross-Reactivity of HRP-Conjugated mAbs to Heterologous p166 Antigen

The ELISA method was applied to evaluate the cross-reactivity of HRP-conjugated mAbs to p166W01 and p166Chn antigens. The results revealed that HRP-conjugated 2B1 at dilutions from 1:400 to 1:25,600 reacted only with p166W01, but not with p166Chn, and HRP-conjugated 4C5 at dilutions from 1:400 to 1:25,600 reacted only with p166Chn. In contrast, HRP-conjugated 3G1 and 5G5 at dilutions from 1:400 to 1:25,600 reacted well with the 2 p166 proteins (Fig. 1). These observations were similar to the previous findings, 2B1 and 4C5 are genotype-specific mAbs, while 3G1 and 5G5 are genotype-common mAbs [9].

Determination of p166W01 Antigen and HRP-Conjugated mAb Titers for the Competitive Binding Assay

p166W01 antigen was used to establish the competitive binding assay of mAbs 2B1, 3G1, and 5G5; the prop-

er titers of mAbs and antigen were determined using ELISA. As shown in Table 1, after square titration, when the dilution of the p166W01 antigen was 1:400, HRP-conjugated 2B1 was 1:6,400, the mean OD value was close to 1.0. Meanwhile, as shown in Table 2, when the dilution of antigen was 1:400, HRP-conjugated 5G5 and HRP-conjugated 3G1 were 1:6,400 and 1:12,800, respectively, and the mean OD value was close to 1.0.

Determination of p166Chn Antigen and HRP-Conjugated mAb Titers for the Competitive Binding Assay

p166Chn antigen was used to establish the competitive binding assay of mAbs 4C5, 3G1, and 5G5, the proper titers of mAbs and antigen were also determined using ELISA. As shown in Table 3, after square titration, when the dilution of the p166Chn antigen was 1:800, HRP-conjugated 4C5 was 1:6,400, and the mean OD value was close to 1.0. Meanwhile, as shown in Table 4, when the dilution of the antigen was 1:800, the dilutions of HRP-conjugated 5G5 and HRP-conjugated 3G1 were 1:6,400 and 1:12,800, respectively; the mean OD value was close to 1.0.

Inhibition of Binding of mAbs to p166W01

The results of inhibition are shown in Table 5; the binding of HRP-conjugated 2B1 to p166W01 antigen was inhibited by unconjugated 2B1 with 68% inhibition. There was no significant inhibition between 2B1 with mAbs 5G5 and 3G1 (27 and 30%). More complete inhibition was obtained from heterologous mAbs 3G1 and 5G5, for the binding of HRP-3G1 to antigen was inhibited by 5G5 with 61% inhibition, HRP-conjugated 5G5 to antigen was inhibited by 3G1 with 99% inhibition. Binding of HRP-3G1 and HRP-5G5 to p166W01 antigen was also almost completely inhibited by themselves (81 and 80%

Table 1. Determination of p166W01 antigen and HRP-conjugated mAb titers

Antigen dilution	Dilution of HRP-conjugated 2B1							negative control
	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	
1:200	2.900	2.225	1.827	1.432	1.138	0.876	0.595	0.076
1:400	2.782	1.754	1.512	1.238	<i>0.958</i>	0.703	0.354	0.061
1:800	1.945	1.301	1.105	0.867	0.775	0.432	0.216	0.034
1:1,600	1.065	0.720	0.856	0.662	0.543	0.245	0.098	0.090
1:3,200	0.832	0.553	0.356	0.213	0.115	0.075	0.034	0.034
1:6,400	0.612	0.298	0.232	0.156	0.089	0.054	0.022	0.023

The OD value in italics is significant, i.e. close to 1.

Table 2. Determination of p166W01 antigen and HRP-conjugated mAb titers

HRP-mAbs	Dilution of HRP-conjugated mAbs							negative control
	1:400	1:800	1:1600	1:3,200	1:6,400	1:12,800	1:25,600	
HRP-5G5	2.659	2.573	2.248	1.665	<i>1.093</i>	0.565	0.241	0.069
HRP-3G1	2.784	2.713	2.618	2.363	1.924	<i>1.077</i>	0.583	0.052

The OD values in italics are significant, i.e. close to 1.

Table 3. Determination of p166Chn antigen and HRP-conjugated mAb titers

Antigen dilution	Dilution of HRP-4C5							negative control
	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	
1:200	2.903	2.683	2.408	1.718	1.273	0.907	0.558	0.096
1:400	2.759	2.562	2.343	1.532	1.057	0.866	0.410	0.045
1:800	2.309	1.953	1.721	1.420	<i>0.956</i>	0.652	0.350	0.056
1:1,600	1.897	1.722	1.456	1.232	0.832	0.563	0.298	0.073
1:3,200	1.55	1.354	1.176	0.929	0.703	0.49	0.121	0.054
1:6,400	1.248	1.033	0.849	0.751	0.465	0.222	0.148	0.029

The OD value in italics is significant, i.e. close to 1.

Table 4. Determination of p166W01 antigen and HRP-conjugated mAb titers

HRP-mAbs	Dilution of HRP-mAbs							negative control
	1:400	1:800	1:1,600	1:3,200	1:6,400	1:12,800	1:25,600	
HRP-5G5	2.639	2.46	2.19	1.604	<i>1.023</i>	0.595	0.245	0.012
HRP-3G1	2.841	2.641	2.312	1.956	1.494	<i>0.908</i>	0.556	0.042

The OD values in italics are significant, i.e. close to 1.

Table 5. Percentage inhibition of binding of HRP-mAbs to p166W01

Antigens/ HRP-mAbs	Unlabeled mAbs		
	5G5	3G1	2B1 (or 4C5)
p166W01			
HRP-5G5	80	99	7.5
HRP-3G1	61	81	29
HRP-2B1	27	30	68
p166Chn			
HRP-5G5	61	76	7.3
HRP-3G1	65	69	6.8
HRP-4C5	25	30	69

inhibition). These results suggest that 3G1 and 5G5 may share the same epitope or overlapping epitope, yet 2B1 probably recognizes a different epitope.

Inhibition of Binding of mAbs to p166Chn

The results of inhibition are shown in Table 5: the binding of HRP-4C5 to p166Chn was inhibited by unconjugated 4C5 with 69% inhibition, there was no significant inhibition (25 and 30%) obtained from the other 2 unconjugated mAbs 3G1 and 4C5. However, a higher inhibition rate was obtained from HRP-conjugated mAbs 3G1 and 5G5 binding to antigen, inhibited by unconjugated 5G5 and 3G1 with 65 and 76% inhibition. Binding of HRP-conjugated 3G1 and HRP-conjugated 5G5 to p166Chn antigen was also almost inhibited by themselves. These results also suggest that 3G1 and 5G5 may share the same epitope or overlapping epitope, while 4C5 probably recognizes a different epitope.

Discussion

Since the novel swine HEV was first isolated [19], anti-HEV antibodies have been reported in many animals, revealed HEV infection among animals and human beings, and this may be transmitted from animals to human beings [20, 21]. The reduction of hepatitis E morbidity and mortality depended on vaccine prevention and effective diagnosis. Knowledge of epitopes of HEV was essential for vaccine preparation and diagnostic development [22]. The detection of specific antibodies in serum is one of the main diagnostic methods, and there is a growing number of serological assays for antibody detection. However, the assays used to detect specific antibodies vary considerably in sensitivity [23]. Some commercial diagnostic assays based on genotype 1 or 2 antigens fail to detect serum an-

tibodies against genotypes 3 or 4 isolates [24]. Little is known about the mechanisms underlying the noncorrespondence of the different diagnostic assays.

At present, an in vitro cell culture system is still unavailable for HEV research. Therefore, pORF₂ containing most immunogenic sites has been widely used for HEV immunological study, with a conformation-dependent character [25]. The structural basis of an immunogen to elicit neutralizing and protective antibodies is the neutralizing epitope, which is the main target of hepatitis E vaccine development [22]. We have previously reported that the p166 protein, a truncated protein of pORF₂, can model HEV neutralizing epitope(s), and antibodies raised against p166 could cross-neutralize different genotypes of HEV [26]. In addition, hepatitis E p239 vaccine derived from genotype 1 HEV was effective in a phase 3 clinical trial conducted in Jiangsu Province, China, where the epidemic HEV isolate is genotype 4 [27]. These findings revealed common neutralizing epitope(s) exist within the different genotypes of HEV. Besides the common neutralizing epitope(s), genotype-specific epitope(s) has also been confirmed to exist, for mAb preparation and characterization [9, 26].

To further identify the characterization of common and genotype-specific neutralizing epitopes, the competitive inhibition assay was established with common neutralizing mAbs, 3G1 and 5G5, and genotype-specific neutralizing mAbs, 2B1 and 4C5, to investigate the competition binding to p166W01 or p166Chn antigen and to determine whether the mAbs recognize independent, similar, or overlapping epitopes.

Initially, ascitic fluids of mAbs 3G1, 2B1, 5G5, and 4C5 were produced, purified, and conjugated with HRP. The ELISA method was applied to compare the binding affinity of HRP-conjugated mAbs with different genotypes of HEV p166. The results revealed that HRP-conjugated 2B1 only reacted with genotype 1 p166W01, but not p166Chn. Similarly, HRP-conjugated 4C5 reacted only with p166Chn. In contrast, HRP-conjugated 3G1 and 5G5 reacted well against the 2 genotype p166 proteins. So, competitive binding assays are based on the genotype 1 and 4 HEV p166 antigens. The results of competitive binding assays are not surprising, i.e. that the binding of 2B1 to p166W01 only competed by itself, while 3G1 and 5G5 could compete each other for the binding to p166W01. Moreover, the binding of 4C5 to p166Chn was similarly only competed by itself, but 3G1 and 5G5 could compete each other for the binding to p166Chn. These findings indicated that 3G1 and 5G5 recognize the same or overlapping epitopes and that 2B1 and 4C5 recognize different ones.

This is the first study that investigates the different characteristics of genotype-common and genotype-specific conformational neutralizing epitopes of HEV. We demonstrated that common and genotype-specific neutralizing epitopes may be independent. This discovery is very beneficial to demonstrate the mechanisms underlying the low concordance of the different diagnostic assays. Further studies are of crucial importance to elucidate the different characteristics of neutralizing epitopes of HEV in the effectiveness of diagnostic development and vaccine preparation.

Acknowledgment

This work was supported by the doctoral scientific research foundation of Anhui Medical University (No. 0110037101).

Disclosure Statement

The authors declare no conflicts of interest.

References

- Debing Y, Moradpour D, Neyts J, Gouttenoire J: Update on hepatitis E virology: implications for clinical practice. *J Hepatol* 2016;65:200–212.
- Oh HW, Cha RR, Lee SS, Lee CM, Kim WS, Jo YW, Kim JJ, Lee JM, Kim HJ, Ha CY, Kim HJ, Kim TH, Jung WT, Lee OJ: Comparing the clinical features and outcomes of acute hepatitis E viral infections with those of acute hepatitis A, B, and C infections in Korea. *Intervirology* 2017;60:109–117.
- Verma N, Sharma M, Biswal M, Taneja S, Batra N, Kumar A, Dhiman RK: Hepatitis E virus induced acute liver failure with scrub typhus coinfection in a pregnant woman. *J Clin Exp Hepatol* 2017;7:158–160.
- Zhou X, de Man RA, de Knecht RJ, Metselaar HJ, Peppelenbosch MP, Pan Q: Epidemiology and management of chronic hepatitis E infection in solid organ transplantation: a comprehensive literature review. *Rev Med Virol* 2013;23:295–304.
- Mushahwar IK: Hepatitis E virus: molecular virology, clinical features, diagnosis, transmission, epidemiology, and prevention. *J Med Virol* 2008;80:646–658.
- Bouwknegt M, Rutjes SA, Reusken CB, Stockhofe-Zurwieden N, Frankena K, de Jong MC, de Roda Husman AM, Poel WH: The course of hepatitis E virus infection in pigs after contact-infection and intravenous inoculation. *BMC Vet Res* 2009;5:7.
- Haider N, Khan MSU, Hossain MB, Sazzad HMS, Rahman MZ, Ahmed F, Zeidner NS: Serological evidence of hepatitis E virus infection in pigs and jaundice among pig handlers in Bangladesh. *Zoonoses Public Health* 2017;64:572–577.
- Nan Y, Zhang YJ: Molecular biology and infection of hepatitis E virus. *Front Microbiol* 2016;7:1419.
- Wen J, Behloul N, Dai X, Dong C, Liang J, Zhang M, Shi C, Meng J: Immunogenicity difference between two hepatitis E vaccines derived from genotype 1 and 4. *Antiviral Res* 2016;128:36–42.
- Zhang H, Dai X, Shan X, Meng J: Characterization of antigenic epitopes of the ORF2 protein from hepatitis E virus genotype 4. *Virus Res* 2009;142:140–143.
- Behloul N, Wen J, Dai X, Dong C, Meng J: Antigenic composition and immunoreactivity differences between HEV recombinant capsid proteins generated from different genotypes. *Infect Genet Evol* 2015;34:211–220.
- Liang JH, Dai X, Dong C, Meng JH: A single amino acid substitution changes antigenicity of ORF2-encoded proteins of hepatitis E virus. *Int J Mol Sci* 2010;11:2962–2975.
- Behloul N, Zhang M, Meng J: Binding preference of anti-HEV antibodies in sera collected in Algeria for antigens derived from HEV genotype 1. *Hepat Mon* 2016;16:e35312.
- Liang JH, Liang LM, Dong M, Han ZG, Dong C, Meng JH: Identification of a novel antigenic epitope on GST fusion-expressed and ORF2-encoded proteins of hepatitis E virus (in Chinese). *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* 2008;24:321–323, 327.
- Zhang J, Dong M, Jiang B, Dai X, Meng J: Antigenic characteristics of the complete and truncated capsid protein VP1 of enterovirus 71. *Virus Res* 2012;167:337–342.
- Xu M, Behloul N, Wen J, Zhang J, Meng J: Role of asparagine at position 562 in dimerization and immunogenicity of the hepatitis E virus capsid protein. *Infect Genet Evol* 2016;37:99–107.
- Lindstrom NM, Call DR, House ML, Moffitt CM, Cain KD: A quantitative enzyme-linked immunosorbent assay and filtration-based fluorescent antibody test as potential tools to screen broodstock for infection with *Flavobacterium psychrophilum*. *J Aquat Anim Health* 2009;21:43–56.
- Zhou EM, Guo H, Huang FF, Sun ZF, Meng XJ: Identification of two neutralization epitopes on the capsid protein of avian hepatitis E virus. *J Gen Virol* 2008;89:500–508.
- Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ, Emerson SU: A novel virus in swine is closely related to the human hepatitis E virus. *Proc Natl Acad Sci USA* 1997;94:9860–9865.
- Caprioli A, Ostanello F, Martelli F: Hepatitis E virus: an emerging zoonotic agent. *Vet Ital* 2005;41:113–127.
- Park WJ, Park BJ, Ahn HS, Lee JB, Park SY, Song CS, Lee SW, Yoo HS, Choi IS: Hepatitis E virus as an emerging zoonotic pathogen. *J Vet Sci* 2016;17:1–11.
- Gu Y, Tang X, Zhang X, Song C, Zheng M, Wang K, Zhang J, Ng MH, Hew CL, Li S, Xia N, Sivaraman J: Structural basis for the neutralization of hepatitis E virus by a cross-genotype antibody. *Cell Res* 2015;25:604–620.
- Vollmer T, Diekmann J, Eberhardt M, Knabbe C, Dreier J: Monitoring of anti-hepatitis E virus antibody seroconversion in asymptotically infected blood donors: systematic comparison of nine commercial anti-HEV IgM and IgG assays. *Viruses* 2016;8:232.
- Bendall R, Ellis V, Ijaz S, Thuraiarah P, Dalton HR: Serological response to hepatitis E virus genotype 3 infection: IgG quantitation, avidity, and IgM response. *J Med Virol* 2008;80:95–101.
- Zhao M, Li XJ, Tang ZM, Yang F, Wang SL, Cai W, Zhang K, Xia NS, Zheng ZZ: A comprehensive study of neutralizing antigenic sites on the hepatitis E virus (HEV) capsid by constructing, clustering, and characterizing a tool box. *J Biol Chem* 2015;290:19910–19922.
- Meng J, Dai X, Chang JC, Lopareva E, Pillot J, Fields HA, Khudyakov YE: Identification and characterization of the neutralization epitope(s) of the hepatitis E virus. *Virology* 2001;288:203–211.
- Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, Wang H, Yang CL, Jiang HM, Cai JP, Wang YJ, Ai X, Hu YM, Tang Q, Yao X, Yan Q, Xian YL, Wu T, Li YM, Miao J, Ng MH, Shih JW, Xia NS: Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet* 2010;376:895–902.