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Immunohistochemistry for hepatitis E virus capsid protein cross-reacts with cytomegalovirus-infected cells - a potential diagnostic pitfall

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Key words

Hepatitis E virus (HEV); HEV ORF2 antibody; cytomegalovirus (CMV) antibody; immunohistochemistry; cross-reactivity

Conflict of interest statement

The authors declare that they have no conflict of interest.

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Authors' contributions

Concept and design of study: DL, AW. Providing liver biopsies and related clinical information, histopathological analysis, and data acquisition: CS, DL, AW. In silico analysis: JGr, JGo. Preparation of figures: DL, AW. Writing of the manuscript: DL, CS, JGr, AW. Critical review and editing of the manuscript: all authors.

Running title

HEV / CMV IHC cross-reactivity

Abbreviations

CMV: Cytomegalovirus; EBV: Epstein Barr virus; FFPE: formalin-fixed, paraffin-embedded; gt: genotype; H&E: Hematoxilin & Eosin; HEV: hepatitis E virus; HHV8: Human herpes virus 8; HSV: Herpes simplex virus; IHC: immunohistochemistry; ORF: open reading frame(s); SARS-CoV2: Severe acute respiratory syndrome coronavirus type 2; VZV: Varicella zoster virus

From This Month's Histopathology

This article highlights a potential diagnostic pitfall in liver pathology describing an unexpected cross-reactivity of the hepatitis E virus ORF2 antibody against CMV proteins. Awareness of the differences in staining patterns will prevent pathologists from misinterpreting positive HEV ORF2 immunohistochemistry in liver specimens.

ABSTRACT

Background and aims

Immunohistochemistry for hepatitis E virus (HEV) ORF2 (capsid) protein is a powerful tool for tissue-based diagnosis of hepatitis E, particularly useful in evaluating abnormal liver values in immunocompromised patients. We here report a previously unobserved reactivity of the HEV ORF2 antibody to human cytomegalovirus (CMV) proteins and contrast the staining patterns encountered in HEV and CMV infection, respectively.

Methods and results

As part of a routine diagnostic workup, the liver biopsy of an immunocompromised patient with elevated liver values was examined histologically for infection with viruses including CMV and HEV. Cytopathic changes were found, suggestive of CMV infection, which was confirmed by immunohistochemistry. Surprisingly, reactivity of a portion of CMV-infected cells with a mouse monoclonal antibody (clone 1E6) against HEV ORF2 protein was also detected. This observation prompted a screening of 22 further specimens (including liver, gastrointestinal, lung, brain and placental biopsies) with confirmed CMV infection/reactivation. Immunoreactivity of CMV-infected cells with HEV ORF2 antibody was observed in totally 18 of 23 specimens. While the HEV ORF2 antibody showed cytoplasmic, nuclear, and canalicular positivity in hepatitis E cases, positivity in CMV-infected cells was limited to the nucleus.

Conclusions

The HEV ORF2 antibody (clone 1E6) shows unexpected immunoreactivity against CMV proteins. In contrast to the hepatitis E staining pattern with cytoplasmic, nuclear and occasional canalicular positivity, reactivity in CMV-infected cells is restricted to the nucleus. Awareness of this cross-reactivity and knowledge of the differences in staining patterns will prevent pathologists from misinterpreting positive HEV ORF2 immunohistochemistry in liver specimens.

INTRODUCTION

Hepatitis E virus (HEV) infection is one of the most common causes of acute hepatitis in the world. In resource-poor countries, hepatitis E may lead to large epidemic outbreaks that are usually caused by genotypes 1 and 2, which are transmitted from human to human by contaminated drinking water. By contrast, HEV genotype 3 (circulating worldwide) and 4 (circulating mainly in China and Southeast Asia), lead to zoonotic infections and are mainly transmitted by consumption of contaminated meat products, e.g. uncooked or undercooked pork or game meat, representing a health threat especially in resource-rich countries.[1] The clinical course of hepatitis E is highly variable and ranges from completely asymptomatic infections or acute, self-limiting hepatitis to acute-on-chronic liver failure in patients with pre-existing liver disease or chronic-active hepatitis in immunocompromised patients.[2]

Although the diagnosis of hepatitis E is usually made by blood testing (detection of antibody and/or sequence of viral RNA by PCR), also histopathology plays a role in diagnosing hepatitis E. We previously demonstrated that the HEV ORF2 (capsid) protein was unequivocally detectable in liver specimens from patients with hepatitis E, with HEV ORF2 immunohistochemistry being as specific and comparably sensitive as PCR for HEV RNA.[3] In case hepatitis E initially has not been considered among the differential diagnoses, or results from serological testing are not available yet, the histological pattern together with the consideration of the immune status of the patient and of the knowledge about a pre-existing liver disease, can hint to hepatitis E.[4] In such cases, immunohistochemistry targeting the HEV ORF2 (capsid) protein is a recognized tool for the histopathological diagnosis of hepatitis E.[5]

Human cytomegalovirus (CMV) is a highly prevalent herpesvirus worldwide.[6] CMV infection usually takes place in childhood with unspecific symptoms and therefore, liver biopsy material is hardly available from those patients. In neonates and immunocompromised patients however, CMV-infection can cause severe disease, including hepatitis.[7-9] Suspected CMV-hepatitis in immunocompromised patients can be tested by serological testing as well as CMV-immunohistochemistry if liver biopsy material is available.[10] Indeed, in our previous study,

CMV-infection had been excluded in all immunocompromised patients by immunohistochemistry.[3]

Having encountered a case of CMV-hepatitis in our routine diagnostics which unexpectedly displayed positivity with the HEV ORF2 antibody, we sought to further study the expression of HEV ORF2 on tissues with proven CMV-infection and to explore the reason for this phenomenon by *in silico* analysis. As the cross-reactivity of monoclonal antibody 1E6 against hepatitis E virus ORF2 capsid with CMV proteins represents a potential diagnostic pitfall, we additionally aimed to describe differences in the immunohistochemical expression pattern to distinguish between the two infections in the liver.

MATERIALS AND METHODS

Biopsy material / Tissue samples

After a first case of CMV-hepatitis showing an unexpected immunoreactivity by HEV ORF2 antibody, cases with CMV-infection or CMV-reactivation were retrieved from the archive of the Department of Pathology and Molecular Pathology, University Hospital Zurich (USZ) and the University Hospital Lausanne (between 2010 and 2021). Collectively, the following tissue specimens with immunohistochemical positivity for CMV were identified: liver 5x, colon 6x, ileum/colon 3x, stomach 3x, lung 3x, brain 1x and placenta 2x.

Ethics

This study was approved by the internal review board of the University Hospital Zurich and the Cantonal Ethics Committee of Zurich, Switzerland (KEK-ZH-Nr. 2013-0504).

Histopathological analysis

H&E slides were reviewed for histopathological changes such as inflammation and typical CMV inclusion bodies (i.e. “owl-eye” changes). IHC slides were reviewed for the exact pattern of HEV ORF2 positivity in CMV-infected tissues and compared to the expression pattern in HEV-infected tissues.

Immunohistochemistry (IHC)

For cases with enough FFPE material, new consecutive tissue slides were cut and stained with hematoxylin and eosin (H&E) as well as incubated with CMV (CMV Blend [8B1.2, 1G5.2 & 2D4.2] mouse monoclonal antibody, prediluted, Cell Marque, USA) and HEV ORF2 (clone 1E6, mouse monoclonal antibody, dilution 1:500, no. MAB8002, Millipore Corporation, USA; direct detection system with OptiView Kit from Ventana) antibodies. Immunohistochemistry was performed according to standard procedures.

Appropriate positive and negative controls were used throughout the incubations.

Furthermore, the HEV ORF2 antibody was applied on tissue samples known to be positive for Herpes simplex virus (HSV), Varicella zoster virus (VZV), Epstein Barr virus (EBV), Human herpes virus 8 (HHV8), adenovirus (ADV) as well as Severe acute respiratory syndrome coronavirus type 2 (SARS-CoV2).

Data availability

Data are available from the authors upon request.

RESULTS

A liver biopsy was performed in a 56-year-old male patient who had presented with icteric sclera and deteriorated condition one month after liver transplantation following fulminant hepatitis B with subtotal liver necrosis. Histology revealed acute hepatitis with microabscesses and viral inclusion bodies, strongly suggestive of an underlying viral infection (**Fig. 1A**). Immunohistochemistry included not only CMV (CMV Blend [8B1.2, 1G5.2 & 2D4.2], mouse monoclonal antibody) but also HEV (clone 1E6, mouse monoclonal antibody). CMV-infection was confirmed with positivity by the CMV antibody. Surprisingly, the same cells showed also a distinct nuclear positivity by the HEV ORF2-antibody (**Fig. 1A**). As the patient had viremia for CMV of 75'794 IE/ml and negative HEV antibodies (IgG and IgM) as well as negative HEV RNA testing at the time of the liver biopsy, the HEV ORF2 positivity was interpreted as cross-reactivity to CMV-infected cells. This observation prompted us to evaluate a panel of liver, gastrointestinal, lung, brain and placenta specimens from various patients with CMV infection or CMV reactivation for reactivity with the HEV ORF2 antibody. Indeed, albeit not in all specimens, the same staining pattern as described above was also found in 17 of 22 of the further cases. Remarkably, in these cases a proportion of cells that were positive in CMV immunohistochemistry also revealed strong positivity for the HEV ORF2 antibody (**Fig. 1B**). More specifically, whereas in hepatitis E cases, the HEV ORF2 antibody displayed cytoplasmic, nuclear and occasional canalicular positivity, the positivity in CMV-infected cells was found to be restricted to the nucleus (**Fig. 1C**). Of note, the HEV ORF2 antibody did not show reactivity beyond background staining in tissues infected with either HSV, VZV, EBV, HHV8, ADV, or SARS-CoV2 (data not shown).

DISCUSSION

Among the different HEV proteins, the HEV ORF2 protein is unique in so far, that as the capsid protein it not only represents the antigenic structure of the virus, but is also produced and secreted in significantly higher amounts compared to the other viral proteins.[11] Antibodies against the HEV ORF 2 protein, including the monoclonal antibody clone 1E6 which was used in this study, are not only valuable tools in HEV basic research, but also helpful tools for the (histopathologic) diagnosis of hepatitis E.[3-5] Thus, knowledge of the cross-reactivity with CMV-infected cells described here is of interest for both viral research and histopathological diagnosis.

A detailed comparison of the staining patterns of HEV- versus CMV-infected hepatocytes with the HEV ORF2 antibody revealed significant differences with respect to subcellular distribution. The fact that CMV-infected hepatocytes show exclusively nuclear staining, whereas HEV infected hepatocytes show both cytoplasmic (most common) and nuclear as well as also canalicular reactivity, helps in daily practice to differentiate true versus cross-reactivity. The knowledge of the described cross-reactivity as well as the knowledge of the staining differences is important for interpretation in daily diagnostic practice.

Our observation that the reactivity is restricted to actual CMV-infected cells suggests that it is not a nonspecific reaction, but in fact reflects a cross-reactivity with CMV proteins. This prompted us to take advantage of *in silico* analyses to test whether the cross-reactivity might be due to a similarity of HEV and CMV epitopes. *In silico* analyses did not reveal any obvious CMV antigen candidate which may explain the cross-reactivity observed with 1E6 mAb. However, one potential candidate identified was the CMV immediate early protein 1 (IE1) which showed the highest partial local blast homology (UniProt entry: P13202; aa 413-426) with the HEV ORF2 1E6 epitope sequence. Remarkably, this viral antigen is thought to transactivate early human CMV genes during infection. The similarity of genes activated early - but not late - during CMV infection is a possible explanation for our observation that five of the 23 CMV-infected tissues of our cohort showed no cross-reactivity. Moreover, CMV IE1 is known to act in the nucleus, reminiscent of the cross-reactive signal observed with mAb 1E6. This is very

well in line with our observation that reactivity is exclusively detectable in nuclei of CMV-infected cells.

In summary, we report a cross-reactivity of HEV ORF2 with CMV-infected cells. Partial homology between HEV and CMV epitopes detected by *in silico* analysis provides a hypothetical explanation for this observation, which however needs further validation. Since HEV-infected hepatocytes show subcellular staining patterns distinct from CMV-infected hepatocytes, further use of the HEV ORF2 antibody for the histopathological diagnosis of hepatitis E can be recommended. Awareness of this cross-reactivity and knowledge of the differences in staining patterns will protect pathologists from misinterpreting positive HEV ORF2 immunohistochemistry in liver specimens.

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FIGURE LEGENDS

FIGURE 1. Histopathological and immunohistochemical findings in biopsy material

(A) Index case of CMV hepatitis with unexpected HEV ORF2 cross-reactivity: Left panel: Acute lobular hepatitis (upper) with micro abscesses (lower) and viral inclusion bodies (insert) (H&E staining; scale bars 200 μm, 50 μm, 10 μm, respectively); Middle (CMV IHC) and right (HEV ORF2 IHC) panel showing positive immunoreaction in the same cells – endothelial cell (upper) and hepatocytes (lower) (scale bars 50 μm).

(B) HEV ORF2 cross-reactivity in other organs with CMV-infection or CMV-reactivation illustrated in lung, placenta, stomach and colon. Comparison between the staining pattern of CMV IHC (left) vs. HEV ORF2 IHC (right) (scale bars: 100 μm (colon), all others 25 μm).

(C) Different HEV ORF2 staining patterns in HEV-hepatitis and CMV-hepatitis: Left panel: Positivity restricted to the nucleus in CMV-hepatitis (scale bars overviews 200 μm and details 20 μm). Right panel: Geographic areas of positive hepatocytes with cytoplasmic and/or nuclear as well as canalicular positivity in HEV-hepatitis (as previously described)[3].

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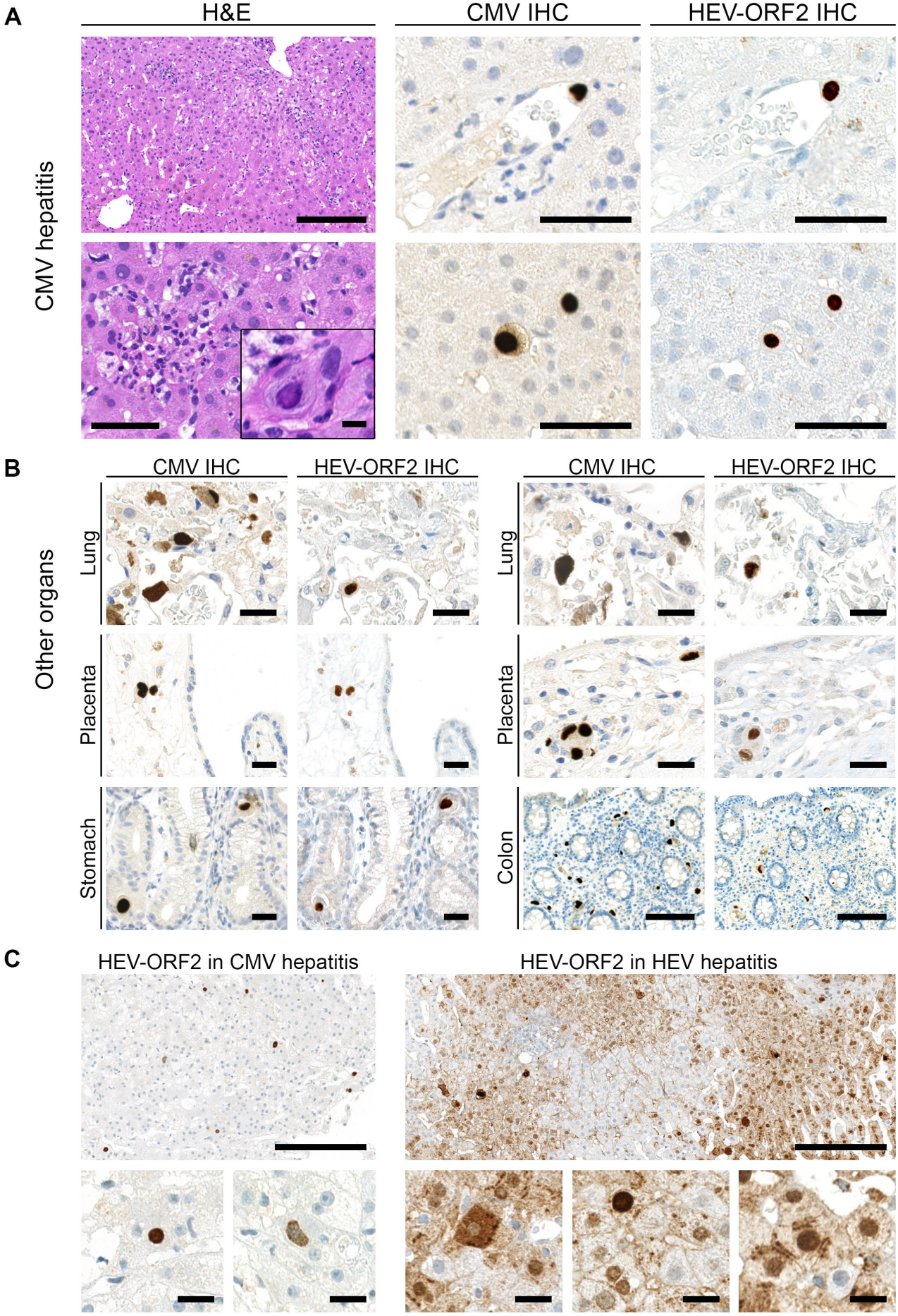
A
H&E
CMV IHC
HEV-ORF2 IHC
CMV hepatitis
B
CMV IHC
HEV-ORF2 IHC
CMV IHC
HEV-ORF2 IHC
Other organs
Lung
Placenta
Stomach
Lung
Placenta
Colon
C
HEV-ORF2 in CMV hepatitis
HEV-ORF2 in HEV hepatitis

Figure 1