Occurrence of Immunoglobulin M Antibodies against Several Bacterial and Viral Pathogens in Acute Hantavirus Infection

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Elevated levels of immunoglobulin M antibodies against various pathogens, most frequently Epstein-Barr-virus and Coxiella burnetii, were detected by immunoassay in 15 of 48 patients (31.3%) with acute Puumala virus infections. Although the mechanisms leading to this IgM response are not clear yet, polyspecific immunoglobulin M antibodies have to be taken into account to avoid misinterpretation of serological results in acute hantavirus infection.

Puumala virus (PUUV), the main causative agent of nephropathia epidemica in central and northern Europe, a disease characterized by fever, headache, and interstitial nephritis with acute renal failure. PUUV belongs to the hantaviruses of the family Bunyaviridae, which are transmitted to humans by inhalation of aerosolized excreta of persistently infected rodents.

In 2010, an epidemic peak of hantavirus infections occurred in Germany which caused 2,017 diagnosed cases, i.e., the highest number of annual infections ever seen in Germany (4). Since October 2011, a marked increase of hantavirus cases has been noted again in Germany (2).

In one patient with clinically and serologically confirmed acute PUUV infection (termed the index patient), we found elevated immunoglobulin M (IgM) antibodies against Coxiella burnetii and enteroviruses without concurrent immunoglobulin G (IgG) antibodies by enzyme immunoassay (EIA). Polyclonal IgM stimulation is well known in several viral infections, like Epstein-Barr virus (EBV), human cytomegalovirus (CMV), and parvovirus B19 infection (1, 3, 6, 7, 9), but it had not been described yet in PUUV infection. Thus, we investigated whether detection of IgM antibodies to pathogens which are not related to PUUV occurs more frequently in acute PUUV infection.

We performed a retrospective analysis of sera collected from 48 patients with acute PUUV infection between April and October 2010. All patients had clinical signs and symptoms of acute PUUV infection and were positive for PUUV-specific IgM and IgG antibodies by immunoblotting (recomLine Bunyavirus IgM/IgG; Mikrogen, Neuwied, Germany) and EIA (Hantavirus Puumala IgM/IgG; Virion Serion, Wuerzburg, Germany). (Note that results from eight patients, including no. 15, 36, 39, and 43, were detected by immunoassay in 15 of 48 patients (31.3%) (Table 2). IgM against EBV (viral capsid antigen [VCA]) IgM positive by CLIA but not confirmed by blotting) in 10 patients (20.8%) and Coxiella burnetii infection (positive by EIA, not confirmed by MIF) in 6 patients (12.5%) occurred most often, followed by IgM against enterovirus (5 patients [10.4%]), Borrelia burgdorferi (IgM positive by CLIA but

Received 24 February 2012 Returned for modification 12 May 2012 Accepted 12 May 2012
Published ahead of print 11 July 2012
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doi:10.1128/CVI.00109-12
not confirmed by blotting in 2 patients [4.2%], and *Leptospira* spp. (1 patient [2.1%]).

In patient no. 41, apart from IgM antibodies against *Coxiella burnetii* and enterovirus detected by EIA, IgM and IgG antibodies against *Borrelia burgdorferi* were positive in the CLIA (Table 2).

### Table 1: Serological follow-up of the PUUV-positive index patient

| Patient serum no. | Day | PUUV* | Coxiella burnetii | Entervirus | MIF (titer) | Enterovirus |
|-------------------|-----|-------|------------------|------------|-------------|-------------|
|                   |     | IgM   | IgG              | IgM (index)* | IgG (U/ml)  | IgM (U/ml) | IgG (U/ml) |
| 24a 0             |     | Negative | Negative | ND  | ND          | ND          | ND          |
| 24b 4             |     | Borderline (PuHa, +/−) | Negative | Positive (151) | Negative | ND          | ND          |
| 24c 21            |     | Positive (PuHa, +/−; Pu, +) | Positive (PuHa, +/− Pu, +) | Positive (248) | Negative | ND          | ND          |
| 24d 42            |     | Positive (PuHa, +/−; Pu, +) | Positive (PuHa, +/− Pu, +) | Positive (219) | Negative | ND          | ND          |
| 24e 57            |     | Positive (PuHa, +/−; Pu, +) | Positive (PuHa, +/− Pu, +) | Positive (154) | Negative | Negative | Positive (>1,000) | Negative |
| 24f 175           |     | Negative | Positive (PuHa, +/−; Pu, +) | Negative (48) | Negative | ND          | ND          |

* In parentheses are the intensities of the detected bands in the recomLine blot abbreviated as follows: PuHa, group-specific antigen; Pu, Puumala virus; Ha, Hantaan virus; and Do, Dobrava virus.

### Table 2: PUUV-positive patients with positive IgM antibodies against nonrelated pathogens

| Patient/ serum no. | Coxiella burnetii | Enterovirus | Epstein-Barr virus | Cytomegalovirus | Leptospira spp. | Borrelia burgdorferi |
|--------------------|------------------|-------------|---------------------|-----------------|-----------------|---------------------|
|                    | IgM (index)* | MIF (titer) | MIF | IgM (U/ml) | IgG (U/ml) | U/ml | Blot* | IgM (index)* | IgM (U/ml) | IgG (U/ml) | U/ml | IgM (index)* | IgG (U/ml) |
| 11a                | + (141)  | −          | −      | + (76) | + (124) | + (121) | −   | ND       | ND       | ND       | ND       | ND       |
| 11b                | + (286)  | −          | −      | + (>/1000) | + (144) | + (>160) | −   | ND       | ND       | ND       | ND       | ND       |
| 24e                | + (154)  | −          | −      | + (>1000) | −      | −       | ND  | ND       | ND       | ND       | ND       | ND       |
| 30                 | + (128)  | −          | −      | +/− (32) | −      | + (98) | −   | ND       | ND       | ND       | ND       | ND       |
| 36                 | + (189)  | ND         | ND    | + (63) | +/− (98) | ND      | ND  | ND       | ND       | ND       | ND       | ND       |
| 41                 | + (127)  | +/− (1:16) | −      | + (87) | −       | −       | ND  | ND       | ND       | ND       | ND       | ND       |
| 5                  | + (120)  | +/− (1:16) | +/− ND | − | −       | −       | ND  | ND       | ND       | ND       | ND       | ND       |
| 26                 | −       | ND         | ND    | + (85) | −       | −       | −   | ND       | ND       | ND       | ND       | ND       |
| 15                 | −       | ND         | ND    | +/− (38) | −      | + (26) | −   | ND       | ND       | ND       | ND       | ND       |
| 49                 | −       | ND         | ND    | +/− (50) | −      | + (26) | −   | ND       | ND       | ND       | ND       | ND       |
| 39                 | −       | ND         | ND    | + (44) | ND       | + ND   | +   | ND       | ND       | ND       | ND       | ND       |
| 10                 | −       | ND         | ND    | + (59) | −       | −       | ND  | + (4.3) | −        | −        | −        | −        |
| 18                 | −       | ND         | ND    | + (26) | −       | −       | ND  | + (2.1) | −        | −        | −        | −        |
| 25                 | −       | ND         | ND    | + (33) | −       | −       | ND  | +/− (1.1) | ND       | ND       | ND       | ND       |
| 14                 | −       | ND         | ND    | + (27) | −       | −       | ND  | ND       | ND       | ND       | ND       | ND       |
| 43                 | −       | ND         | ND    | + (47) | −       | −       | ND  | ND       | ND       | ND       | ND       | ND       |

* +, positive; −, negative; +/−, borderline; ND, not determined. The interpretative criteria of the serological tests are as follows. For *Coxiella burnetii* (EIA phase II), an index of >110 is positive and an index of 90 to 110 is borderline. For enterovirus (EIA) IgM, >50 U/ml is positive and 30 to 50 U/ml is borderline. For enterovirus (EIA) IgG, >100 U/ml is positive and 80 to 100 U/ml is borderline. For EBV (CLIA), >20 U/ml is positive. For *Leptospira* spp. (EIA) IgM, >20 U/ml is positive and 15 to 20 U/ml is borderline. For *Borrelia* (CLIA) IgM, an index of >1.1 is positive and an index of 0.9 to 1.1 is borderline. For *Borrelia* (CLIA) IgG, >15 U/ml is positive and 10 to 15 U/ml is borderline. Positive and borderline results are shaded in gray.

* The index represents the cutoff index determined according to the recommendations of the manufacturer.

* In the negative EBV IgM blots, no antigen-specific bands were detected.

* In parentheses are the intensities of the detected bands in the recomLine blot abbreviated as follows: PuHa, group-specific antigen; Pu, Puumala virus; Ha, Hantaan virus; and Do, Dobrava virus.

* The index represents the cutoff index determined according to the recommendations of the manufacturer.

* In the negative EBV IgM blots, no antigen-specific bands were detected.
though an acute enterovirus infection in addition to the PUUV infection cannot be ruled out, it appears likely that the serological results represent cross-reactions due to unspecific IgM and maybe IgG stimulation. Unfortunately, neither a follow-up serum sample nor material from the investigated sample was available for further analysis.

Follow-up sera were available in another patient (patient 11) apart from the index patient. Serum 11b was collected 2 days after serum 11a and showed a marked increase of IgM antibodies against *Coxiella burnetii*, enterovirus, and EBV by immunoassay (Table 2). Although apart from IgM IgG antibodies against enterovirus were also detected, the observed marked increase of IgM but not IgG antibodies in conjunction with the marked increase of IgM against the other pathogens investigated suggests polyclonal IgM stimulation rather than enterovirus infection. IgM and IgG antibodies against the group-specific antigen and PUUV were detected with high intensity (+++/+++ ) in both sera.

For laboratory diagnosis of hantavirus infection, detection of viral RNA in blood and serology are available. Since detection of viral RNA is in most cases of Puumala virus infection only successful during the first days of illness, investigation of hantavirus-specific IgM and IgG antibodies in patients’ serum is recommended to diagnose hantavirus infection in central Europe. Hantavirus-specific IgM antibodies and in most cases also IgG antibodies appear already early during the disease, i.e., within the first days of illness (5, 8). Exact identification of the causal virus can, however, only be achieved by analyses of viral RNA, stressing the importance of RNA detection during the early phase of disease in patients with a severe clinical course or for epidemiological reasons.

As shown in our study, isolated or implausibly elevated levels of IgM antibodies against several bacterial and viral pathogens, which do not result from coinfection with the respective pathogens, can quite frequently be detected by immunoassays in patients with acute PUUV infection. Polyspecific IgM antibodies against other pathogens can occur early in PUUV disease and may be short-lived. Thus, follow-up sera should be recommended in all ambiguous cases in order to avoid misinterpretation of serological results. False-positive IgM responses against *Coxiella burnetii* may lead to misdiagnosis of acute Q fever in patients with acute hantavirus infection due to a similar clinical picture in some patients. This is especially relevant in regions like southern Germany, where both hantavirus and *Coxiella burnetii* are endemic. Confirmatory tests for *Coxiella burnetii* serology (MIF) appear, however, able to resolve the unspecific IgM stimulation detected by EIA.

Hantavirus serology may be valuable in patients with isolated IgM response against *Coxiella burnetii*, but may also be warranted in cases with isolated IgM antibodies against *Leptospira* spp. since the clinical pictures of leptospirosis and nephropathia epidemica can be quite similar. Nevertheless, further studies that include a larger number of cases are necessary to determine the frequency of false-positive leptospirosis serology in hantavirus infection since we only found one patient with a positive IgM response in the *Leptospira* EIA, and the EIA used has a specificity of only 90%, as stated by the manufacturer. Unfortunately, there is no more serum available for further investigation of this case.

The mechanisms leading to the observed IgM antibodies against various pathogens are not yet elucidated. However, as described for parvovirus B19 infections, polyclonal B cell stimulation may be suggested (7, 9). Further studies are necessary to investigate the underlying mechanisms in order to optimize our understanding and interpretation of serological tests in hantavirus infection.

ACKNOWLEDGMENTS

We thank Sonja Rothenberger and Andreas Essig, Institute of Medical Microbiology and Hygiene, University Hospital of Ulm, Ulm, Germany, for performing the *Coxiella burnetii* MIF.

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