NOTES

Antibody Response to Lipopolysaccharide in Patients Colonized or Infected with an Endemic Strain of *Acinetobacter* Genomic Species 13 Sensu Tjernberg and Ursing

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The levels of antilipopolysaccharide (anti-LPS) antibodies in patients colonized with an endemic *Acinetobacter* strain were compared to those in patients with bloodstream infections. Seropositivity and seronegativity correlated with positive and negative blood cultures, respectively, indicating that determination of the level of anti-LPS antibodies is useful for diagnosing *Acinetobacter* infections.

Members of the genus *Acinetobacter* have gained importance as nosocomial pathogens (1). To date, difficulties in the unambiguous identification of certain *Acinetobacter* spp. (2), we have recently started to generate and characterize monoclonal antibodies (MAbs) against *Acinetobacter* O antigens (4, 5) with the aim to develop an O-serotype-based identification scheme for these bacteria. Since lipopolysaccharide (LPS) is a potent antigen (7), we were also interested in determining the potential diagnostic value of anti-LPS antibodies generated during infection with *Acinetobacter* spp.

During a 6-month period, all patients admitted to the surgical intensive care unit (ICU) of a tertiary care hospital were surveyed for the presence of an endemic strain of *Acinetobacter* genomic species 13 sensu Tjernberg and Ursing (13TU). Tracheal aspirates and swabs from multiple body sites were obtained on admission and twice weekly thereafter and submitted for culture. Blood culture samples were obtained in cases of suspected bacteremia and inoculated as described previously (9). Additional specimens were obtained when clinically indicated. Isolates were identified to the genus level as described previously (9). They were identified to the species level by ribotyping and epidemiologically characterized by pulsed-field gel electrophoresis as described elsewhere (8). Their LPS O-antigen type was determined as described below.

Sera were obtained from 34 colonized patients from 13 of whom paired sera were available (Table 1). From the remaining patients, a single serum sample was available. Colonization was defined as the isolation of *Acinetobacter* organisms from one or more sites on one or more occasions in the absence of clinical symptoms or signs of infection. Bacteremia was defined in accordance with standard criteria (3). Control sera were obtained from 22 healthy donors. All samples were stored at −20°C.

Enzyme immunoassay (EIA) was performed as described elsewhere (6). Since MAb S48-13, prepared against LPS of *Acinetobacter* sp. 13TU strain 108 (5), reacted with all patient isolates (see below), purified LPS of this strain was used as a solid-phase antigen (5 µg/ml, diluted in phosphate-buffered saline [PBS, pH 7.2]). Serial twofold dilutions of serum in PBS supplemented with 2.5% casein (Sigma) were tested for immunoglobulin (Ig) antibodies starting at a dilution of 1:400. Prior to determination of IgM antibodies, sera were absorbed with an IgG-absorbing reagent (Mastsorb; Mast Diagnostika). Horseradish peroxidase-conjugated goat anti-human IgG, IgM, and IgA (Fcγ, μ chain, and α chain specific, respectively; Dianova) diluted 1:1,400 in PBS–2.5% casein were used as secondary antibodies. LPS of two *Acinetobacter* strains which share common determinants in the core region of their LPS with strain 108 but which have differing O-antigen types were used as controls for patient sera. Antibody titers were defined as the reciprocal value of the highest serum dilution yielding an optical density at 405 nm of ≥0.2. Cutoff titers (COV) were determined for each Ig class by the formula COV = highest blood donor antibody titer in the respective Ig class + 1 titer step. Seropositivity was defined as an antibody titer COV in two Ig classes for single sera and as a fourfold increase in titer in any Ig class for paired sera. The χ² test was used to determine the statistical significance of differences between serodiagnosis and blood culture results, and *P* = 0.001 was considered significant. Western and dot blot assays were performed as described earlier (6, 10), with proteinase K-treated bacterial lysate of strain 108 as the antigen. Sera were diluted 1:500 in blot buffer (10) supplemented with 10% nonfat dry milk. Alkaline phosphatase-conjugated goat anti-human IgG, IgM, and IgA (heavy plus light, μ, and α chain specific, respectively; Dianova) diluted 1:1,000 in the same buffer were used as secondary antibodies.

Patient isolates were determined as belonging to genomic species 13TU by ribotyping. All isolates showed the same pulsed-field gel electrophoresis pattern and therefore were considered to be of the same clonal origin (data not shown; the ribotype and pulsed-field gel electrophoresis patterns of some strains have been published elsewhere [8]). The strains were indeed of the same O-antigen type, as determined by dot blot assay using MAB S48-13 (data not shown). Blood donors had IgG, IgM, and IgA LPS antibody titers of 1,600, 800, and 400,
respectively (data not shown). COV were thus set to 3,200, 1,600, and 800 for the respective Ig classes.

Patients were colonized with the endemic strain within a mean of 8.6 days (range, 2 to 31 days) after admission to the surgical ICU. For three patients, no colonization prior to onset of bacteremia could be determined. Nineteen patients developed *Acinetobacter* bacteremia (Table 1) within a mean of 22.6 days after colonization. For the remaining patients, the endemic strain was isolated from respiratory tract specimens, as well as from other body sites. The antibody titer in all Ig classes

### TABLE 1. Reactivity of patient sera in EIA and in Western blot assay

| Patient no. | Serum sample no. | Time of collection<sup>a</sup> | Blood culture positivity<sup>b</sup> | Antibody titer of patient serum in EIA<sup>c</sup> | Serodiagnosis<sup>d</sup> of patient serum in Western blot assay<sup>e</sup> | Antibody reactivity of patient serum in Western blot assay<sup>f</sup> |
|-------------|------------------|-------------------------------|--------------------------------|---------------------------------|-------------------------------------------------|----------------------------------|
| 1           | 1                | 11                            | + (32)                         | 3,200 400 800                   |                    | +                              |
| 2           | 1                | 0                             | + (4)                          | >400 <400 400                   |                    | /--                             |
| 2           | 1                | 12                            |                                | 1,600 800 3,200                  |                    | +                               |
| 3           | 1                | 17                            | + (27)                         | 12,800 800 1,600                 |                    | +                               |
| 2           | 2                | 27                            |                                | 51,200 3,200 25,600              |                    | +                               |
| 4           | 1                | -1                            |                                | <400 400 800                    |                    | /--                             |
| 2           | 2                | 27                            |                                | 400 <400 800                    |                    | /--                             |
| 5           | 1                | 2                             |                                | 12,800 <800 800                 |                    | +                               |
| 2           | 1                | 5                             |                                | 12,800 <800 800                 |                    | +                               |
| 2           | 9                |                                |                                | 102,400 1,600 1,600              |                    | +                               |
| 3           | 12               |                                |                                | 204,800 1,600 1,600              |                    | +                               |
| 6           | 1                | 14                            | + (31)                         | 6,400 800 3,200                  |                    | +                               |
| 2           | 34               |                                |                                | 1,638,400 25,600 204,800         |                    | +                               |
| 3           | 57               |                                |                                | 819,200 6,400 102,400            |                    | +                               |
| 7           | 1                | 9                             | + (48)                         | 1,600 800 3,200                  |                    | /--                             |
| 2           | 28               |                                |                                | 25,600 3,200 1,600               |                    | +                               |
| 3           | 49               |                                |                                | 3,200 400 400                    |                    | /--                             |
| 8           | 1                | 51                            | + (44)                         | 409,600 25,600 26,500            |                    | +                               |
| 9           | 1                | 67                            |                                | 409,600 51,200 12,800            |                    | +                               |
| 10          | 1                | -1                            |                                | <400 400 400                     |                    | /--                             |
| 2           | 32               |                                |                                | 102,400 6,400 25,600             |                    | +                               |
| 11          | 1                | 18                            | + (13)                         | 1,600 400 400                    |                    | +                               |
| 2           | 30               |                                |                                | 1,600 800 1,600                  |                    | +                               |
| 12          | 1<sup>e</sup>    | 7                             | + (14)                         | 800 400 <400                     |                    | /--                             |
| 2           | 21               |                                |                                | 1,600 800 400                    |                    | +                               |
| 3           | 61               |                                |                                | 1,600 <400 400                   |                    | +                               |
| 13          | 1<sup>f</sup>    | -6                            | + (0)                          | <400 <400 <400                   |                    | /--                             |
| 14          | 1                | 45                            |                                | 1,600 12,800 3,200               |                    | +                               |
| 15          | 1                | 5                             |                                | 400 800 <400                     |                    | /--                             |
| 16          | 1                | 27                            | + (0)                          | 25,600 3,200 12,800              |                    | +                               |
| 17          | 1                | 21                            |                                | 400 800 400                      |                    | /--                             |
| 18          | 1                | 5                             | + (0)                          | 6,400 400 3,200                  |                    | +                               |
| 19          | 1                | -4                            |                                | 400 <400 <400                    |                    | /--                             |
| 20          | 1                | 3                             |                                | 1,600 400 400                    |                    | /--                             |
| 21          | 1                | 27                            | + (20)                         | 25,600 6,400 6,400               |                    | +                               |
| 22          | 1                | 2                             |                                | <400 <400 <400                   |                    | /--                             |
| 23          | 1                | -6                            |                                | 400 <400 400                     |                    | /--                             |
| 24          | 1                | 12                            | + (11)                         | 12,800 800 1,600                 |                    | +                               |
| 25          | 1                | 2                             |                                | 400 800 400                      |                    | /--                             |
| 26          | 1                | 88                            | + (87)                         | 102,400 12,800 3,200             |                    | +                               |
| 27          | 1                | 21                            | + (12)                         | 25,600 400 3,200                 |                    | +                               |
| 28          | 1                | 28                            | + (21)                         | 3,200 800 12,800                 |                    | +                               |
| 29          | 1                | 17                            | + (6)                          | 25,600 800 3,200                 |                    | +                               |
| 30          | 1                | 12                            |                                | 204,800 51,200 102,400           |                    | +                               |
| 31          | 1                | 5                             | + (2)                          | 1,600 800 3,200                  |                    | +                               |
| 32          | 1                | 7                             |                                | <400 <400 <400                   |                    | /--                             |
| 33          | 1                | 1                             |                                | <400 <400 <400                   |                    | /--                             |
| 34          | 1                | 2                             |                                | <400 <400 <400                   |                    | /--                             |

*<sup>a</sup>* The number of days postcolonization is indicated. A dash (--) indicates serum sample collection prior to colonization.

*<sup>b</sup>* +, positive blood culture; --, negative blood culture. The number of days postcolonization is in parentheses. A zero indicates that no prior colonization was determined.

*<sup>c</sup>* IgG, IgM, and IgA titers of serum sample 1 of patient 12 was 100.

*<sup>d</sup>* The IgG, IgM, and IgA titers of serum sample 1 of patient 13 were 200, 100, and 100, respectively.

*<sup>e</sup>* The IgG, IgM, and IgA titers of serum sample 1 of patient 12 was 100.

*<sup>f</sup>* The IgG, IgM, and IgA titers of serum sample 1 of patient 13 were 200, 100, and 100, respectively.
was 400 for all patients ($n = 4$) whose sera were collected prior to colonization or on the day colonization was documented ($n = 1$). Only for patient 4 was an IgA titer of 800 observed prior to colonization. However, since swabs were collected only twice a week the possibility cannot be excluded that this patient was colonized up to 3 days prior to a positive bacteriological culture. Of the 34 patients investigated, 22 were diagnosed as seropositive and 12 were as seronegative. Serodiagnosis was found to be consistent with the outcome of blood cultures ($P = 0.000037$); 18 seropositive patients were blood culture positive, and 11 seronegative patients were blood culture negative. Patient 8 was considered seropositive despite the absence of a fourfold increase in antibody titer in any of the Ig classes, since titers were considered to be peak values due to the late time points at which the samples were collected. Discrepant results were obtained in five cases. Four seropositive patients were blood culture negative. These patients were colonized at multiple body sites, including the respiratory tract, and their chest X-rays were suggestive of nosocomial pneumonia. Thus, these patients probably had *Acinetobacter* respiratory tract infections but their blood cultures remained negative. Seronegativity was diagnosed in one patient who had documented *Acinetobacter* bacteremia. This discrepancy is explained by the time point of serum collection, which was probably too early for serodiagnosis. In control experiments with LPS of two *Acinetobacter* strains which share common determinants in the core region of their LPS with strain 108 but which have differing O-antigen types, all 34 patients were seronegative, indicating that patient sera are LPS O serotype specific. Western blot analyses showed that seropositive patients had antibodies against the O antigen in all of the Ig classes tested, as well as IgM antibodies against the core lipid A moiety (Table 1).

Sensitivity, specificity, positive predictive value, and negative predictive value, calculated with regard to the serodiagnosis of *Acinetobacter* infection using blood culture positivity as the only parameter for documented infection, were 94.7, 73.3, 81.8, and 91.7%, respectively. These values indicate that serological assays such as the one described herein can be used to complement diagnosis by bacteriological cultures.

Bacteria of the genus *Acinetobacter* are known to colonize the skin and mucous membranes of patients, particularly in the ICU. This can lead to difficulties in determining the clinical significance of a specimen from which *Acinetobacter* bacteria are isolated (1). In this study, we addressed the question of whether determination of the level of anti-LPS antibodies in the sera of patients may be of diagnostic relevance. Seropositivity and seronegativity were indeed found to correlate with the recovery of the endemic *Acinetobacter* strain from blood cultures, indicating that the level of anti-LPS antibodies can be used to determine whether a patient is infected with an (endemic) *Acinetobacter* strain of a particular serotype, hence allowing clinicians to assess the significance of a specimen from which *Acinetobacter* bacteria are cultured.

Further studies need to be performed in order to demonstrate the possible benefit of serological assays in the diagnosis of *Acinetobacter* infections other than bloodstream infections, in particular, respiratory tract infections in mechanically ventilated patients.

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