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Antibody Response to Enterobacterial Lipoprotein of Patients with Varied Infections Due to *Enterobacteriaceae*[1,2] (39647)

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The cell wall of members of the family *Enterobacteriaceae* is complex, both structurally and biochemically. It consists of lipopolysaccharide, nearest to the cell surface; protein and phospholipid of the outer membrane; and lipoprotein (LP) and peptidoglycan (murein) components of the innermost layer.

Lipoprotein is part of the rigid layer of the cell wall (1). The protein portion of the molecule consists of repetitive amino acid sequence (2) with its N-terminal end covalently linked to the lipid component (3) and its C-terminal end covalently bound to the murein layer (4). Approximately 250,000 lipoprotein molecules are linked to the murein (5). The murein-lipoprotein complex can be isolated in pure form after vigorous isolation procedures, such as treatment with 4% boiling dodecylsulfate, and lipoprotein can be further cleaved from the murein by treatment with trypsin (4, 6). When the protein is released from the murein, a morphological distortion of the wall occurs, which suggests that this protein plays an important structural role in the organization of the cell wall. Preliminary studies suggest that the lipoprotein chemically and antigenically is similar among the members of the family *Enterobacteriaceae* (5, 7).

In view of the possible biologic importance of antigens common to enterobacteriaceae, either for diagnostic purposes or for immunization, the present study was initiated to determine the antibody response to lipoprotein of patients with various enterobacterial infections. This investigation was also considered important because patients with these infections respond differently to another antigen common to enterobacteriaceae, the common enterobacterial antigen (CA) (8).

Subjects, materials, and methods. The following groups of patients from Children's Hospital and Roswell Park Memorial Institute, Buffalo, New York, were included in the study: (1) 33 patients with peritonitis, complicating appendicitis; (2) 33 patients with cystic fibrosis, complicated by enterobacterial respiratory tract infection; (3) 25 patients with enteritis, due to *Salmonella* or *Shigella;* (4) 25 patients with urinary tract infection, due to enteric gram-negative organisms; and (5) 33 patients with malignant disease, complicated by gram-negative bacteremia. In the first four groups, the age of the patients ranged from 1 month to 21 years, the majority from 1 to 14 years. The patients with malignant disease were all adults, ranging in age from 20 to 81 years. From these patients a total of 597 serum specimens were examined; from most subjects, at least 2 specimens and from many, multiple specimens were available for study. In addition, serum from 25 healthy children, from 7 to 14 years old, and 25 healthy adults were included for control purposes; the sera were kindly supplied by the Virology Laboratory at Children's Hospital.

Cultures were obtained from the appropriate sites of infection of all patients and microorganisms were isolated by conventional means. Antigens (O) from the patients' own microorganisms were prepared according to a previously described procedure (9, 10).

Two preparations of lipoprotein (LP) extracted from the cell wall of *E. coli* B were kindly made available by Dr. Volkmar Braun of the Universität Tübingen, Tübingen, Germany. One preparation was murein-lipoprotein, subjected to lysozyme degradation of the murein; the other was

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lipoprotein released by trypsin from the murein. Because of availability, these studies were carried out with the former preparation and the specificity of the antibodies was documented in selected sera with the latter preparation. The materials were received in powder form and treated as follows. To 5.0 mg of lipoprotein (LP) was added 0.5 ml of 0.1 *N* NaOH; this mixture was incubated at 56° for 1 hr with occasional shaking. For neutralization, 0.5 ml of 0.1 *N* HCl was added. This stock solution contained 5000 μg/ml of LP. Further dilutions were prepared in phosphate hemagglutination buffer (PHB) (Difco; pH 7.3). All antigens were stored at −20°.

Serum specimens were collected from patients and healthy subjects and stored at −20° prior to use. The passive hemagglutination test was used to titrate antibodies to LP and to the O antigens of the infecting microorganisms. A 2.5% suspension of human (blood group O) erythrocytes was prepared and washed three times in PHB. For modification with LP, either preparation in a concentration of 50 μg/ml was added to the sediment of the washed red blood cells to yield a 2.5% concentration of the latter. The mixture was incubated at 37° in a waterbath for 30 min. The erythrocytes were again washed three times to remove unattached antigen and resuspended to the original concentration. Modification of erythrocytes with O antigen was accomplished by the procedure previously described in detail (9, 10). To titrate antibodies, serial twofold dilutions of serum in PHB were made in V-shaped wells of microtiter plates (Flow Laboratories, Rockville, Maryland), using microtiter diluters of 0.025 ml capacity (Cooke Engineering, Alexandria, Virginia). Then, 0.025 ml of PHB was added to each well followed by 0.025 ml of antigen-modified erythrocytes. The plates were incubated at 37° for 1 hr. Hemagglutination was determined by the settling pattern of the erythrocytes.

Results. The lipoprotein (LP) antibody levels of healthy subjects and of patients with varied enterobacterial infections were determined. Whenever multiple specimens from individual patients were available, the highest titer was recorded. The results are summarized in Table I.

Perusal of the table shows that none of the healthy children and only one of the healthy adults had antibodies against LP. Nor did patients with salmonellosis, shigellosis, or urinary tract infection have detectable levels of these antibodies. In contrast, 18% of children with peritonitis complicating appendicitis, 24% of patients with enterobacterial infection of the respiratory tract complicating cystic fibrosis, and 42% of adult subjects with bacteremia complicating malignancy, had LP antibodies in the serum. Twelve of the 28 patients with LP antibodies had elevated titers in the first available serum specimen and no further increase was observed in subsequent specimens. In contrast, an increase in the titer between acute and convalescent specimens could be documented in 16 out of the 28 patients. Such an increase occurred in 5 out of 6 subjects with peritonitis and 10 out of 14 patients with bacteremia. It is of interest to note that in patients with cystic fibrosis, associated with chronic infection, an increase in the titer of

TABLE I. LP ANTIBODY RESPONSE OF VARIOUS GROUPS OF PATIENTS WITH ENTEROBACTERIAL INFECTION.

Groups of subjects	Number of subjects	LP hemagglutinin titers[a] Numbers of subjects (%) <2	2–8	16–64	Total number of subjects with LP antibody response
Healthy children	25	25 (100%)	0	0	0 (0%)
Healthy adults	25	24 (96%)	1 (4%)	0	1 (4%)
Peritonitis	33	27 (82%)	4 (12%)	2 (6%)	6 (18%)
Cystic fibrosis	33	25 (76%)	3 (9%)	5 (15%)	8 (24%)
Enteritis	25	25 (100%)	0	0	0 (0%)
Urinary tract infection	25	25 (100%)	0	0	0 (0%)
Malignancy with bacteremia	33	19 (58%)	5 (15%)	9 (27%)	14 (42%)
Total	199	170 (85%)	13 (7%)	16 (8%)	29 (15%)

[a] Highest titered serum specimen.

LP antibodies was documented in only 1 out of 8 cases; in the others, elevated titers did not change. These elevated titers persisted from 3 to 30 months. Analysis of the relationship between LP antibody response and age revealed that these antibodies were produced by children as well as adults, the youngest child being only 8 months old, the others ranging in age from 1 to 18 years. Of the 28 patients with a significant LP antibody level the enterobacteriaceae isolated included *E. coli, Klebsiella, Enterobacter,* and *Citrobacter.* This observation provides suggestive evidence of the similarity of LP produced by various enteric bacteria, as proposed by Braun (5, 7).

Since these studies were carried out with the lysozyme-treated murein-lipoprotein preparation, which still retains fragments of murein at the C-terminal end of the protein, the specificity of the antibodies required clarification. To this end, 10 serum specimens containing antibodies against the antigen in elevated titers and 5 sera without these antibodies were tested in parallel with the murein-lipoprotein, as above, and with a preparation of lipoprotein cleaved from the murein by trypsin. In all instances the titrations yielded essentially identical results, the titers of the positive sera differing at most by one twofold dilution. Therefore, it is concluded that the antibodies demonstrated in the patients of this study were directed against the lipoprotein rather than against murein.

Finally, it was shown that an antibody response to the O antigens of the infecting microorganisms occurred more frequently than to the LP: 88% of patients with peritonitis, 88% of patients with enterobacterial infection complicating cystic fibrosis, 96% of patients with enteritis, 84% with urinary tract infection, and 85% of the patients with bacteremia complicating malignancy had a significant O antibody response.

Discussion. The lipoprotein (LP) portion of the cell wall of enterobacteriaceae, extensively studied by Braun and associates (4, 6), appears to be shared by various members of this family as revealed by analysis of the amino acid sequence (5) and immunologic properties (7). Such common antigens of microorganisms offer the opportunity of exploring immunization with a single antigen against infection by various pathogens sharing this antigenic determinant. On the other hand, if such an antigen is responsible for immunologic injury, such pathologic lesions may be engendered by seemingly unrelated microorganisms. Studies of the immune response to another common antigen of enterobacteriaceae, the common enterobacterial antigen (CA), have revealed that striking differences exist in the antibody response of patients with varied enterobacterial infections (8). It was deemed of interest, therefore, to investigate the immune response of such patient groups to the enterobacterial LP. Using the passive hemagglutination test, it could be shown that striking differences exist in the LP antibody response of patients with various enterobacterial infections. Such antibodies could not be demonstrated in 25 healthy children and in only 4% of healthy adults. In contrast, 42% of patients with bacteremia due to enterobacteriaceae complicating malignancy produced these antibodies. Twenty-four percent of patients with enterobacterial infection of the respiratory tract complicating cystic fibrosis and 18% of subjects with purulent peritonitis following appendicitis had LP antibodies. On the other hand, children with enteritis due to *Shigella* or *Salmonella* or with infection of the urinary tract did not have LP antibodies in the serum. Age alone did not account for these differences. It is of interest to note that in patients with similar infections the pattern of the immune response to LP differs strikingly from that to CA. Against CA, patients with peritonitis developed antibodies significantly more frequently and in higher titers than patients with the other infections studied (8). It remains for future investigations to identify the reasons for these differences in the immune response to two common antigens of enterobacteriaceae. Also, it will be of interest to learn whether lipoprotein autoantibodies present in certain patients with cancer or other diseases cross-react with LP of bacterial origin (cf. 11).

Summary. The passive hemagglutination test was used to determine the presence of antibodies against the lipoprotein (LP) component of the cell wall of members of the

family *Enterobacteriaceae* in the serum of healthy subjects and of patients with varied enterobacterial infections. LP antibodies were not demonstrated in the serum of healthy children and in only 4% of healthy adults. In contrast, 18% of patients with peritonitis complicating appendicitis, 24% of children with enterobacterial infection of the respiratory tract complicating cystic fibrosis, and 42% of patients with enterobacterial bacteremia complicating malignancy had these antibodies. LP antibodies were not detected in the serum of patients with *Salmonella* or *Shigella* enteritis or enterobacterial infection of the urinary tract. The specificity of the antibody was documented by the observation that with 10 serum specimens containing antibodies essentially identical titers were obtained with either the murein-lipoprotein or the lipoprotein preparation cleaved from murein as indicator antigens. The reason for these striking differences in the immune response to this common antigen remains to be elucidated.

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