INFECTION AND IMMUNITY, May 1981, p. 967-968
0019-9567/81/040967-02$02.00/0

Vol. 32, No. 2

Specific *Pseudomonas* Immunoglobulin E Antibodies in Sera of Patients with Cystic Fibrosis

JASMIN SHEN,[1] ROBERT BRACKETT,[2] THOMAS FISCHER,[1] ALAN HOLDER,[1] FRANK KELLOGG,[1] AND J. GABRIEL MICHAEL[1]*

Departments of Microbiology and Pediatrics, University of Cincinnati Medical Center, Cincinnati, Ohio 45267,[1] and Warner-Lambert Co., Detroit, Michigan 48232[2]

Immunoglobulin E antibodies to *Psuedomonas aeruginosa* were demonstrated in patients with cystic fibrosis colonized with the bacterium.

We recently reported that immunization of CBA and BDF_1 mice with gram-negative bacteria resulted in formation of reaginic immunoglobulin E (IgE) antibacterial antibodies (1). These antibodies were directed against surface protein antigens of *Pseudomonas aeruginosa, Escherichia coli,* and several other enterobacterial species. We investigated whether IgE antibacterial antibodies are formed in humans. Patients suffering with cystic fibrosis (CF), who are often chronically infected with *P. aeruginosa*, were selected for this investigation.

Sera were obtained from patients with CF who had sputum cultures positive for *P. aeruginosa* and from children with asthma without history of pseudomonas infection (Children's Hospital, Cincinnati, Ohio). The ages of the donors of serum samples were from 3 to 16 years. The sera selected for this study, taken from CF patients and asthmatics, contained over 100 U of IgE per ml. The sera from healthy donors had IgE levels below that value.

Total IgE levels were measured by a commercially developed radioimmunoassay (PRIST, Pharmacia Diagnostics, Piscataway, N.J.). The manufacturer kindly donated the kits for this assay. Specific antibacterial IgE antibodies were estimated by calculating the reduction in total serum IgE after adsorption of the sample with bacteria. Briefly, sera were incubated with 10% suspensions of acetone-dried bacteria at 37°C for 3 h. Subsequently, the bacteria were removed by centrifugation, and IgE levels before and after adsorption with bacteria were determined. Reduction in IgE levels in the serum was taken as an evidence for removal of specific antibacterial antibodies. The specificity of the antibody was ascertained by adsorption with a different bacterial species. IgE antibody to short ragweed was measured by the radioallergosorbent test with a commercially prepared kit (Pharmacia Diagnostics). Antibodies directed against bacterial lipopolysaccharide antigens were measured by the passive hemagglutination technique as described by us and others (5). Human O erythrocytes were coated with lipopolysaccharide prepared from seven serological types of *P. aeruginosa* as defined by Fisher et al. (2). Serial dilutions of sera, untreated and treated with 2 mercaptoethanol, were mixed with lipopolysaccharide-coated erythrocytes, and agglutinin titers were determined after overnight incubation. Treatment with 2-mercaptoethanol degrades IgM antibodies.

To establish the extent of the normal immune response to *P. aeruginosa*, agglutinins to seven immunotypes of the bacterium were measured. As shown in Table 1, agglutinin titers to all of the pseudomonas serotypes were significantly higher in CF patients than they were in normal individuals or in asthmatics, both in untreated and in 2-mercaptoethanol-treated sera. To determine whether antimicrobial IgE antibodies are present in sera of CF patients and asthmatics, these sera were adsorbed with *P. aeruginosa* or *E. coli* suspensions (acetone-dried bacteria), and residual IgE in adsorbed sera was determined (Table 2). The reduction in total IgE after adsorption with *P. aeruginosa* was significantly greater in the CF group compared to asthmatic patients. The significance of the differences between these groups was determined by analysis of covariance ($P = 0.0062$). To determine whether a difference in the reduction of IgE exists between *P. aeruginosa* and *E. coli*, a paired t test was performed for both groups, those with CF and those with asthma. A log transformation was made of the response variables to stabilize the variance. The paired t test for the patients with CF was significant ($P < 0.0001$). Thus, the average reduction with *P. aeruginosa* was significantly greater than the average reduction with *E. coli*. The paired t test for the patients with asthma did not yield a significant test statistic ($P = 0.2899$). To ascertain that the bacteria removed only antibacterial IgE antibodies, several sera from the CF group (no. 15, 23, and 39) were tested by the radioallergosorbent test before and after adsorption with *P. aeruginosa* cells for antiragweed antibodies. No apparent reduction in the antiragweed antibody titer after adsorption with bac-

TABLE 1. *Agglutinin titers to seven immunotypes of P. aeruginosa*

Population	n	2-Mercaptoethanol treatment	Agglutinin titer to immunotype: 1	2	3	4	5	6	7
Normal	7	−	2.3 ± 0.0[a]	3.3 ± 0.3	2.9 ± 0.2	4.5 ± 0.4	2.3 ± 0.0	4.1 ± 0.2	2.7 ± 0.2
Asthmatics	10	−	2.9 ± 0.2	2.3 ± 0.2	2.0 ± 0.3	3.8 ± 0.4	1.7 ± 0.0	3.6 ± 0.3	2.0 ± 0.2
CF Patients	10	−	7.6 ± 0.7	7.9 ± 0.4	4.7 ± 0.3	9.0 ± 0.7	5.6 ± 0.5	8.4 ± 0.5	5.6 ± 0.7
Normal	7	+	2.2 ± 0.0	3.0 ± 0.4	2.3 ± 0.0	2.6 ± 0.2	2.3 ± 0.0	2.3 ± 0.0	2.7 ± 0.2
Asthmatics	10	+	2.5 ± 0.2	2.0 ± 0.0	1.7 ± 0.0	3.3 ± 0.2	1.4 ± 0.0	3.3 ± 0.2	1.8 ± 0.3
CF Patients	10	+	6.3 ± 0.9	4.8 ± 0.7	3.1 ± 0.1	6.7 ± 0.8	4.4 ± 0.3	6.2 ± 0.8	4.5 ± 0.6

[a] Mean ± standard error of the mean agglutinin titer to $\log_2$. CF patients versus asthmatic and normals, difference in pseudomonas agglutinin titers: $P < 0.01$ with every serotype.

TABLE 2. *Antibacterial IgE antibodies in sera of CF patients and asthmatics*

Population/patient no.	Total IgE (IU/ml)	IgE (IU/ml) bound to: *P. aeruginosa* 1210	*E. coli* O127
CF			
7	260	49.9	12.0
13	170	27.0	4.2
15	340	49.9	9.9
18	260	40.5	7.8
19	110	29.1	3.4
20	540	183.6	0
23	1,000	294.0	83
26	100	17.4	2.2
28	105	13.0	7.0
39	740	149.4	60
Asthma			
1	420	29.8	0
2	660	0	0
3	670	0	0
4	940	9.4	10.3
5	388	11.2	11.2
6	2,100	84.0	71.4
7	560	28.0	43.9
8	510	21.9	35.2
9	670	43.5	31.5
10	420	19.7	31.9

teria was detected.

As expected, CF patients chronically infected or colonized with *P. aeruginosa* produced significant amounts of antibody of IgM and IgG classes to this organism. These antibodies were of multiple specificities, indicating the patients' exposure to many serotypes. Impairment of the pulmonary defenses through reduced phagocytic activity by alveolar macrophages and polymorphonuclear leukocytes and poorly functioning mucociliary clearance is believed to be responsible for chronic bacterial infections in CF patients (3). CF patients have been reported to have elevated serum IgE levels to several allergens, but antibacterial antibodies have not been reported (4, 6). Although in this study the specific pseudomonas antigens responsible for IgE production have not been identified, we have shown that surface protein antigens, common to all *P. aeruginosa* strains, provide a vigorous stimulus for reaginic antibody formation in mice (1). The role of IgE in CF pathogenesis is not known. Some reports suggest that respiratory allergy may provide a degree of protection against pulmonary infections (4). Other studies contradict this claim and state that atopy causes a higher incidence of infections and deterioration of patients' health, as evidenced by chest X-rays (6). Indeed, histamine released from mast cells during IgE-mediated degranulation has been reported to inhibit certain functions of polymorphonuclear leukocytes, including chemotaxis (7), thus impairing influx of polymorphonuclear leukocytes to the lungs and reducing the effectiveness of bacterial elimination. Our data indicate that it is essential to investigate further the relationship between the immunological state and the disease in patients with CF.

We thank Joan Shillis and Barbara Underwood for assistance in agglutinin titer determination.

This work was supported by Public Health Service grant AI 13877 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. **Danneman, P. J., and J. G. Michael.** 1976. Reaginic antibody production to protein antigens of *Escherichia coli* and *Pseudomonas aeruginosa* by mice. Infect. Immun. **14**:694–702.
2. **Fisher, M. W., H. B. Devlin, and F. J. Gnabasik.** 1969. New immunotype schema for *Pseudomonas aeruginosa* based on protective antigens. J. Bacteriol. **98**:835–836.
3. **May, J. B., N. C. Herrick, and D. Thompson.** 1972. Bacterial infection in cystic fibrosis. Arch. Dis. Child. **47**:908–913.
4. **Rachelefsky, G. S., A. Osher, R. F. Dooley, B. Ank, and E. R. Stiehm.** 1974. Coexistent respiratory allergy and cystic fibrosis. Am. J. Dis. Child. **128**:355–359.
5. **Veit, B. C., and J. G. Michael.** 1972. The lack of thymic influence in regulating the immune response to *Escherichia coli* 0127 endotoxin. J. Immunol. **109**:547–553.
6. **Warner, J. O., B. W. Taylor, A. P. Norman, and J. F. Soothill.** 1976. Association of cystic fibrosis with allergy. Arch. Dis. Child. **51**:507–511.
7. **Zurier, R. B., G. Weissmann, and S. Hoffstein.** 1974. Mechanism of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP autonomic agonists and agents which affect microtubule function. J. Clin. Invest. **53**:297–309.