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THE IMMUNE RESPONSE OF THE HOST: AN AID TO ETIOLOGY, PATHOGENESIS, DIAGNOSIS, AND EPIDEMIOLOGY OF BACTERIAL INFECTIONS**

The antibody response of patients with various infections has been utilized as an aid to diagnosis since the early days of scientific microbiology, as exemplified by the Widal, Wassermann, and Weil-Felix tests. A variety of serologic techniques have been used to this end. In our laboratories we have studied the antibody response by means of the passive bacterial hemagglutination test on patients with a variety of bacterial infections, including enteropathogenic *E. coli* enteritis, salmonellosis, shigellosis, urinary tract infection, and *Pseudomonas aeruginosa* infection. This method is based on the fact that various bacterial antigens, polysaccharide in nature, readily become attached to the surface of erythrocytes, resulting in the acquisition of a new serologic specificity. These antigen-modified red blood cells are agglutinated in the presence of homologous bacterial antibodies. It is the purpose of this paper to illustrate the potential usefulness of such studies as an aid to etiology, pathogenesis, diagnosis, and epidemiology.

SUBJECTS, MATERIALS, AND METHODS

Selected patients from Children's Hospital and its out-patient department were studied bacteriologically and immunologically. Microorganisms were identified by conventional methods. Serum specimens were collected at various intervals and tested for antibodies to the isolates by means of the passive hemagglutination test.[1] For control purposes, 5 O groups each of salmonellae, shigellae, and enteropathogenic *E. coli* were used in the multivalent hemagglutination procedure. Briefly, human erythrocytes of blood group O were modified with bacterial antigens by incubation of the cells with supernates of agar-grown cultures for 30 minutes in a waterbath at 37°C. The cells were washed, in order to remove residual antigen, and then added to equal amounts (0.2 ml) of serum in serial two-fold dilutions. The mixtures were incubated for 30 minutes at 37°C. Hemagglutination was read grossly after centrifugation at 1300 g. for 2 minutes.

The hemagglutination test is at least as sensitive as the bacterial agglutination method and has the following additional advantages: (1) It can be employed for screening and control purposes as a multivalent procedure and (2) it allows the documentation of an antibody response with certain rough strains, unsuitable for bacterial agglutination.

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RESULTS

Antibody response and suspected pathogens. Two decades ago, epidemiologic evidence suggested the etiologic role of certain serotypes of *E. coli* in epidemic and endemic diarrheal disease of infants. It was reasoned that, if a specific immune response could be documented, it would provide additional support for this concept. For these reasons, the hemagglutination test was adapted to the study of antibodies to O antigens of various enterobacteriaceae, including enteropathogenic O groups of *E. coli* (E.E.C.). In more than half of the infants with E.E.C. enteritis a specific antibody response could be documented.[2]

This approach is also useful with respect to other suspected pathogens, notably those present on mucous membranes of the gastrointestinal, respiratory, or urinary tracts. It is clear that the mere isolation of such microorganisms from feces, sputum, or voided urine does not by itself prove that the isolates are responsible for clinical infection.

The findings on a patient with cystic fibrosis, from whose sputum *E. coli* O75 was repeatedly isolated, are summarized in Table 1. A marked increase in the antibody titer to *E. coli* can be documented, thus suggesting a pathogenic role of the isolate. It is well known that patients with cystic fibrosis characteristically harbor, in the respiratory tract, pathogenic staphylococci and that *Pseudomonas aeruginosa* frequently becomes established in the course of this genetically conditioned disease. Thus, it was of interest to determine whether these patients respond immunologically to the O antigens of this species. A study by Diaz and Neter[3] revealed that the majority of patients with either mucoid, or non-mucoid *Pseudomonas aeruginosa,* or both, in the respiratory tract developed O specific antibodies in significant titers, thus supporting the concept of these microorganisms as pathogens.

This immunologic approach is applicable to a variety of other clinical problems as well. Another illustration involves a patient with the clinical diagnosis of appendicitis. At laparotomy a normal appendix was discovered; bacteriologic study revealed *Shigella sonnei* in the feces. The fact that a significant increase in the titers of homologous antibodies could be

TABLE 1. ANTIBODY RESPONSE TO *E. Coli* O75 OF PATIENT WITH CYSTIC FIBROSIS

Dates of serum specimens	*Hemagglutinin titers (reciprocal)*	
	E. coli O75	*Proteus mirabilis*
5-16-70	40	40
6-25-70	640	40

documented within a 3-day period, as shown in Table 2, clearly indicates that this patient had acute clinical shigellosis. Without this information, the finding of *S. sonnei* did not necessarily explain the nature of the illness, since it could have represented a previously existing carrier state.

The potential value of detecting a specific immune response is also illustrated by the following observations. *Shigella sonnei* was isolated from a patient with chronic ulcerative colitis. Alone, this finding does not constitute evidence for a diagnosis of clinical shigellosis complicating the basic disease. However, demonstration of a significant rise in the titer of *Shigella* antibodies does provide such evidence (Table 3). An immunologic approach appears to be particularly useful when dealing with opportunistic pathogens, organisms which are assuming an ever increasing role in present-day infections.

Immune response and epidemiology. A. Immune response and subclinical infection. Studies carried out during the past few years on family members of patients suffering from either shigellosis or salmonellosis revealed that these infections are far more widespread than is generally recognized, as indicated by the immunologic findings.[4,5,6,7] The specific antibody response was frequently detected in siblings, parents, or other contacts. Infection may be either overt or subclinical. In addition, a rise in the titer of group specific antibodies quite frequently can be demonstrated even when cultural examination of the feces fails to yield positive results. This is not surprising, since the duration of the antibody response usually exceeds, by a considerable length of time, that of the excretor status in the majority of patients with these enteric infections. The immunologic approach can help establish

TABLE 2. PSEUDOAPPENDICITIS AND SHIGELLOSIS

Dates of serum specimens	*S. sonnei Hemagglutinin titers (reciprocal)*
1-23-70	40
1-26-70	2560

TABLE 3. IMMUNE RESPONSE TO *Shigella Sonnei* OF PATIENT WITH ULCERATIVE COLITIS

Dates of serum specimens	*Hemagglutinin titers (reciprocal)*	
	Shigella sonnei	*E. coli* O14
9- 8-70	20	40
9-11-70	20	40
9-24-70	640	40

a retrospective diagnosis as well as foster recognition of subclinical infection, and thus provides more information on the true incidence of these common maladies.

B. Immune response and onset of infection. The epidemiologic events leading to family outbreaks of shigellosis and salmonellosis frequently remain obscure. By documenting the immune response, primary and secondary cases can be distinguished. Findings on a representative family outbreak of *Shigella sonnei* infection are summarized in Table 4. It is evident that the index case (A.P.) had the infection prior to that of both a sibling (M.P.) and the mother, since on May 10 the antibody titer was already significantly elevated in patient (A.P.) but only later increased in the other two family members. A third sibling, (D.P.), failed to show immunologic evidence of infection during this time period. The results of a similar study on a family with *S. typhimurium* infection are presented in Table 5. It is clear that infection of the mother and of one contact preceded that of the index case (R.M.). A secondary infection was also present in another contact. That these antibody responses are specific is evident from the observations described in the table, that parallel increases in the titers of antibodies to *Salmonella* group C_1 and to 5 serogroups of shigellae did not occur during the same time period.

C. Immune response and double infection. When bacteriologic examination of a patient with diarrheal disease reveals the presence of two potential pathogens, the assumption is frequently made that both are responsible for the illness. It is conceivable, however, that the disease is due to one of

TABLE 4. IMMUNE RESPONSE AND ONSET OF INFECTION (*Shigella Sonnei* DYSENTERY)

Subjects and dates of serum specimens	*Hemagglutinin titers (reciprocal) S. sonnei*
A.P.	
5-10	640
5-19	640
M.P.	
5-10	10
5-19	320
Mother	
5-10	40
5-19	640
D.P.	
5-10	10
5-19	10

these microorganisms in a carrier of the other. Based on these considerations, immunologic studies have been performed on patients harboring two potential pathogens in the intestinal tract. A specific immune response could often be documented to only one of the isolates, suggesting that the disease was not due to double infection. In other instances, however, as shown in Table 6, a specific antibody response to two distinct O groups of salmonellae, which were isolated from a single fecal specimen, were noted. That the high titers of *Salmonella* O antibodies were not due to a cross-reaction is evident from the results of absorption experiments, also shown in the table. Both *Salmonella* O groups completely removed only the homologous antibodies. This immunologic approach may also be useful in the study of patients excreting both potentially pathogenic viruses and bacteria.

Duration of illness and immune response. It is generally recognized that documenting a rise in antibody titer is often more meaningful than a single antibody titration. That the second blood specimen need not be taken seven or more days after the first, is based on the consideration that the interval necessary for documentation of an immune response depends upon the on-

TABLE 5. SEROLOGIC EPIDEMIOLOGY OF SALMONELLOSIS

	Hemagglutinin titers (reciprocal)		
	Salmonella groups		*Shigella*
	B	C_1	
R.M.			
10-18	40	<10	<10
11-7	320	<10	<10
Mother			
10-18	160	40	40
11-7	320	20	40
Contact			
10-18	160	20	20
11-7	160	20	20
Contact			
10-18	<10	10	20
11-7	640	20	10

TABLE 6. IMMUNOLOGIC DIAGNOSIS OF DOUBLE INFECTION BY SALMONELLAE

	Hemagglutinin titers (reciprocal)					
	Salmonella groups				*Shigella*	*E.E.C.*
Serum	*B*	C_1	C_2	*D*	*5 types*	*5 types*
Not absorbed	640	40	1280	160	40	10
Absorbed with						
Salmonella B	<40	—	1280	—	—	—
Salmonella C_2	160	—	<40	—	—	—

set of infection and, therefore, of the antigenic stimulus. In a number of instances such an increase in antibody titers could be clearly shown with two specimens taken 48 hours apart. As shown in Table 7, even a 24 hour interval may suffice to prove an immune response in patients whose infection commenced five or more days prior to the procurement of the first blood specimen. It is evident also from the table that a very significant increase took place during the ensuing six days.

TABLE 7. SPECIFIC RISE IN ANTIBODY TITERS WITHIN 24 HOURS IN PATIENT WITH SALMONELLA ENTERITIS 4 DAYS AFTER ONSET OF CLINICAL ILLNESS

Dates of blood specimens	*Salmonella group D antibody titers*
4-11-67	<10
4-12-67	80
4-18-67	10,240

SUMMARY AND CONCLUSIONS

A study of the immune response of patients with a variety of bacterial infections was carried out, utilizing the passive hemagglutination test. The pathogens included, among others, salmonellae, shigellae, enteropathogenic *E. coli,* and *Pseudomonas aeruginosa.* Documentation of a specific antibody response was shown to help clarify the role of suspected pathogens, to establish the presence of subclinical infection, to make possible the diagnosis of recent infection when cultural examination yields negative results, and to establish the existence of dual infection. A significant increase in titer may be documented with two blood specimens taken only a few days or even 24 hours apart, depending upon the onset of the disease.

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