JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 1975, p. 298-301
Vol. 1, No. 3

Printed in U.S.A.

Antibody Response of Patients with Malignancies to Bacteremia with Gram-Negative Bacteria

MICHAEL J. SURGALLA, ERWIN NETER,* AND JOHN E. FITZPATRICK

*Department of Laboratory Medicine, Roswell Park Memorial Institute, Buffalo, New York 14203; and the Laboratory of Bacteriology, Children's Hospital, Buffalo, New York 14222**

Received for publication 16 December 1974

Antibody response, determined by means of the indirect bacterial hemagglutination test, was studied in 58 consecutive patients with various malignancies whose blood culture yielded growth of *Enterobacteriaceae* or *Pseudomonas* and from whom serum specimens were obtained. Of these patients 59% had a significant antibody response. The invading microorganisms were *Escherichia coli* in 33 and *Klebsiella* in 19 subjects, an antibody response being documented with essentially equal frequency (60 and 57% of the subjects, respectively). Two patients had positive blood cultures for both *E. coli* and *Klebsiella*, one of whom had a significant response to one isolate only. A specific antibody response was documented in 67% of the subjects from whom blood for antibody titration was obtained at least 5 days after the blood culture, but from only 21% of patients whose serum was procured during the first 5 days after the blood culture. Similarly, such an antibody response was identified in 73% of subjects with two consecutive serum specimens, but in only 28% of the patients with a single serum specimen for antibody titration. Documentation of the immune response may be of diagnostic aid in differentiating between infection and contamination even in patients with underlying malignancy and under potentially immunosuppressive therapy.

Documentation of the specific antibody response aids in the establishment of the clinical significance of bacterial isolates and in epidemiological studies of infections. This supporting evidence is particularly important when contamination of specimens cannot be excluded with certainty. It is also a helpful tool when dealing with opportunistic pathogens, whose pathogenic potential depends more on host factors than on virulence factors of the microorganisms. The present study was undertaken to determine the antibody response of patients with malignancy to gram-negative bacteria isolated from the blood. Such a study was deemed particularly desirable since cancer chemotherapy and the disease itself may interfere with the production of bacterial antibodies (1, 3, 7, 19, 20, 22), and since infection still plays a major role in the subjects even today (2, 6, 8-10, 21).

MATERIALS AND METHODS

In all, 58 consecutive subjects with positive blood cultures were available from whom at least one blood specimen for antibody titration was obtained subsequently. For reasons beyond our control, including death of patients or unavailability of serum specimens, some patients with bacteremia, notably due to *Pseudomonas aeruginosa*, could not be included in the study. Of these 58 patients, 33 had solid tumors and the remaining 25 had malignant disease of the bone marrow or reticuloendothelial system (RES). In the former group, carcinoma of the colon and rectum was present in eight subjects; of the uterus and cervix in seven; of the prostate in five; of the bladder in three; of the ovary in three; of the breast in two; of the testis, lung, stomach, or mouth in one each; a single patient with malignant melanoma is included in this group. In 28 of these patients the disease had disseminated beyond the site of origin. Of the 25 patients with malignant diseases of the bone marrow or RES, 15 had acute leukemia, five malignant lymphoma unrelated to Hodgkin's disease; two had multiple myeloma; and one each had chronic leukemia, Hodgkin's disease, or reticuloendothelial sarcoma.

Of the 25 patients with malignant diseases of the bone marrow or RES, 24 (96%) had received multiple drug combination cytotoxic chemotherapy in contrast to 17 (52%) of 33 patients with solid tumors.

All but three of the 58 subjects were adults. The youngest patient was a nine-year-old child with reticulum cell sarcoma and the other two patients, both 16 years of age, had acute leukemia. Of the others, 15 patients were between 20 and 50 years of age; 21 subjects between 50 and 59; and 19 subjects between 60 and 81 years of age.

Of the 58 patients, eight succumbed within 7 days after the blood culture was taken. Somatic O antigens were prepared from the isolates according to a previously published method (14, 17). Blood specimens for

antibody titration were collected as soon as possible after the blood cultures were found to be positive and a second specimen approximately a week later. Antibodies to the patient's own organisms were quantitated by means of the passive hemagglutination test, as described previously (14, 17). In addition, antibodies against the common enterobacterial antigen were quantitated in parallel (18). Antibody levels against five serogroups of enteropathogenic *Escherichia coli*, five serogroups of *Salmonella*, and five serotypes of *Shigella* were included for control purposes (9, 14). The antibody response was considered to be positive when either there was at least a fourfold increase in the antibody titers to the O antigens of the patients' own microorganisms, or when the titers of these antibodies were at least fourfold higher than those against the control antigens (for examples see Table 3). In no instance was there an increase in the titers of antibodies against control antigens in the absence of an increase in the antibody titers against the patients' microorganisms. Nor did antibody titers against the control antigens increase in parallel with those against the patients' microorganisms.

RESULTS

The antibody response of the 58 patients, whose blood cultures were positive for *Enterobacteriaceae* or *Pseudomonas aeruginosa*, was studied. The results summarized in Table 1 show that 34 out of 58 (59%) patients did have a significant antibody response to the O antigens of their own strains present in the blood stream. Of the various species, *E. coli* and *Klebsiella* accounted for 50 (86%) of the 58 subjects and more than half of these patients did have a significant immune response. In contrast, of the three patients from whom *Serratia* was isolated none developed antibodies in significant titer.

As expected, a specific immune response was observed far more often in patients from whom at least two serum specimens, rather than one, were available, and whose serum specimens were obtained more, rather than less, than 5 days after the blood cultures (Table 2).

TABLE 1. *Antibody response to their isolates of patients with positive blood cultures and malignancies*[a]

Microorganisms	Patients positive	Patients negative
Escherichia coli	20	13[b]
Klebsiella	10[b]	9[b]
Serratia	0	3
Pseudomonas	1	1
Enterobacter sp.	1	0
E. cloacae	1	0
E. liquefaciens	1	0

[a] For explanation see Materials and Methods.
[b] From two patients, both *E. coli* and *Klebsiella* were recovered.

Table 2 also shows that a significant antibody response was observed somewhat more frequently in patients with solid tumors (69.7%) than in those with bone marrow or RES malignancies (44%). Further, a significant immune response could be documented even in patients whose leukocyte counts were $<1{,}000/mm^3$ (Table 2).

The results of antibody studies on three patients, representative of the three patterns of the immune response, are shown in Table 3.

Of the strains of *E. coli*, 21 were typable and 12 were not, and 16 strains belong to the O groups known to cause urinary tract infection in 40 to 60% of the patients ([E. Neter, Kidney Int., in press] 16). Analysis of the available data

TABLE 2. *Antibody response to their isolates of various groups of patients with positive blood cultures and malignancies*[a]

Patient groups	Patients positive (%)	Patients negative (%)
Patients with:		
Two serum specimens	29 (72.5)	11 (27.5)
One serum specimen	5 (27.8)	13 (72.2)
Patients with serum specimens taken after blood culture:		
>5 days	31 (67.4)[b]	15 (32.6)[b]
<5 days	3 (21.4)	11 (78.6)
Patients with:		
Bone marrow or RES malignancies	11 (44)	14 (56)
Solid tumors	23 (69.7)	10 (30.3)
Patients with leukocyte counts:[c]		
$<1{,}000/mm^3$	13 (56.5)	10 (43.5)
$>1{,}000/mm^3$	21 (60)	14 (40)

[a] For explanation see Materials and Methods.
[b] From two patients, two species of microorganisms each were recovered from the blood cultures.
[c] Findings on the days of the blood cultures.

TABLE 3. *Patterns of antibody responses of patients with positive blood cultures and malignancies*

Patients	Dates of specimens	Antibody titers (reciprocals)			
		Patients' microorganisms	Mixtures		
			Shigella	*Salmonella*	*Escherichia coli*
A. P.	July 1974	80	20	80	40
	Aug 1974	2560	20	80	20
H. B.	Jan 1974	640	80	40	40
M. M.	May 1974	20	20	40	20
	June 1974	40	20	40	20

indicates that 20 out of 33 (61%) patients with *E. coli* bacteremia showed a significant immune response. When considering only typable strains, 12 out of 21 patients (57%) showed a significant immune response. The O groups which represent 40 to 60% of urinary tract infection due to *E. coli* also account for a significant percentage of infections outside the intestinal tract, including meningitis, sepsis, pneumonia, peritonitis, etc. (E. Neter, Kidney Int., in press). So far as the present series of patients is concerned, these O groups (O4, O6, O7, O75) account for 16 out of 33 patients (48%) or 16 out of 21 patients (76%) when considering only typable strains. Of the latter 16 patients, 10 mounted a significant antibody response.

An increase in the titer of homologous antibodies between two serum specimens taken a few days apart indicates that the antigenic stimulation took place recently rather than several weeks or months previously. Of the 30 patients from whom two serum specimens were obtained and whose antibody titers were elevated, 77% showed a significant increase, indicating that the infection documented by the blood culture was of recent origin. A localized infection caused by the same microorganisms, of course, also may have stimulated antibody production.

A study of the antibody response of these patients to the common enterobacterial antigen revealed that only three showed a significant increase in the titers of these antibodies, the increase being fourfold in two, and eightfold in the other case. In contrast, a patient with *Shigella sonnei* bacteremia, after renal transplantation, produced such antibodies in surprisingly high titers while under immunosuppressive therapy (15).

DISCUSSION

The immune response of the host as an aid to the specific diagnosis of infectious diseases has been used for more than half a century since the Widal, Wassermann, and Weil-Felix tests were introduced into the armamentarium of the microbiology laboratories. Documentation of the specific humoral immune response has been used for the elucidation of the pathogenicity of various suspected pathogens, notably, viruses. Studies over the past decade carried out in our laboratories have revealed that this approach can be fruitful for the study of enteric and urinary tract infections also, and allows for differentiation between contamination and infection or colonization when suspected pathogens are recovered from the respiratory and intestinal tracts or from blood cultures and other clinical specimens (4, 5, 7, 11–13, 16). The present study was carried out to determine whether patients with malignancy, many under treatment with potentially immunosuppressive drugs, produced O antibodies against gram-negative bacteria recovered on blood culture. Such an investigation was deemed to be particularly important since impairment of the immune system in patients and experimental animals with malignancies has been documented (1, 3, 7, 19, 20, 22), and since infection still plays a major role in morbidity and mortality of these subjects (2, 6, 8–10, 21). By the use of conservative criteria for the interpretation of the antibody titrations, some 59% of these patients had a diagnostically significant antibody response, shown either by an increase in the titers of homologous O antibodies or by the fact that antibody titers against the patients' own isolates were significantly higher than the titers of antibodies against control antigens. The percentage increased to 73% for those patients from whom two consecutive blood specimens were available for antibody study. Thus, investigations on the antibody response of patients with malignancy are of value despite the possible immunosuppressive effects of the underlying disease and/or of the cytotoxic chemotherapy.

ACKNOWLEDGMENTS

We express our appreciation to Betty P. Patterson, Helga von Langendorff, Robert J. Botzer, Laurie S. Morchan, and Verna Mordaunt for excellent assistance.

This study is supported in part by Public Health Service research grant AI 00658 from the National Institute of Allergy and Infectious Diseases.

LITERATURE CITED

1. Aisenberg, A. C. 1966. Manifestations of immunologic unresponsiveness in Hodgkin's disease. Cancer Res. **26**:1152–1160.
2. Armstrong, D., L. S. Young, R. D. Meyer, and A. H. Blevins. 1971. Infectious complications of neoplastic disease. Med. Clin. N. Am. **55**:729–745.
3. Ceglowski, W. S., and H. Friedman. 1968. Immunosuppressive effects of Friend and Rauscher leukemia disease viruses on cellular and humoral antibody formation. J. Nat. Cancer Inst. **40**:983–995.
4. Diaz, F., L. Mosovich, and E. Neter. 1970. Serogroups of *Pseudomonas aeruginosa* and the immune response of patients with cystic fibrosis. J. Infect. Dis. **121**:269–274.
5. Diaz, F., and E. Neter. 1970. *Pseudomonas aeruginosa*: serogroups and antibody response in patients with neoplastic diseases. Am. J. Med. Sci. **259**:340–345.
6. Feld, R., G. P. Bodey, V. Rodriguez, and M. Luna. 1974. Causes of death in patients with malignant lymphoma. Am. J. Med. Sci. **268**:97–106.
7. Geller, W. 1953. Study of antibody formation in patients with malignant lymphomas. J. Lab. Clin. Med. **42**:232–237.
8. Hughes, W. T., S. Feldman, and F. Cox. 1974. Infectious diseases in children with cancer. Ped. Clin. N. Am.

21:583-615.
9. Ingaki, J., V. Rodriguez, and G. P. Bodey. 1974. Causes of death in cancer patients. Cancer 33:568-573.
10. Klastersky, J., D. Daneau, and A. Verhest. 1972. Causes of death in patients with cancer. Eur. J. Cancer 8:149-154.
11. Neter, E. 1971. The immune response of the host: an aid to etiology, pathogenesis, diagnosis, and epidemiology of bacterial infections: Yale J. Biol. Med. 44:241-246.
12. Neter, E. 1974. Opportunistic pathogens: immunological aspects, p. 37-48. *In* J. E. Prier and H. Friedman (ed.), Opportunistic pathogens. University Park Press, Baltimore.
13. Neter, E., A. M. Drislane, A. H. Harris, and G. T. Jansen. 1959. Diagnosis of clinical and subclinical salmonellosis by means of a serologic hemagglutination test. N. Engl. J. Med. 261:1162-1165.
14. Neter, E., E. A. Gorzynski, R. M. Gino, O. Westphal, and O. Lüderitz. 1956. The enterobacterial hemagglutination test and its diagnostic potentialities. Can. J. Microbiol. 2:232-244.
15. Neter, E., C. Merrin, M. J. Surgalla, and Z. Wajsman. 1974. *Shigella sonnei* bacteremia. Unusual antibody response from immunosuppressive therapy following renal transplantation. Urology 4:198-200.
16. Neter, E., O. R. Oberkircher, M. I. Rubin, J. M. Steinhart, and I. Krzeska. 1970. Patterns of antibody response of children with infections of the urinary tract. Pediatr. Res. 4:500-509.
17. Neter, E., O. Westphal, O. Lüderitz, and E. A. Gorzynski. 1956. The bacterial hemagglutination test for the demonstration of antibodies to enterobacteriaceae. Ann. N.Y. Acad. Sci. 66:141-156.
18. Neter, E., and H. Y. Whang. 1972. The common antigen of gram-negative enteric bacteria, p., 14-21. *In* A. Nowotny (ed.), Cellular antigens. Springer-Verlag, New York.
19. Solowey, A. C., and F. T. Rapaport. 1965. Immunologic responses in cancer patients. Surg. Gynecol. Obstet. 121:756-760.
20. Tanapatchaiyapong, P., and S. Zolla. 1974. Humoral immunosuppressive substance in mice bearing plasmacytomas. Science 186:748-750.
21. Umsawasdi, T., E. A. Middleman, M. Luna, and G. P. Bodey. 1973. Klebsiella bacteremia in cancer patients. Am. J. Med. Sci. 265:473-482.
22. Zolla, S., D. Naor, and P. Tanapatchaiyapong. 1974. Cellular basis of immunodepression in mice with plasmacytomas. J. Immunol. 112:2068-2076.