Characterization of the Antibody Response to Acetone-Killed Typhoid Vaccine

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LABORATORY EVALUATION of the efficacy of typhoid vaccine has generally produced results which show little correlation with those obtained in field-trial evaluation. Hence, extensive field trials have been deemed necessary to evaluate the various typhoid vaccines (*1–3*). Recent advances in immunological methodology have demonstrated distinct classes of immunoglobulins with differing biological activities (*4*).

The application of these techniques has resulted in the detection of patients with immunoglobulin deficiencies who can produce normal levels of gamma M antibody but who cannot synthesize gamma G antibody (*5*). These patients are extremely susceptible to various bacterial infections, a sensitivity suggesting that while gamma M antibody is able to agglutinate with antigen in the laboratory, it is unable to protect against infection. Furthermore, immunogenic agents of unequivocal effectiveness, such as tetanus toxoid, diphtheria toxoid, and poliomyelitis vaccine, result in the formation of gamma G (7S) antibody (*6–8*). These observations suggested that characterization of the class of antibody response might make it possible to predict in the laboratory how effective a vaccine will be.

LoSpalluto and associates demonstrated that the O antibody formed in response to heat-killed phenolized typhoid-paratyphoid vaccine was entirely of the gamma M immunoglobulin type (*9*). Since acetone-killed typhoid vaccine was shown to be more effective than heat-killed phenolized vaccine in field trials in British Guinea and Yugoslavia (*1*, *2*), a study of the quality of antibody produced in response to acetone-killed typhoid vaccine was undertaken to determine whether the vaccine resulted in the formation of gamma G (7S) anti-O antibody. Such data might account not only for this vaccine's greater effectiveness, but also support the hypothesis that immunoglobulin characterization correlates better with protection than do other laboratory procedures for evaluation of the vaccine's clinical effectiveness.

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Materials and Methods

Normal, healthy volunteers received two subcutaneous injections of 0.5 ml. of freshly suspended acetone-killed typhoid vaccine (lot No. P–5, manufactured at the Walter Reed Army

Institute of Research), 4 weeks apart. Blood was drawn before and at the 1st, 2d, 4th, 6th, and 8th weeks after the first injection of vaccine; the serum was then separated and stored at −5° C. Antibody titrations were performed by making twofold serial dilutions of the serums, to which equal volumes of antigen were added. Two sets of dilutions were made with each serum. To one set, saline-suspended antigen was added; to the other, antigen suspended in 2-mercaptoethanol (0.05 M) in saline. The mixtures were incubated for 2 hours at 37° C., kept overnight at 5° C., and read the next day. Titers were expressed as reciprocals of the highest dilutions showing gross agglutination. Sucrose density gradient ultracentrifugation of serums was done by the method of Kunkel and associates (*10*). Antibody activity in the fractions was assessed as just described. Immunoelectrophoresis was done by the microtechnique of Scheidegger, using specific goat antihuman gamma A, gamma G, and gamma M globulins from the Hyland Laboratories, Los Angeles, Calif. (*11*).

Results

All of the volunteers had undergone previous immunization and had some antibody at the outset of the study (see table). In all volunteers except one, the control anti-H titers were reduced only one or two dilutions by treatment with 2-mercaptoethanol (2-ME). The small reduction suggests that the control antibody was predominantly composed of gamma G (7S) globulin. After antigenic stimulation, anti-H titers increased in all the volunteers. In 7 of the 12, a fourfold or greater increase in 2-ME resistant titer (7S) occurred at 1 week. Thus,

Agglutination titers at various periods after volunteers received acetone-killed typhoid vaccine

Volunteer	0 week		1 week		4 weeks		8 weeks	
	Saline	2-ME	Saline	2-ME	Saline	2-ME	Saline	2-ME
	Anti-H titers							
1	2, 560	1, 280	10, 240	1, 280	10, 240	1, 280	5, 120	640
2	1, 280	640	5, 120	640	10, 240	640	5, 120	640
3	640	320	10, 240	5, 120	5, 120	640	10, 240	1, 280
4	640	320	5, 120	1, 280	2, 560	1, 280	5, 120	640
5	160	40	2, 560	640	2, 560	640	1, 280	320
6	80	40	2, 560	320	5, 120	1, 280	10, 240	2, 560
7	2, 560	640	5, 120	1, 280	10, 240	1, 280	2, 560	640
8	640	320	1, 280	640	5, 120	160	2, 560	320
9	640	160	20, 480	1, 280	5, 120	1, 280	5, 120	640
10	320	160	2, 560	640	20, 480	2, 560	5, 120	1, 280
11	1, 280	160	10, 240	2, 560	40, 960	2, 560	20, 480	1, 280
12	640	320	1, 280	320	10, 240	640	1, 280	320
	Anti-O titers							
1	40	0	80	0	80	0	80	±
2	40	0	160	40±	80	40	80	40
3	40	0	80	0	40	0	80	0
4	80	0	320	0	160	0	320	0
5	40	0	160	0	80	0	160	0
6	80	0	320	0	160	0	320	0
7	40	0	160	0	80	0	80	0
8	320	0	640	0	320	0	640	0
9	40	0	80	0	80	0	160	0
10	160	0	320	0	160	0	160	0
11	160	0	640	0	640	0	640	0
12	160	0	80	0	160	0	160	0

NOTE: Titers recorded are the reciprocal of the highest dilution showing gross agglutination among two sets of dilutions—one to which saline and another to which 0.05 M 2-mercaptoethanol had been added. The first tube in each titration was a 1 to 20 dilution; hence, 0 represents < 1 to 20.

secondary challenge with typhoid H antigen resulted in increases in both the 2-ME sensitive (19S) antibody and the 2-ME resistant (7S) antibody. The anti-O antibody levels with one exception were all 2-ME sensitive (19S). The rises in anti-O titer were less than the increases in anti-H titers.

The volunteer who had some 7S anti-O antibody in his blood received a third injection (0.5 ml.) of vaccine, and further attempts were undertaken to characterise the 2-ME resistant antibody. While the antibody was not sensitive to 0.05 M 2-ME, it was sensitive to 0.1 M 2-ME. Ultracentrifugation studies revealed that although most of the antibody appeared to be in the gamma M fractions, a small amount was present in those fractions shown by immunoelectrophoresis to contain gamma G antibody.

Discussion

Although anti-typhoid immunization has long been practiced, its effectiveness has remained controversial. The newer acetone-killed typhoid vaccine has been shown to produce better protection than either the alcohol-killed or the heat-killed phenolized vaccine (*1–3*, *12*). While serum anti-O and anti-H antibody levels in response to heat-killed phenolized vaccine do not differ from those in response to the acetone-killed vaccine (*2*, *13*), we thought the difference in their protective capacity might relate to differences in the class of antibody formed. Pike and Schulze have shown that rabbits hyperimmunized with acetone-killed vaccine do produce gamma G (7S) anti-O antibody (*14*). Thus, we believed that the acetone-killed vaccine might yield 7S antibody to typhoid O antigen in human subjects. If it did, this antibody might account for the greater efficacy of the acetone-killed vaccine over the other types. With the exception of one subject, however, the vaccine did not yield 7S antibody, and the antibody responses were both quantitatively and qualitatively similar to the responses after immunization with heat-killed phenolized vaccine reported by LoSpalluto and associates (*9*).

Although clinical and experimental observations suggested that gamma G antibodies might be more protective than gamma M antibodies, it is apparent from the present studies that the greater protection afforded by the acetone-killed typhoid vaccine as compared with the heat-killed phenolized typhoid vaccine cannot be ascribed to a capacity to stimulate the formation of gamma G (7S) antibody.

Summary

Clinical and experimental observations suggested that gamma G antibodies might provide more protection against infective organisms than gamma M antibodies. Acetone-killed typhoid vaccine has been shown to produce better protection than the heat-killed phenolized type. In an attempt to relate these two observations, 12 volunteers were immunized with acetone-killed typhoid vaccine to determine if gamma G anti-O antibodies would be formed rather than the gamma M anti-O antibodies that develop in response to heat-killed phenolized vaccine. Differences in the antibodies formed might account, it was believed, for differences in the protective capacity of the two types of vaccine. The antibody responses to injection of acetone-killed vaccine, however, proved to be quantitatively and qualitatively similar to those observed after immunization with heat-killed phenolized vaccine.

REFERENCES

(*1*) Ashcroft, M. T., Ritchie, J. M., Nicholson, C. C., and Stuart, C. A.: Antibody responses to vaccination of British Guiana schoolchildren with heat-killed phenolized and acetone-killed lyophilized typhoid vaccines. Amer J Hyg 80: 221–228, September 1964.

(*2*) Yugoslav Typhoid Commission: A controlled field trial of the effectiveness of acetone-dried and inactivated and heat-phenol-inactivated typhoid vaccines in Yugoslavia. Bull WHO 30: 623–630 (1964).

(*3*) Yugoslav Typhoid Commission: Field and laboratory studies with typhoid vaccines. Bull WHO 16: 897–910 (1957).

(*4*) Fudenberg, H. H.: The immune globulins. Ann Rev Microbiol 19: 301–338 (1965).

(*5*) Rosen, F. S., and Bougas, J. A.: Acquired dysgammaglobulinemia. Elevation of the 19S gamma globulin and deficiency of the 7S gamma globulin in a woman with chronic progressive bronchiectasis. New Eng J Med 269: 1336–1340, Dec. 19, 1963.

(*6*) Vaughan, J. H., and Butler, V. P.: Current status of the rheumatoid factor. Ann Intern Med 56: 1–11, January 1962.

(7) Robbins, J. B.: Studies on the interaction of immunoglobulins toward protein antigens with biological activity. *In* Molecular and cellular basis of antibody formation, edited by J. Sterzl. Academic Press, New York, 1965, p. 241.
(8) Svehag, S. E., and Mandel, B.: Formation and properties of poliovirus-neutralizing antibody. 1. 19S and 7S antibody formation: differences in kinetics and antigen dose requirement for induction. J Exp Med 119: 1–19, January 1964.
(9) LoSpalluto, J., Miller, W., Jr., Dorward, B., and Fink, C. W.: The formation of macroglobulin antibodies. 1. Studies on adult humans. J Clin Invest 41: 1415–1421, July 1962.
(10) Kunkel, H. G., Rockey, J. H., and Tomasi, T.: Methods of separation and properties of antibodies of high molecular weight. *In* Problems in microbiology. Rutgers University Press, New Brunswick, N.J., 1960, p. 30.
(11) Scheidegger, J. J.: Un micro-methode de l'immuno-electrophorese. Int Arch Allerg 7: 103–110 (1955).
(12) Marmion, D. E., Naylor, G. R. E., and Stewart, I. O.: Second attacks of typhoid fever. J Hyg 51: 260–267, June 1953.
(13) Ashcroft, M. T., Ritchie, J. M., and Nicholson, C. C.: Controlled field trial in British Guiana school children of heat-killed-phenolized and acetone-killed lyophilized typhoid vaccines. Amer J Hyg 79: 196–206, March 1964.
(14) Pike, R. M., and Schulze, M. L.: Production of 7S and 19S antibodies to the somatic antigens of *Salmonella typhosa* in rabbits. Proc Soc Exp Biol Med 115: 829–833, March 1964.

Legal Note . . . Air Pollution Control

Regulation imposing limitations upon smoke emissions but exempting incinerators in private dwellings held reasonable classification and valid exercise of police power. *Lees v. Bay Area Air Pollution Control District,* 48 Calif. Reporter 295 (1965), (rehearing denied February 16, 1966).

The Bay Area Air Pollution Control District established a regulation imposing limitations upon the emission of dense smoke from certain sources including apartment house incinerators, but specifically exempted incinerators in one- and two-family dwellings. The plaintiffs, owners of apartment houses with more than two families, sued to challenge the constitutionality of the regulation on the grounds that it would require costly and extensive modifications of their incinerators and would therefore confiscate their property without just compensation, and it created an unreasonable and arbitrary classification by exempting one- and two-family dwelling incinerators. In the alternative the plaintiffs asked that the court direct the district to grant them a variance from the regulation.

The action was an appeal from the decision of a lower court which had rejected the plaintiffs' arguments and had denied their request for a variance. The District Court of Appeals of California (1st District Division 3) affirmed, holding that the control district's adoption and enforcement of the regulation was a lawful and proper exercise of the State's police power.

The court, rejecting the plaintiffs' contention that the regulation was unconstitutional because it confiscated their property without just compensation, noted that where the police power is legitimately exercised a property owner has no right to compensation if, in the exercise of that power, the owner's property is either damaged, taken, or destroyed. In upholding the reasonableness of the regulation, the court declared:

> Generally, an exercise of the police power will be upheld if the act, ordinance, or regulation is not arbitrary, unreasonable, or discriminatory. It is common knowledge today that the atmosphere of almost every metropolitan area, including the area embraced within the limits of the . . . District, is subject to pollution from noxious gases discharged by vehicles, industrial establishments and incinerators. The evident purpose of the District's regulation is to protect the purity of the air that it may be free from harmful contamination. The District exists for that very purpose. Its regulation is not only reasonable, but indeed is essential, and represents a lawful and proper exercise of the police power. . . .

Finally, the court concluded that it was neither arbitrary nor unreasonable for the district to select the larger problem of incineration at multifamily apartment houses for immediate attention and to look to the lesser problem of incinerator operation at one- and two-family dwellings at a later day.—HOWARD WALDERMAN, *attorney, Public Health Division, Office of the General Counsel, Department of Health, Education, and Welfare.*