Differences in Antibody Responses of Mouse Strains to Enterobacterial Common Antigen[1] (34881)

EUGENE A. GORZYNSKI, ERWIN NETER, AND JULIAN L. AMBRUS

Public Health Division, Erie County Laboratory, Buffalo, New York, 14202; Department of Microbiology, State University of New York at Buffalo, Buffalo, New York 14214; and Microbiology Laboratory, Roswell Park Memorial Institute, Springville, New York, 14141

An antigen (CA) common to members of the family *Enterobacteriaceae* was first described by Kunin *et al.* in 1962 (1). This heat-stable antigen readily conditions erythrocytes for agglutination by CA antibodies. Only a few members of the family, notably *Escherichia coli* 014, induce CA antibodies in high titers in the rabbit upon intravenous (iv) injection of bacterial suspensions or supernates therefrom. With other members, CA antibodies are not engendered in significant titers unless antigen-treated erythrocytes are administered (2) or the antigen is given subcutaneously (sc), with or without Freund's adjuvant (3). The poor immunogenicity of CA produced by organisms other than *E. coli* 014 is due to the simultaneously present O antigen (lipoplysaccharide). Removal of this latter antigen results in a highly immunogenic ethanol-soluble fraction (4). Recombination of this fraction with O antigen (lipopolysaccharide) or its lipid A component renders the antigen less immunogenic (5).

It is well known that animal species vary in their responses to antigenic stimuli. For example, capsular polysaccharides of pneumococci are immunogenic in certain species, such as man and mouse, whereas in other species, such as the rabbit, they act as haptens. Differences in antibody responses to various antigenic stimuli have also been reported within animal species, *e.g.*, between inbred strains of mice (6–11).

[1] Supported in part by Grant T-516 from the American Cancer Society, General Research Support Grant FR-05648-03, Health Research, Inc., Roswell Park Division, and Research Grants 40847 and 00658 from the National Institute of Allergy and Infectious Disease, U.S. Public Health Service.

The present investigation has shown that four mouse strains (C57BL/6Ha, DBA/2 Jax, CBA/St, and Swiss albino) differ significantly in their capacity to produce antibodies against CA, either with or without incomplete Freund's adjuvant. The C57BL/6Ha mouse strain was used because it is known to produce antibodies readily. There was no basis for selecting the other strains.

Materials and Methods. Smooth strains of *E. coli* 0111, 014, and 086 were grown on brain veal agar (Difco) in Kolle flasks for 18 hr at 37°. The growth was suspended in 25 ml of phosphate buffer (pH 7.3; Difco) and heated for 1 hr in water bath at 100°. Supernatant fluid was obtained after centrifugation at 23,500*g* for 20 min and was frozen until used. It contains both CA and O antigen and will be referred to as HKS.

For the preparation of ethanol-soluble fraction of *E. coli* 0111, a method previously described in detail (4) was employed. Briefly, a mixture of *E. coli* 0111 HKS (1 vol) and 95% ethanol (8.5 vol) was kept at 22° for 18 hr. The mixture was centrifuged at 2° (23,500*g*) for 15 min; the decanted supernatant fluid was dried overnight in a partial vacuum at 22°. The material was dissolved in distilled water to the original concentration. This ethanol-soluble fraction, which contains CA and only trace amounts of O antigen, will be referred to as ES.

C57BL/6Ha, DBA/2Jax, and CBA/St mice (male, 4–7 weeks old), which had been continuously inbred by brother–sister matings for more than 20 generations, were used. Random-bred Swiss albino mice were obtained from a local dealer. The mice were housed in groups of 10 and given food and

water *ad libitum*. *E. Coli* 014 HKS or 0111 ES was injected intraperitoneally (ip) 3 or 4 times at 5-day intervals. Each immunizing dose contained 0.25 ml of antigen in 0.5 ml of buffer or incomplete Freund's adjuvant (Difco). Puncture of the retro-orbital sinus was used for collecting blood at weekly intervals for approximately 10 weeks. Serum specimens were kept frozen until used.

Titration of CA antibodies was carried out by means of hemagglutination tests, as previously described (4). Briefly, serum in 2-fold serial dilution (0.1 ml) was mixed with mouse erythrocytes (0.1 ml) that had been modified with CA from *E. coli* 086 HKS. After incubation at 37° and centrifugation at 1300*g* for 2 min, the resulting hemagglutination was read grossly. The specificity was documented by inhibition of hemagglutination by CA from *E. coli* 014 HKS (0.1 ml), mixed with the serum (30 min at 37°) prior to addition of antigenically modified erythrocytes.

Results. In the first series of experiments, 10 mice each of 4 strains were injected ip with *E. coli* 014 HKS or 0111 ES, with or without adjuvant. Blood samples were obtained prior to the first injection (day 0), 3 days after the second injection (day 8), 5 days after the third injection (day 15), and 21 and 52 days after the fourth injection (days 36 and 67, respectively). The results, summarized in Table I, indicate a better CA antibody response by CBA/St and C57BL/6Ha mice than DBA/2Jax and Swiss albino strains. CA antibody specificity was confirmed by hemagglutination-inhibition tests. The ethanol-soluble CA from *E. coli* 0111 was a better immunogen than was *E. coli* 014 (HKS). The reason for this finding is not clear, because hemagglutination-inhibition tests showed that the latter contained at least as much CA as did the former. Maximal titers were noted 5 days after the third injection of antigen (day 15). Table I shows also that CA antibody production did not persist, since titers had declined to 1:8 or less by 52 days after the fourth injection (day 67). It is of interest to note also that adjuvant did not enhance the immunogenicity of CA. In fact, it may have interfered with antibody production.

Since a secondary response with CA, whose chemical composition is as yet unknown, was documented previously in the rabbit (12), an attempt was made to induce a booster response in the mouse. Accordingly, CA immunized mice were given one injection ip of *E. coli* 0111 ES on day 67, when CA antibody titers had declined significantly. Serum specimens obtained 3, 7, and 15 days thereafter revealed no change in antibody titers.

These data indicate a difference between mouse strains in the antibody response to CA.

Discussion. The present study confirms and extends previous reports dealing with strain differences of mice in the immune response to antigens. Our results indicate that C57BL/6Ha and CBA/St mice produce CA hemagglutinatinins more readily and to significantly higher titers than do DBA/2Jax and Swiss albino mice. As early as 1949 Davidsohn and Stern (6) found, with sheep erythrocytes, that C57BL mice were excellent and DBA were poor antibody producers. Convincing evidence that the immune responses of inbred mice to certain antigens are genetically controlled traits was presented by McDevitt and Chinitz (8). These investigators showed that all strains with the same genotype for the major histocompatibility (H-2) locus exhibited the same pattern of immune response to synthetic polypeptide antigens. It must be kept in mind, however, that differences in immune responses under genetic control may depend on: the particular route of administration, the age of the animal, and the nature of the antigen employed. For example, Fink and Quinn (7) reported differences in antibody response to egg albumin, depending upon the route of immunization: C57BL/6 and DBA, among others, responded well to intramuscular (im) injection, whereas BALB responded well to ip but not to im immunization. Playfair (11) found that differences in antibody response of mice was age-dependent: neonatal NZB strains responded better than did neonatal C57BL or BALB/c strains to sheep, pig, or chicken erythrocytes. Auzins and Rowley (10) noted that, although BALB/c and Swiss white mice responded to 3 antigenic components of *S. typhimurium* C5 vaccine, only the latter pro-

TABLE I. Hemagglutinin Responses of Mice to CA Preparations of *E. coli* 014 and 0111.

E. coli CA preparations[c]	No. of injections	Day		Mouse strains							
				CBA/St		DBA/2Jax		C57BL/6Ha		Swiss albino	
				CA hemagglutinin titers							
		Last ip	Serum	—[a]	—[b]	—[a]	—[b]	—[a]	—[b]	—[a]	—[b]
014 HKS	2	5	8	0		0		0		0	
	3	10	15	0		0		0		0	
	4	15	36	0		0		1	16	0	
	4	15	67	0		0		0		0	
	4	67	*d*	2	8	1	8	0		0	
014 HKS + adjuvant	2	5	8	0		0		0		0	
	3	10	15	0		0		0		0	
	4	15	36	0		0		0		0	
	4	15	67	0		0		0		0	
	4	67	*d*	0		0		0		0	
0111 ES	2	5	8	0		0		0		0	
	3	10	15	5	18 (8–64)	0		9	25 (8–128)	5	9 (8–16)
	4	15	36	1	16	0		6	11 (8–32)	0	
	4	15	67	1	8	0		2	8	0	
	4	67	*d*	2	8	0		5	8	0	
0111 ES + adjuvant	2	5	8	0		0		0		0	
	3	10	15	0		0		5	49 (32–128)	0	
	4	15	36	0		0		0		0	
	4	15	67	1	8	0		2	8	0	
	4	67	*d*	2	11 (8–16)	1	8	3	10 (8–16)	0	

[a] 10 mice were used; values are number of mice with serum titer ≥8.

[b] Geometric means of antibody titers of responders; values in parentheses are ranges of titers.

[c] 0.5 ml ip.

[d] 3, 7, and 15 days after a single ip injection of *E. coli* 0111 ES.

duced antibodies against antigen 5. The role of the particular antigen is evident from the study of Cerottini *et al.* (9) who showed that NZB/W mice responded better to SRBC than did A/J mice although the latter strain was superior to NZB/W in response to keyhole limpet hemocyanin and bovine serum albumin. It is reasonable to assume that the differences of 4 strains of mice in the antibody responses to CA of *Enterobacteriaceae,* noted in the present study, are on a genetic basis.

Information on the biologic significance of CA and CA antibody is incomplete. However, it was shown that this antibody is present in low titer in human serum from healthy adults and increases in titer during convalescence of children with enterobacterial enteritis (13). In addition, CA antibodies opsonize enteric bacteria for phagocytosis by rabbit leukocytes (14) and protect rabbits against pyelonephritis experimentally produced with a CA containing microorganism, *Proteus mirabilis* (15). Further studies are needed to determine the protective effect of CA antibodies against heterologous bacterial infections.

Summary. The genetic influence of various mouse strains on antibody response to the common antigen (CA) of *Enterobacteriaceae* was determined. Four strains of mice, three inbred and one random-bred, were injected ip with CA of *E. coli* 014 or 0111, with or without incomplete Freund's adjuvant. CA antibody titers, determined by hemagglutination tests, revealed the following: CBA/St and C57BL/6Ha mice responsed significantly better than did DBA/2Jax and Swiss albino mice; ethanol-soluble CA from *E. coli* 0111 was a better immunogen than was CA from *E. coli* 014; adjuvant did not enhance immunogenicity of CA in responding mice; highest titers were noted 5 days after the third injection of CA, persisted for 2 weeks, and declined by the end of 2 months; and a subsequent injection of CA failed to elicit a secondary response.

The capable technical assistance of Mrs. Richard Anderson and Mr. Harry Babbit is gratefully acknowledged.

1. Kunin, C. M., Beard, M. V., and Halmagyi, N. E., Proc. Soc. Exp. Biol. Med. **111**, 160 (1962).

2. Neter, E., Whang, H. Y., Suzuki, T., and Gorzynski, E. A., Immunology **7**, 657 (1964).

3. Gorzynski, E. A., Whang, H. Y., Suzuki, T., and Neter, E., Proc. Soc. Exp. Biol. Med. **114**, 700 (1963).

4. Suzuki, T., Gorzynski, E. A., and Neter, E., J. Bacteriol. **88**, 1240 (1964).

5. Whang, H. Y., Lüderitz, O., Westphal, O., and Neter, E., Proc. Soc. Exp. Biol. Med. **120**, 371 (1965).

6. Davidsohn, I., and Stern, K., Cancer Res. **9**, 426 (1949).

7. Fink, M. A., and Quinn, V. A., J. Immunol. **70**, 61 (1953).

8. McDevitt, H. O., and Chinitz, A., Science **163**, 1207 (1969).

9. Cerottini, J. C., Lambert, P. H., and Dixon, F. J., J. Exp. Med. **130**, 1093 (1969).

10. Auzins, I., and Rowley, D., Immunology **17**, 579 (1969).

11. Playfair, J. H. L., Immunology **15**, 35 (1968).

12. Whang, H. Y., and Neter, E., J. Immunol. **98**, 948 (1967).

13. Whang, H. Y., and Neter, E., J. Pediat. **63**, 412 (1963).

14. Domingue, G. J., and Neter, E., J. Bacteriol. **91**, 129 (1966).

15. Domingue, G. J., Rountree, C., Hall, R., and Butcher, B., Bacteriol. Proc. **1969**, 74.

Received Feb. 26, 1970. P.S.E.B.M., 1970, Vol. 134.