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Production of Incomplete Vi Antibody in Mice. (25921)

SIDNEY GAINES, JULIUS A. CURRIE AND JOSEPH G. TULLY
(Introduced by Maurice Landy)
Dept. of Microbiology, Walter Reed Army Inst., Washington, D. C.

Landy(1) reported that Swiss Bagg mice respond to isolated Vi antigen by producing specific antibody demonstrable by the standard hemagglutination test(2), and that the passive protection afforded by this antibody was sufficient to account for the immunogenic activity of the antigen. These findings have been confirmed for BALB/c mice (an inbred variant of the Bagg), the strain employed most extensively in our laboratory. Recently, however, it was observed that specific antibody could not be demonstrated in the serum of Vi-immunized Cinnamon mice, a strain not previously employed by us for serological studies. Inasmuch as Cinnamon, as well as BALB/c mice, could be protected actively against challenge with virulent *Salmonella typhosa* by administration of Vi antigen, this observation was unexpected, and suggested that response to antigenic stimulation might differ with the strain of mouse employed. The present study was therefore designed to elucidate and compare responses of these 2 strains of mice to Vi antigen. Evidence was obtained that the type of antibody evoked by Vi antigen does indeed vary with the mouse strain. The antibody developed in Cinnamon mice immunized with Vi antigen was of the incomplete type, but was nonetheless protective against challenge with *S. typhosa*.

Materials and methods. Mice. The animals ranged in weight from 11-16 g, and were of mixed sex. BALB/c mice were obtained from Microbiological Associates, Inc., Bethesda, Md., and Cinnamon mice from Dept. of Lab. Animal Medicine, Walter Reed Army Inst. of Research. *Vi Antigen.* The purified Vi antigen was prepared from the Vi strain of *Escherichia coli* (5396/38) by the method of Webster *et al.*(3). Immediately prior to use, antigen was dissolved in 0.85% NaCl to give desired concentration. All inoculations were made intraperitoneally. *Sera.* Specimens for use in passive tests and for serological study were prepared as follows: mice were inoculated with 10 μg of Vi antigen, bled 6 days later, and the serums pooled. *Absorption of sera.* 0.3 ml of packed bacterial cells (killed by heating at 60°C for 1 hr) was added to each ml of serum, the suspension incubated at 37°C for 1 hr, at 4°C overnight, and clarified by high speed centrifugation. *Passive protection tests.* Tests for serum potency were based on use of graded immunizing doses and a constant challenge dose, and were performed in Cinnamon mice. Serum was given intraperitoneally 1 hour prior to challenge with a saline suspension of 3 x 10^7 *S. typhosa* Ty2V (fully virulent form) *via* the same route. ED_{50}s were determined by the method of Reed and Muench(4). *Serological procedures.* The hemagglutination test for complete (saline) Vi antibody was performed as described by Landy and Lamb(2). The procedure for demonstration of incomplete Vi antibody was the same except that serum dilutions were made in 15% bovine serum albumin (BSA) obtained from Armour Pharmaceutical Co.

Results. During our studies on the role of Vi antigen in virulence of *S. typhosa* for mice,

TABLE I. Response of Cinnamon and BALB/c Mice to Purified Vi Antigen.

Vi antigen (μg)	Vi hemagglutinin titers (saline)*	
	Cinnamon serum†	BALB/c serum†
10	Neg‡	1:240
1	"	1:120
.1	"	1:60
.01	"	1:7.5
.001	"	Neg

* Serum diluted in .85% saline.
† Pooled from 20 mice bled 6 days following a single inj. of Vi antigen.
‡ Neg = < 1:7.5.

it was observed that Cinnamon mice failed to produce demonstrable Vi antibody in response to administration of this antigen, in contrast to the response observed in BALB/c mice. These findings are shown in Table I. As little as 0.01 μg of purified Vi antigen elicited production of complete (saline) Vi antibody in BALB/c mice, while as much as 10 μg of antigen failed to stimulate production of this antibody by Cinnamon mice. As indicated previously, both Cinnamon and BALB/c mice could be actively immunized against challenge with virulent *S. typhosa*. Based on quantity of Vi antigen required to protect half the animals, the extent of protection afforded the 2 mouse strains was essentially the same. Employing a challenge of 3.36 x 10^7 Ty2V cells, the ED_{50}s of Vi for Cinnamon and BALB/c mice were 0.0078 and 0.0098 μg respectively. In the case of the BALB/c animals, the amount of Vi antibody produced was sufficient to account for the immunogenic activity of this antigen, but there was no demonstrable antibody to account for the protection afforded Cinnamon mice following administration of Vi antigen. Consequently, the serum of Vi-inoculated Cinnamon mice was examined for its capacity to protect passively. Groups of mice were inoculated intraperitoneally with graded doses of pooled serum from Cinnamon mice previously immunized with Vi antigen; 1 hour later, they were challenged with virulent typhoid bacilli (strain Ty2). For control purposes, other groups of mice were passively immunized with pooled "immune" BALB/c serum and similarly challenged. Results of a typical experiment (Table II), show that serum obtained from Vi-immunized Cinnamon mice gave protection to the same extent as that afforded by serum from Vi-immunized BALB/c mice.

In the absence of demonstrable Vi antibody, the protective capacity of serum from Cinnamon mice suggested the presence of an atypical antibody, which might still account for the observed protection. The findings (Table III) clearly show that although saline antibody is absent (Table I), an incomplete type of Vi antibody is present in the serum of Vi-immunized Cinnamon mice. It is noteworthy that the titer of serum from Vi-inoculated BALB/c mice diluted in albumin was invariably higher than the saline titer.

TABLE II. Passive Protection Provided by Sera from Vi-immunized Cinnamon and BALB/c Mice.

Mouse serum*	Serum dose (ml)			ED_{50} (ml)†
	.05	.01	.002	
Cinnamon	9/10‡	6/10	2/10	.007
BALB/c	10/10	7/10	"	.005

* Pooled from 20 mice bled 6 days following a single inj. of 10 μg of Vi antigen.
† Challenge: 3 × 10^7 *S. typhosa* Ty2V.
‡ Survivors/No. inj.

From the findings given in Tables II and III, it is apparent that incomplete antibody accounted for the protection afforded mice by immunization with Vi antigen. To ascertain the specificity of this protection, serum of immunized Cinnamon mice was subjected to specific absorption procedures to remove its antibody and protective capacity. Serum from Vi-immunized BALB/c mice was treated in the same way, and these absorbed sera were then examined to determine whether they were still protective and serologically reactive. Table IV reveals that absorption of both Cinnamon and BALB/c sera with a Vi-containing organism markedly reduced

TABLE III. Production of Incomplete Vi Antibody by Cinnamon and BALB/c Mice.

Vi antigen (μg)	Vi hemagglutinin titers (BSA)*	
	Cinnamon serum	BALB/c serum
10	1:240	1:1920
1	1:60	1:960
.1	1:15	1:480
.01	Neg	1:120
.001	"	1:7.5

* Serum diluted in 15% bovine serum albumin (BSA).

TABLE IV. Specificity of Protection Afforded by Cinnamon and BALB/c Sera. Effect of absorption.

Treatment of serum	Cinnamon serum* Dose (ml) .05	.01	.002	ED_{50} (ml)†	BALB/c serum* Dose (ml) .05	.01	.002	ED_{50} (ml)†
Unabsorbed	9/10‡	6/10	2/10	.007	10/10	7/10	2/10	.005
E. coli (Vi-containing)	2/10	0/10	0/10	>.05	2/10	1/10	1/10	>.05
S. typhosa Ty2W (non-Vi variant)	9/11				10/10			

* Pooled from 20 mice bled 6 days following a single inj. of 10 μg of Vi antigen.
† Challenge: 3×10^7 *S. typhosa* Ty2V.
‡ Survivors/No. inj.
Challenge controls: 10 mice were inj. with normal Cinnamon serum, 10 with normal BALB/c serum, and 10 with saline prior to challenge. There were no survivors.

protective capacity of both sera. On the other hand, absorption with a W form (non Vi-containing) *S. typhosa* culture was without effect on the protection afforded by these sera. Similarly, both incomplete and complete Vi antibody were absorbed from these sera by treatment with Vi-containing bacilli, but not by absorption with non-Vi cells (Table V). The findings that protective activity and antibody content of these sera could be removed by absorption with Vi-containing organisms provide evidence for the specific nature of the responses of both Cinnamon and BALB/c mice to administration of Vi antigen.

Discussion. Incomplete antibodies have been detected in experimental animals and in human beings, but much of the investigative work on this type of antibody has been along serological lines. Relatively little is known of the protective capacity of sera containing only incomplete antibody, despite observations that sera which apparently contain immune antibodies as shown by protection tests, fail to show serological activity (agglutinins or precipitins). The presence of incomplete antibody in serologically non-reactive sera has been suggested by some workers(5) as a possible explanation for the passive protection provided by such sera. Landy(1) reported that serum of mice injected with a heat-phenol vaccine prepared from a Vi-containing strain of *S. typhosa* afforded a limited amount of protection to Bagg strain mice in absence of antibody detectable *in vitro*. Based on the results of absorption experiments, he attributed the protective activity of the serum to Vi antibody, and stated that such antibody differed in some way from the readily demonstrable Vi antibody produced in response to more potent immunizing agents such as acetone-killed and dried Vi-containing organisms or isolated Vi antigen. On the other hand, Tyler and Swingle(6), investigating the effect of photo-oxidation of anti-pneumococcal rabbit serum and anti-diphtheric horse serum concluded that "univalent" type antibody is ineffective for passive protection against bacteria, but may function as protective antibody against toxins. The present study, however, has shown that incomplete Vi antibody effectively protects Cinnamon mice against challenge with virulent typhoid bacilli.

TABLE V. Specificity of Vi Antibody in Cinnamon and BALB/c Sera. Effect of absorption.

Absorbant	Cinnamon serum* Vi antibody titer† Saline	BSA	BALB/c serum* Saline	BSA
Unabsorbed	Neg	1:240	1:240	1:1920
E. coli (Vi-containing)	"	Neg	Neg	Neg
S. typhosa Ty2W (non-Vi variant)	"	1:240	1:240	1:1920

* Pooled from 20 mice bled 6 days following a single inj. of 10 μg of Vi antigen.
† Determined by Vi hemagglutination test. Serum dilutions made in 0.85% saline or 15% bovine serum albumin.

The protection afforded by normal and certain so-called immune sera against a variety of virulent organisms, and enhancement of phagocytic activity by such sera in the absence of readily detectable antibody may also be due to specific incomplete antibody. Tests

by diagnostic, clinical laboratories on sera of patients suffering from infectious diseases seldom, if ever, include determinations for incomplete antibody. If present, such antibody might provide a more useful measure of the progress of the infection. Potuzník and Gavlík(7) have published data comparing incomplete and complete Vi antibody in serum of typhoid patients and carriers, and found that incomplete Vi antibody appeared in higher titers in almost every case, particularly among the carriers.

It is generally believed that during the many years of its use, typhoid vaccine has afforded man a significant degree of protection against natural infection. The fact that Vi antibody, as determined by the usual procedures, appears in a relatively small percentage of immunized individuals has led a number of investigators to question the importance of Vi antibody in protection of man against this disease. In the light of the present work, however, it might be profitable to reexamine sera from typhoid-vaccinated individuals for incomplete Vi antibody. Such a study is currently in progress in this laboratory.

Summary. Immunization with purified Vi antigen elicited incomplete Vi antibody in the Cinnamon strain, and complete Vi antibody in BALB/c mice. Incomplete Vi antibody alone accounted for protection afforded by serum from Cinnamon mice against challenge with fully virulent typhoid bacilli. Absorption tests verified the specificity of incomplete Vi antibody and protection it afforded.

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Growth and Passage of Variola Virus in Mouse Brain.* (25922)

ARTHUR BROWN, VIRGINIA ELSNER AND JULIUS E. OFFICER
(Introduced by A. N. Gorelick)
Virus and Rickettsia Division, Fort Detrick, Frederick, Md.

Variola virus has been distinguished from other pox viruses by its poor growth on primary inoculation into laboratory animals, and its failure to become adapted to laboratory animals on subsequent passage(1,2,3). Furthermore, it has been reported that mice inoculated intracerebrally (IC) with variola virus succumb in erratic death patterns(4), and that lethality disappears after the first passage(3). Under proper conditions to be described here, it will be shown that these distinguishing characteristics may not serve as absolute markers for differentiating variola from the other pox viruses. Data are presented which show that variola virus does grow well when inoculated IC in mice, and that it can be serially passaged.

Materials and methods. The Yamada strain of variola virus was used. To determine whether multiplication of virus occurred, a large number of Swiss mice were inoculated IC. At selected sampling intervals, the brains of 3 sacrificed mice were pooled, homogenized as 20% suspensions in heart infusion broth, and assayed for virus titer on the chorio-allantoic membrane (CAM) of 11-day-old embryonated eggs(5). Ten eggs were used for each serial 10-fold dilution. Titers were expressed

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