BRIEF NOTE

The Effect of Oral and Parenteral Immunization with Killed Vaccines on the Fecal Shedding of Mice Fed Yersinia enterocolitica

Ikuo UCHIDA, Ken-ichi KANEKO and Nobuo HASHIMOTO

Department of Veterinary Public Health, Faculty of Veterinary Medicine, Hokkaido University, Sapporo 060

(Received for publication November 30, 1981)

Maruyama et al. [9] demonstrated that Yersinia enterocolitica O3, O5B, O9 and O8 were excreted in mouse feces in large numbers for several weeks after their oral or intravenous administration. In contrast, Ueno et al. [10] reported that the fecal excretion of intragastrically inoculated O3 organism was inhibited in the mice which had been previously vaccinated with the viable O3 organism. The mechanism of this inhibition, however, is still obscure. Of particular interest was the question of whether or not the challenged organism is inhibited from being excreted in the feces by vaccination with killed cells. In the present study, the authors intended to demonstrate the inhibition of the O3 organism by oral inoculation with killed cells and to show the zero effect of serum agglutinin on the inhibition.

The bacterial strain used was Y. enterocolitica O3, Wauters biovar 4, authors' laboratory no. SD1416-11, which was isolated from the rectal content of a brown rat captured in a slaughterhouse by Kaneko et al. [7]. It had a property of temperature sensitive dependence upon Ca²⁺. The strain was maintained in equal volumes of calf serum and 10% lactose water solution at −80°C. The organism was grown on Trypticase soy agar (BBL) plates at 25°C for 48 hours and suspended in physiological saline then autoclaved at 121°C for 2.5 hours. The suspension was washed 3 times by centrifugation and diluted with sterile distilled water to a final concentration of 2 mg per ml (wet weight). It was used as a heat-killed vaccine. Formaldehyde was mixed with the suspension of the viable organism to a final concentration of 0.3%. The suspension was then diluted with sterile distilled water to the same concentration as the heat-killed vaccine after all the cells were killed and washed. It was used as a formalin-killed vaccine. The heat-killed or formalin-killed vaccine was given orally to the mice for 4 weeks as drinking water. The control group of mice drank sterile distilled water for 4 weeks. The mice were challenged intragastrically with 10⁷ viable cells one week after the last vaccination by a gastric feeding tube. Mice injected subcutaneously with 4 doses of 400 μg formalin-killed cells (wet weight) at 4-day intervals were also challenged intragastrically with 10⁷ viable cells 9 days after the last injection by the gastric feeding tube. The challenge organism was prepared from the stock culture.
which was diluted with physiological saline to a concentration of $10^7$ viable cells per 0.1 ml.

Female, 4-weeks old SPF ICR mice (Japan CLEA, Tokyo) were used. Feces from the mice were suspended in physiological saline and diluted in concentration of from $10^1$ to $10^{4.5}$% at tenfold. Salmonella-Shigella agar (Eiken) plates were used as the selective media. Identification of the colonies grown on the plate was done by the slide agglutination test using rabbit O antisera. Determination of O and OH agglutinin titer was done according to Kaneko et al. [7] using the heat-killed cells and the formalin-killed cells as O antigen and OH antigen respectively. The sera were incubated at 56°C for 30 minutes before the test.

Fig. 1 shows the fecal excretion of O3 organism in the mice which had been orally inoculated with heat-killed or formalin-killed cells after intragastric challenge with $10^7$ viable cells of the organism. The mice which had been given water containing the heat-killed or formalin-killed cells for 4 weeks were then challenged intragastrically, and the number of excreted organisms in the feces was calculated at 7-day intervals. In this case, no fecal excretion of the organism was apparent in 6 out of the 8 mice which had been vaccinated with either the heat-killed or the formalin-killed cells; however, it was detected in the other 2 mice for 5 weeks or more. On the other hand, the fecal excretion was observed for 2 weeks or more in all the control mice which had received sterile distilled water. The incidence of fecal excretions lasting for more than 2 weeks in the mice which had been vaccinated with killed cells was significantly lower than that in the unvaccinated mice by Fisher's exact test ($P=0.03$). Serum agglutinin against O antigen was not detected during the oral vaccination of killed cells,
but it was observed in 3 out of the 8 mice which had been vaccinated with formalin-killed cells one and three weeks after the administration of viable cells at a low titer of 1:20.

The influence of serum agglutinin on the fecal excretion of the organism was examined. Mice which had received 4 doses of 400 µg of formalin-killed cells were challenged intragastrically with $10^7$ viable O3 cells, and the excretion of the organism from their feces was examined. As shown in Fig. 2, all of the mice subcutaneously inoculated with formalin-killed cells excreted the organism for 2 weeks or more, although they had already developed an O agglutinin titer of 1:20 to 1:160 and an OH agglutinin titer of 1:80 to 1:320 against the organism at the time of challenge. The agglutinin titer was not boosted by the challenge of viable cells. Moreover, one mouse excreted the organism for more than 200 days in spite of the OH agglutinin titer of 1:320.

The results of the present study demonstrated inhibition of fecal excretion in mice which had been inoculated orally with killed cells. Acquired resistance to enteropathogenic bacteria in host by killed oral vaccine has been reported [1, 3–5]. Collins and Carter [2] reported on the successful oral vaccination of killed cells in experimental salmonellosis. It was reported that no long excretion of O3 organism in the feces was observed after the rechallenge with viable cells [10]. Since the inhibition of challenged viable cells to colonize was demonstrated by previous oral vaccination of both viable and killed cells, the inhibition was considered to be due to host immunity.

It is reported that serum antibody alone plays no role in Salmonella or Shigella infection [5, 6]. In the present study, a small number of mice showed serum agglutinin titers of as low as 1:20 after oral inoculation of viable cells; these were apparently not the results of the oral inoculations of killed cells. It was demonstrated that even high agglutinin titers of 1:160 or 1:320 developed by subcutaneous injection of the killed cells could not protect against fecal excretion of the intragastrically challenged organism. These facts indicate that the inhibition of the challenged organism from colonizing in mice is not due to serum antibody. Future investigations are warranted to confirm that the mechanism of the inhibition is due to gut immunity. Because the fecal excretion of the challenged viable cells was inhibited by drinking not only the formalin-
killed cells but also the heat-killed cells
(Fig. 1) H antigen was not considered to
be the responsible factor of the inhibition.
Since Lawtone et al. [8] reported that both
of V and W antigens are not stable after
heating at 80°C for 30 minutes, the V and
W antigens seemed to be not responsible
for the inhibition in spite of a possibility
of the used strain’s producing these antigens.
It was, however, suggested that heat stable
substances of the organism might be related
to the inhibition.

References
Immun. 6, 451–458.
Dawkins, A. T., Heiner, G. G., and Woodward,
Libonati, J. P., Formal, S. B., and Gangerosa,
[6] Honjo, S., Takasaka, M., Fujiwara, T., Kaneko,
M., Imaizumi, K., Ogawa, H., Mise, K., Nakamura,
318.
[8] Lawton, W. D., Erdman, R. T., and Surgalla,
5, Karger, Basel, 317–323.
[10] Ueno, H., Kaneko, K., and Hashimoto, N.

要 約
マウスにおける結膜および非結膜免疫の Yersinia enterocolitica 排菌に対する効果（短報）：内田郁夫・金子賢一・橋本信夫（北海道大学獣医学部獣医公衆衛生学講座）——Yersinia enterocolitica O3 菌
の121°C 2 時間半加熱死菌あるいはホルマリン死菌で縫口免疫したマウスでは、胃内接種された同生菌
の扁平への排菌が阻止された。また O3 菌のホルマリン死菌を皮下免疫することによって、O 納集素価
1:160, OH 溶集素価 1:320 を保有するマウスでは、胃内接種された同生菌の扁平への排菌は阻止され
なかった。