Correlation between the Indirect Hemagglutinating Antibody and Protection of Mice against *Clostridium chauvoei*

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**ABSTRACT.** The indirect hemagglutination (IHA) titers of sera against sonic extract of *Clostridium chauvoei* paralleled the protection of mice immunized with blackleg vaccine. The titers of 1:8 or higher correlated with sufficient protection of mice to the challenge exposure. These results suggest that the IHA test may be useful as an aid for evaluation of protective potency of blackleg vaccine.—**KEY WORDS:** *Clostridium chauvoei*, IHA test, mouse protection.


*Clostridium chauvoei*, which causes blackleg in cattle and sheep, has been shown to possess a cellular protective antigen which exists both on the cell wall [1, 2] and flagella [5]. Our previous study showed that an indirect hemagglutination (IHA) test using a sonic extract as an antigen mainly detects the antibodies against flagella of *C. chauvoei* [4]. The present study records a correlation between the IHA antibody and protection of mice against *C. chauvoei*.

Strain C6H of *C. chauvoei* obtained from the Research Institute for Microbial Diseases, Osaka, Japan, was used for preparation of the IHA antigen [4]. Strain Okinawa of *C. chauvoei* was used for preparation of challenge inoculum in the mouse protection test [3].

The IHA test was performed as previously reported [4]. Formalin-treated cells of strain C6H were sonicated with a Tomy model UR-200P sonicator (Tomy Co., Ltd., Tokyo, Japan) at 20 KHz/sec at 105 W for 10 min and centrifuged at 4,000×g at 4°C for 30 min. Tanned glutaraldehyde-fixed sheep red blood cells (SRBC) were sensitized with the resulting supernatant (sonic extract) at a protein concentration of 200 μg/ml. The major protein component of the extract was same as the flagella of strain Okinawa [4], when observed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The sensitized SRBC were suspended to yield a 2% suspension, and then added to equal volume of two-fold serum dilutions (0.025 ml) in U-bottom plates. The plates were shaken and allowed to stand for 2 hr at room temperature. The IHA titer was expressed as the reciprocal of the highest dilution of serum showing a definite positive pattern (flat sediment) [4].

Blackleg vaccine prepared from formalin-treated whole culture of strain Okinawa was used throughout the experiments, and designated as the vaccine. Supernatant fluid of the vaccine was separated by centrifugation at 6,500×g for 20 min at 4°C. The resulting cells were washed three times with 0.15 M phosphate-buffered saline (PBS) solution, pH 7.2, and resuspended in PBS to a concentration of the same optical density of the vaccine at 540 nm (washed cells).

Mouse protection test was performed according to the method of the potency test on blackleg vaccine described in Minimum Requirements for Biological Products for
Animal Use in Japan [3]. Twenty ddY mice, weighing about 20 g, were intraperitoneally injected four times (0.25 ml each injection) at 2-day intervals. Ten days after the final injection, ten mice randomly selected in each experimental group were intramuscularly challenge-exposed with 0.25 ml of 3% calcium chloride solution containing $2.3 \times 10^2$ spores of strain Okinawa (100 minimum lethal doses). Protection rate of the mice to the challenge exposure was determined from the survival rate. Blood samples were collected from the remaining 10 mice in each group, and the sera were titrated by the IHA test.

Mice were injected with the vaccine, supernatant fluid, or washed cells and challenge-exposed (Fig. 1). The vaccine, which contained both cellular and extracellular antigens, possessed a high protective ability and IHA antibody-producing ability. Washed cells did not differ significantly both in protection and the antibody-producing abilities from the vaccine ($P>0.05$). A low level of IHA antibodies was detected in mice injected with supernatant fluid, although a half number of them were protected against the challenge exposure.

Mice were immunized with serial 1:5 dilutions of the vaccine and challenge-exposed. The result showed that IHA titers decreased linearly with the dilution and roughly paralleled protection as indicated by logarithmic rate (Fig. 2).

Twenty mice of each immunization group were injected with 6 different lots of the vaccines. Again, a direct relationship between IHA titers and protection was evident.

![Fig. 1. Relationship between IHA antibody and protection of mice injected with three different fractions of blackleg vaccine. IHA titer represents the mean of ten mice±standard error.](image1)

![Fig. 2. Relationship between IHA antibody and protection of mice injected with diluted vaccines. IHA titer represents the mean of ten mice±standard error.](image2)

![Fig. 3. Correlation between IHA antibody and protection of mice injected with different lots of vaccines. IHA titer represents the mean of ten mice±standard error.](image3)
(Fig. 3). The minimum requirement for protective potency of blackleg vaccine in Japan requires survival rate of 60% or higher in immunized mice against the challenge exposure [3]. About ninety percent (34/38) of the mice injected with the vaccine, which induced survival rate of 60% or higher, had IHA titers of 1:8 or higher. On the other hand, 83% (15/18) of the mice injected with the vaccine, which induced survival rate of less than 60%, had IHA titers of less than 1:8.

In a previous paper [4], sonic extract of C. chauvoei was used for sensitization of tanned glutaraldehyde-fixed SRBC for the IHA test. The antigen predominant in the extract adsorbed onto the SRBC appeared to be flagella [4]. The result of the present study would correlate well with the previous finding, since the protective antigenicity of the blackleg vaccine resides to the bacterial cell including flagella (Fig. 1).

The important role of the flagella of C. chauvoei in immune resistance was previously demonstrated [5]. The degree of antibody response to the extracted antigen, as measured by the IHA test, correlates well with protection of mice against the challenge exposure (Figs. 2 and 3).

The conclusion can be made that the IHA test, using sonic extract of C. chauvoei as an antigen, may be useful for evaluation of blackleg vaccine, as an additional aid to the mouse protection test.

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REFERENCES


要約

気腫疽菌免疫処理抗原に対する間接赤血球凝集抗体と予防液のマウス感染防御能との相関関係：田村 豊，牧江弘孝，田中まゆみ（農林水産省動物薬品検査所）——気腫疽菌の免疫処理抗原に対する間接赤血球凝集（IHA）抗体価は，気腫疽予防液を注射したマウスの防御と平行し，IHA 抗体価 1:8 以上を示すマウスは強毒株の攻撃に対し60％以上耐過生残した。気腫疽予防液力価評価法として，IHA 反応は有用であることが示された。