Serological and Bacteriological Studies on the Staphylococcal Exfoliative Skin Disease in Guinea Pigs

Chiaki ISHIHARA

Laboratory of Animal Experiments, Institute of Immunological Science, Hokkaido University, Kita-15, Nishi-7, Kita-ku, Sapporo 060, Japan.

(Received for publication: February 29, 1980)

A skin disease showing marked erythema and subsequent epidermal exfoliation has recently been observed in our breeding colony of guinea pigs. The distribution of staphylococcal antibodies and the frequency of Staphylococcus aureus isolation were studied in 5 guinea pig groups, three of which were in the clinical stages of the exfoliative skin disease, and two control groups. The mean titers of anti-alpha-toxin antibodies exhibited peak levels in the group of the middle clinical stage (titer of 24.0), when compared to those in the groups of the early (titer of 3.5) or the late clinical stages (titer of 4.1). The percentage of positive sera (titers ≥ 5) for this antibody was also greater in the group of the middle clinical stage (100%) than in the groups of the early (55%) or the late clinical stages (64%). The agglutinating antibody was detected only in the groups of the early and the middle clinical stages. Staphylococcus aureus was isolated from 73% of the animals in the early stage, and from only 11% of those in the late stage.

Introduction

Skin diseases in guinea pigs caused by staphylococcal infections have been reported in a few papers. Bumble-foot, a chronic inflammation and ulceration of the foot pad, has been described as a staphylococcal skin disease [3,12]. Recently, a skin disease which was characterized by marked erythema and subsequent epidermal exfoliation was observed in this breeding colony of guinea pigs [4]. The high frequency of the disease in animals near parturition, the restriction of erythema and desquamation of the ventral skin and brief course of the disease [4] were clinically distinct from those of staphylococcal pododermatitis [3, 12], dermatophytosis [9] or arthropod infestations [10]. The present author isolated Staphylococcus aureus from the skin lesions, and the skin symptoms were reproduced by infections of the isolate [4]. The author also made attempts to study the etiology of this exfoliative skin disease. This report describes serological and bacteriological correlations between Staphylococcus aureus and the exfoliative skin disease observed in the colony of guinea pigs.

Materials and Methods

Guinea pigs: The breeding colony of Strain 13 guinea pigs was maintained by monogamous and polygamous pair breeding systems, producing 30 youngs per month from 80 female and 70 male breeders. The animals were housed in cages of galvanized metal (45×65×25cm) with
Fig. 1. Exfoliative skin disease in the early stage undergoing desquamation of skin flakes on day 7 from onset of the disease. The hair on the ventral abdomen has been clipped.

woven wire floors (7.2×7.2mm with wire mesh), or in aluminum cages (32×48×22 cm) with longitudinal, stainless steel bar floors (6.4mm diameter bars placed 8.1 mm apart). The guinea pigs were fed a commercially prepared pelleted feed GC-4 (Oriental Yeast Co. Ltd., Tokyo), and water ad libitum. The cages were cleaned and steamed once a week. This colony had a history of exfoliative skin disease since April, 1977.

From January to August, 1978, thirty-one retired female breeders from 1 to 3 years of age were submitted to the experiment. They were divided into 4 groups on the basis of the clinical course and development of the exfoliative skin disease. Eleven of the animals showed early stage symptoms, which occurred about 2 weeks after the onset of the disease. This group showed marked erythema, cracks in the skin, and a subsequent formation of dried skin flakes of the abdomen (Figure 1). Five guinea pigs were grouped into the middle stage in which alopecia on the abdomen was observed 2 to 4 weeks after onset of the disease. Eleven animals were grouped into the late stage in which the alopecia on the abdomen and the other clinical signs apparently reduced. The remaining 6 animals showed no skin symptoms of the disease during this study.

Eighteen female guinea pigs of Hartley strain, weighing 300 grams, and free of staphylococcal infection, were obtained from the Shizuoka Agricultural Co. Assoc., Hamamatsu, and used as controls.

Preparation of alpha-toxin: Staphylococcus aureus strain 0012, which was isolated from a guinea pig with the exfoliative skin disease, was used for production of the alpha-toxin. The strain did not produce beta- or delta-toxins. A partially purified alpha-toxin was prepared by the method of Nugent et al [8]. The culture supernatant fluid was mixed with sucrose (5%w/vol), applied to a column (2.5×45 cm) of sephadex G-100, and then eluted with 0.03M phosphate buffer, pH 6.5, containing 0.85% NaCl.

Hemolysis inhibition test: The antibodies against the staphylococcal alpha-toxins were examined by the hemolysis inhibition test [11], in which to 0.25ml each of the two-fold serial serum dilutions in 0.85% saline containing 0.01% MgSO4, an equal quantity of alpha-toxin (4 hemolytic units) was added. The resulting mixture was incubated in a water bath at 37°C for 30 minutes. After that, 0.5ml of 1% rabbit erythrocyte suspension was added to the mixture, which was incubated at 37°C for 30 minutes. The degree of hemolysis was determined by the naked eye, and the titer was expressed as the reciprocal of the highest
Fig. 2. Distribution of antibodies against staphylococcal alpha-toxins or cells in the sera from 4 groups of retired breeders and from staphylococcal disease-free controls.

serum dilution which showed a complete inhibition of hemolysis. None of the sera obtained from the control animals showed anti-alpha-toxin titers of 5 or more in this study; therefore, an antibody titer of less than 5 was considered to be negative.

Agglutination test: The antibodies against the staphylococcal cells were examined by the agglutination test [11]. The antigen used for this test was prepared from Staphylococcus aureus strain 0012, which was cultivated in a brain heart infusion broth at 37°C for 18 hours. A bacterial mass, which was precipitated from the culture by centrifugation, was suspended in 0.85% saline containing 0.5% formalin at a concentration of McFarland No. 5. In this test, an equal quantity of the antigen was added to each 0.5ml of the two-fold serial serum dilutions in saline. The resulting mixtures were placed in a water bath at 37°C for 2 hours, and then at 4°C overnight before reading. The antibody titer was expressed as the reciprocal of the highest dilution that showed a clear agglutination. As none of sera from the control animals showed agglutination at the lowest serum dilution (×100), an antibody titer of less than 100 was considered to be negative.

Staphylococcus aureus isolation: Four groups of guinea pigs, with the exception of the middle clinical stage group, were studied. Forty-four animals were sacrificed by cardiac puncture, and the skin, pharynx, trachea, lung, heart, liver, kidney and spleen were aseptically collected. The samples were individually homogenized and suspended in saline at a concentration of 10% (w/vol).

The nasal cavity was washed with 2ml of saline, and the washings were harvested. One-tenth ml of each specimen was aerobically cultured at 37°C on mannitol salt agar and No. 110 agar (Eiken Chemicals Co. Ltd.). In order to identify Staphylococcus aureus, the recognizable colonies of staphylococci developed on these agars were cloned and tested by Gram stain,
Table 1. Isolation of *Staphylococcus aureus* from 3 groups of retired breeders and from staphylococcal disease-free controls.

<table>
<thead>
<tr>
<th>Guinea pig group</th>
<th>No. of positive guinea pigs/No. of guinea pigs examined (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retired breeders in the early clinical stage</td>
<td>8 / 11 (73)</td>
</tr>
<tr>
<td>‡ in the late clinical stage</td>
<td>1 / 9 (11)</td>
</tr>
<tr>
<td>‡ without skin symptoms</td>
<td>1 / 6 (17)</td>
</tr>
<tr>
<td>Staphylococcal disease-free controls</td>
<td>0 / 18 (0)</td>
</tr>
</tbody>
</table>

catalase, glucose oxidation and fermentation by using oxidation fermentation media (Difco), acid phosphatase, and coagulase for rabbit plasma [1]. The isolates which showed glucose fermentation, positive catalase reaction, positive phosphatase activities, Gram positive cocci and positive coagulase activities were identified as *Staphylococcus aureus*.

**Results**

*Serological investigation*: In the present investigation, one serum sample from each guinea pig was examined for either the anti-alpha-toxin antibody or the anti-staphylococcal cell antibody. The distribution of these antibodies in the sera from the 5 groups of guinea pigs is shown in Figure 2. The occurrence of sera exhibiting the positive anti-alpha-toxin antibody with a mean antibody titer of 3.5 was 55% (6/11) in the early clinical stage group. This antibody was detected most frequently in the group of the middle clinical stage (100%) with a mean titer of 24.0. In the group of the late clinical stage, this antibody was detected in 64% of the sera (7/11) and revealed a mean titer of 4.1. Even in the group without skin symptoms, 33% of the sera (2/6) were positive for this antibody.

The agglutinating antibody was detected only in the groups of the early (5/11) and the middle (2/5) clinical stages. Although the occurrence of sera positive for the anti-staphylococcal cell antibody was not high, the frequency of positive sera or the distribution of the titers seemed to be similar to that of the anti-alpha-toxin antibody. Antibodies against staphylococcal alpha-toxins or cells were not detected in the sera from the control animals free of staphylococcal infection.

*Staphylococcus aureus isolation*: The results of *Staphylococcus aureus* isolation from the 4 groups are shown in Table 1. *Staphylococcus aureus* was isolated from 10 of the 44 guinea pigs. The majority of the isolates were cultured from the animals in the early clinical stage. Among 11 guinea pigs of this group, 8 (73%) were positive for *Staphylococcus aureus* isolation. Five specimens from the skin and pharynx, two tracheal specimens, and four nasal washings were positive in these animals. In the group of the late clinical stage, only one of 9 guinea pigs was positive for *Staphylococcus aureus*. The bacteria were cultured from the pharynx specimen in this animal. The pharynx specimen of 1 guinea pig of the group without skin symptoms was positive. The bacteria were not isolated from 18 animals of the control group.

**Discussion**

The present results demonstrated that exfoliative skin disease in guinea pigs was likely to be caused by staphylococcal infections. The occurrence of the anti-alpha-toxin antibody and the distributions of its titers correlated with both the clinical course and the development of the exfoliative skin disease. The percentage of animals with this antibody in each group studied was higher in the group of
the middle clinical stage (100%) than in the group of the early (55%) or the late (64%) clinical stages. The distribution of the titers also exhibited peak levels in the group of the middle stage (mean titer of 24.0), when compared to those in the groups of the early (mean titer of 3.5) or the late (mean titer of 4.1) clinical stages. The occurrence of the agglutinating antibody was relatively low in the 4 groups of retired breeders; however, the distribution of the titers in these groups appeared to be similar to that of the anti-alpha-toxin antibody.

The frequency of *Staphylococcus aureus* isolation from affected skin was relatively low, but it correlated to the clinical course and development of the disease. The 73% isolation rate of the early clinical stage group was higher than that of the late clinical stage group (11%), or the group without skin symptoms (17%). In the diseased animals, the lesions were found in the superficial layer of the skin; these lesions disappeared from the skin when desquamation of the skin flakes occurred [4]. The localization of the bacteria in these superficial lesions and the prompt decline of the bacteria upon desquamation of the skin flakes may have caused the relatively low isolation rate even in the early clinical stage (73%). With regard to the role of the anti-alpha-toxin antibody, Easmon and Glynn reported that an inflammatory response caused by the toxin-antibody reaction might play a protective role in local infections of *Staphylococcus aureus* in mice [2]. However, it was not clear whether this antibody behaved similarly in the staphylococcal skin infections of the guinea pigs used in this study.

The strains of *Staphylococcus aureus* isolated in the present study were not found in phage group 2 [4], and were not shown to cause exfoliation when inoculated into new-born mice. The exfoliative skin disease in guinea pigs, therefore, seems to be distinct from the staphylococcal scalded skin syndrome in infants and children [5, 6, 7]. However, the disease in the guinea pigs appeared to be a model for the localized skin infection occurring in human being and animals which is characterized by mild superficial infection including a blister formation.

### References


モルモットにみられたブドウ球菌性皮膚剝離症の
血清学的および細菌学的検索

石原 智明
北海道大学免疫科学研究所附属免疫動物実験施設

モルモットの繁殖集団に紅斑とそれにづく皮膚剝離を主徴とする病気が認められた。この病気と、その病原と思われるブドウ球菌との関係を明らかにするために本症の血清学的・細菌学的検討をおこなった。モルモットをその臨床像から、発症初期、中期、および後期の3群に分け、各群の個体について抗体保有状態とブドウ球菌分離状況をしらべた。その結果、ブドウ球菌α溶血毒に対する各群の平均抗体価は初期3.5、中期24.0、および後期4.1と中期群で最も高く、また抗体陽性率（抗体価5倍以上）も初期55％、中期100％、後期64％と中期群が他の2群より高かった。ブドウ球菌に対する凝集抗体は発症初期および中期群に認められ、後期群は陰性であった。またブドウ球菌分離率は発症初期群が73％であったのに対し、後期群では11％と低かった。このようにモルモットの本症においては、ブドウ球菌に対する抗体保有状態およびブドウ球菌分離成績は、いずれも本症の臨床像とよく相関していた。