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DEVELOPMENT OF TWO INBRED STRAINS OF RATS AND CHARACTERISTICS OF THEIR SKIN REACTIONS

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SUMMARY Two inbred strains of rat (Donryu and Sprague-Dawley strains) were developed. The skin reactions of these strains immunized with *M. tuberculosis*, hen egg albumin (OVA) or hen egg lysozyme and challenged with the purified protein derivative (PPD) or each antigen were even and uniform. The Donryu strain showed a typical Arthus reaction with petechiae and edema and a negligible delayed skin reaction, whereas the Sprague-Dawley strain showed a poor Arthus reaction and a typical delayed skin reaction with central necrosis and induration. The Arthus reaction or delayed skin reaction could be passively transferred to recipient rats of each strain by immune sera or sensitized peritoneal exudate cells (PEC), respectively.

INTRODUCTION

Usually, immune responses are influenced by differences in antigens, immunization methods, animal species and strains even of a single species. Consequently, when fixed antigens and immunization methods are employed to elucidate some unknown mechanism, the experimental animals must be homogeneous. To obtain sufficient homogeneity, in other words to allow the coefficient of relationship of the animals to be as nearly 100% as possible, successive litter- or sib-matings have been carried out and many inbred strains of mice have been developed (Dinsley, 1963; Kondo, 1970; Lane-Petter and Pearson, 1971).

We tried to obtain a homogeneous strain of rats, because rats have the advantages over mice that they are bigger and easier to operate on and have more blood, and that their immunoglobulins (Arnason et al., 1964; Banovitz and Ishizaka, 1967) seem to be somewhat simpler than those of mice (Fahey et al., 1964a; 1964b; Potter, 1972).

We obtained two inbred strains of rats (Donryu and Sprague-Dawley) which elicit very homogeneous skin reactions to test antigens. This paper describes these two inbred strains and their characteristic skin reactions to PPD, OVA and lysozyme.

MATERIALS AND METHODS

1. *Animals*

A litter of non-inbred Donryu rats (2 male and 5 female) was supplied by Takeda Chemical Industrial Co., Ltd., Osaka, Japan. Their sister-brother mating was begun in 1970 and 3 sublines (C112, D110 and D111) have been maintained for 24 generations up to the present.

A pair of non-inbred Sprague-Dawley rats (from a closed colony) was obtained from the Breeding Station for Laboratory Animals, Osaka University, Osaka in 1972. Litter mating of their offspring was repeated and one subline (B301) has now been maintained for 14 generations.

As experimental animals, female Donryu rats in the 24th generation and female Sprague-Dawley rats in the 14th generation of about 4 months old (weight 240 ± 10 g) at the time of priming were used.

All these rats were bred and maintained in The Quarters for Experimentally Infected Animals, in The Research Institute for Microbial Diseases, Osaka University.

2. *Antigens*

Mycobacterium tuberculosis, strain Aoyama B, was kindly provided by Dr. Kyuya Fujii, Department of Tuberculosis Research II, The Research Institute for Microbial Diseases, Osaka University, and killed by heating at 100 C for 60 min.

Five times crystallized hen egg albumin (OVA) and hen egg lysozyme were purchased from ICN Pharmaceutical, Inc., Life Sciences Group, Cleveland, Ohio and Eizai Co., Ltd., Tokyo, respectively.

3. *Immunization and skin testing*

Five rats of each group were sensitized by subcutaneous injection in opposite inguinal regions on days 0 and 14 with 0.5 ml volumes of water in oil (incomplete Freund's adjuvant) emulsion containing 1 mg of heat-killed *M. tuberculosis*, OVA or lysozyme.

Skin-testing was carried out on day 28 by intradermal injection of 0.05 ml volumes of 0.1% of each antigen (PPD, OVA or lysozyme) in physiological phosphate buffered saline on a shaved flank. The Arthus reaction was read at 3 hr and the delayed hypersensitivity reaction at 24, 48 and 72 hr after skin testing.

Each skin-test site was observed and palpated at the time of reading, and the width and thickness of the area of inflammation, if present, were measured with sliding metric calipers. The presence of petechiae or central necrosis was observed at the same time. None of the 3 antigens used causes inflammation of more than 2 mm in diameter in unsensitized rats, so we designate a reaction of more than 5 mm diameter as positive. These rats were usually clearly either non-sensitive (no reaction) or sensitive (reaction of 20 ± 4 mm mean diameter and 8.0 ± 1.0 mm mean thickness of folded skin), so results are reported as such here.

4. *Passive transfer*

Three donors of Sprague-Dawley rats immunized with *M. tuberculosis* or lysozyme as described above were boosted with 0.2 ml volumes of incomplete Freund's adjuvant containing 200 μg of each antigen in an axillar region 2 weeks after skin-testing. These donors were injected intraperitoneally with 10 ml volumes of paraffin oil another 2 weeks later. They were killed by total bleeding from the carotid artery 4 days after injection of oil and their sera were pooled. Peritoneal exudate cells (PEC) were harvested immediately after bleeding by washing the peritoneal cavity with 20 ml of heparinized minimum essential medium (MEM) using a 20 ml syringe with a 18 gauge needle. The washing fluids containing PEC from 3 donors immunized with each antigen were pooled and the paraffin oil was removed using separatory funnels. PEC was further washed 3 times with 50 ml volumes of MEM and finally resuspended with 1.5 ml of MEM. Volumes of 0.5 ml of this suspension were injected intraperitoneally into two recipient Sprague-Dawley rats after suspending to 3 ml of MEM (PEC alone) or into another 2 recipients after mixing with 3 ml of pooled immune serum (PEC+serum). Two other recipients were injected with 3 ml of immune serum (serum alone) to test the transfer or Arthus hypersensitivity.

After these procedures, over 60% of the transferred nucleated cells were viable by the eosin exclusion test. Thus, two recipients received slightly less than the equivalent of one donor's PEC (usually, 4×10^7 to 5×10^7 viable nucleated cells per recipient).

Recipients were challenged by intradermal injection of 0.05 ml volumes of 0.1% of each antigen at 24 hr after transfer. The site of each skin test was observed and the reaction was measured as described above.

RESULTS

1. *Skin reaction of Donryu rats*

When Donryu rats immunized with *M. tuberculosis* were challenged with PPD, neither the Arthus nor delayed skin reaction was detected.

Donryu rats immunized and challenged with

FIGURE 1. Skin reaction at 24 hr of 3 Donryu rats immunized with OVA. Only pigmentation and necrosis caused by the Arthus reaction remain.

FIGURE 2. Skin reaction at 24 hr of 3 Donryu rats immunized with lysozyme. Only pigmentation caused by the Arthus reaction remains. The degree of inflammation is less than that with OVA shown in Fig. 1.

OVA showed a skin reaction with petechiae and edema which began to appear after 2 hr and was maximal about 3 to 4 hr after challenging. This Arthus reaction disappeared gradually and as shown in Fig. 1, only pigmentation and necrosis caused by the reaction remained 24 hr after skin testing. The skin reactions in 5 Donryu rats after 3 hr ranged from 20 to 23 mm in diameter, the folded skin was 9 to 12 mm in thickness and the reactions were very uniform.

When lysozyme was used as antigen, the skin reactions in 5 Donryu rats after 3 hr ranged from 13 to 16 mm in diameter and from 8 to 9 mm in thickness of folded skin and were very homogeneous and even. These reactions were also accompanied by petechiae and edema and began to appear after 2 hr and were maximal about 3 hr after skin testing. These Arthus reactions disappeared faster than those with OVA. As shown in Fig. 2, only pigmentation caused by the Arthus reaction remained 24 hr after challenging.

2. *Skin reaction of Sprague-Dawley rats*

The skin reaction to PPD of Sprague-Dawley rats immunized with *M. tuberculosis* started to appear after 36 hr and was maximal after about 48 hr. This reaction was accompanied by induration and lasted until 72 hr after challenge. Figure 3 shows skin reactions of 3 Sprague-Dawley rats at 48 hr: the reactions in 5 rats ranged from 18 to 20 mm in diameter and from 12 to 13 mm in thickness of folded skin and were homogenenous and uniform. No inflammatory reaction appeared within 30 hr after skin testing.

Five Sprague-Dawley rats were immunized with OVA and tested with the same antigen. Neither an Arthus nor a delayed skin reaction was detected in this strain of rats.

When lysozyme was used as antigen, no inflammatory reaction appeared within 30 hr, but a skin reaction with induration began to appear around 36 hr and reached a maximum about 72 hr after challenging. The skin re-

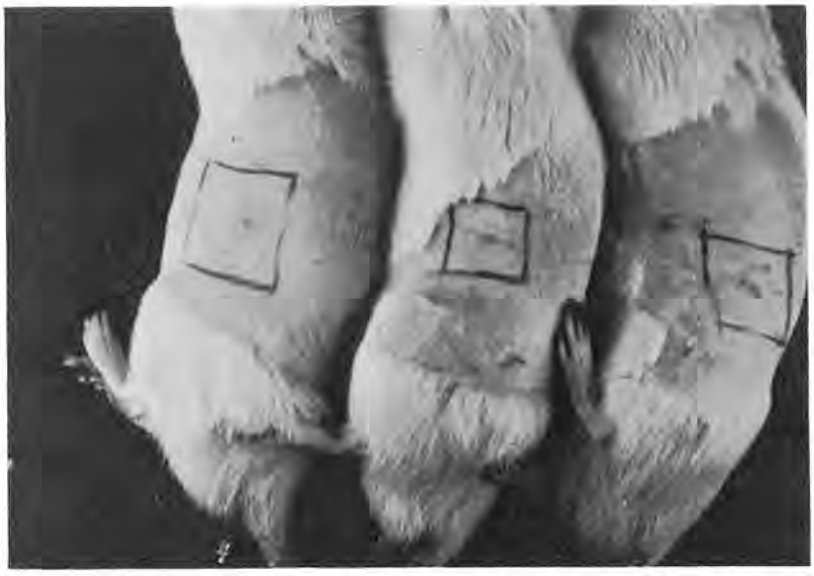

FIGURE 3. Skin reaction at 24 hr of 3 Sprague-Dawley rats immunized with *M. tuberculosis* and challenged with PPD. The indurations were very homogeneous and were accompanied by slight erythema caused by the remaining Arthus reaction.

FIGURE 4. Skin reaction at 72 hr of 2 Sprague-Dawley rats immunized with lysozyme. The indurations of these rats were very homogeneous. The degrees of inflammation were less than those with PPD shown in Fig. 3.

TABLE 1. *Summary of the skin reactions of the two inbred strains of rats sensitized with various antigens*

Antigen	Donryu rats		Sprague-Dawley rats	
	Arthus	Delayed	Arthus	Delayed
M. tuberculosis[a]	−	−	−	+
OVA	+, P[b]	−	−	−
Lysozyme	+, P	−	−	+

[a] Skin test with PPD.

[b] P : petechiae.

actions of 5 Sprague-Dawley rats after 72 hr ranged from 10 to 12 mm in diameter and 8 mm in thickness of folded skin and were uniform (Fig. 4).

The data summarized in Table 1 show that Donryu rats gave an Arthus reaction to OVA or lysozyme but did not give any detectable delayed skin reaction to *M. tuberculosis*, OVA or lysozyme with the immunization schedule described above. On the contrary Sprague-Dawley rats showed delayed skin reactions to *M. tuberculosis* and lysozyme but no Arthus reaction to any antigen tested.

3. *Passive transfer of the immune state of Sprague-Dawley rats*

Sprague-Dawley rats immunized with *M. tuberculosis* or lysozyme were used as donors. Recipient Sprague-Dawley rats of the same generation were divided into three groups of 2 females each, and were sensitized intraperitoneally with PEC alone, PEC+immune serum, and immune serum alone, respectively, in an approximate ratio of donor to recipient of one to two. Then they were challenged with PPD or lysozyme 24 hr later.

1) Transfer of the immune state to *M. tuberculosis*

The groups of recipients which received serum and serum+PEC (4.5×10^7 of nucleated cells) from donors immunized with *M. tuberculosis*, showed skin reactions accompanied by edema and slight petechiae. These reactions began to appear after 2 hr and were maximal about 3 to 5 hr after challenging, and the maximal reactions at 4 hr were 20 mm in diameter and 12 mm in thickness of folded skin. The reaction of recipients injected with serum alone disappeared leaving only pigmentation within 8 hr. However, the reaction of rats injected with PEC+serum lasted until 72 hr and was accompained by induration of 18 mm diamter which was maximal about 24 hr after challenging.

The recipients injected with PEC (4.5×10^7 of nucleated cells) without any serum showed the inflammatory skin reaction with induration and central paleness but little edema. This reaction appeared after about 3 hr, gradually increased to a maximum after about 24 hr and lasted until 72 hr. No detectable petechiae was observed in these recipients. These results, summarized in Table 2, show that the Arthus and delayed skin reactions could both be transferred to syngeneic recipients with serum or PEC or both.

TABLE 2. *Transfer of the immune state to M. tuberculosis in Sprague-Dawley rats*[a]

Transfer with	Skin reaction	
	at 3 hr	at 24 to 48 hr
PEC	±	+, N[b]
PEC+serum	+, P[c]	+, N
Serum	+, P	−

[a] Skin test with PPD.
[b] N: typical delayed central necrosis with paleness.
[c] P: petechiae.

2) Transfer of the immune state to lysozyme

The same system was used as for the experiment in 1) except that PEC and serum were obtained from donors immunized with lysozyme instead of *M. tuberculosis*. Table 3 shows the results. The Arthus reaction with petechiae and edema could be transferred with serum or with PEC+serum, and the delayed reaction with induration and pale central necrosis could be transferred with PEC or PEC+serum. The times of appearance of the skin reactions we similar to those in the experiment on transfer of the immune state to *M. tuberculosis*, but the reactions were slightly less, the Arthus reaction being 15 mm±2 mm in diameter and the induration of the delayed skin reaction being 12 mm±1 mm in diameter.

TABLE 3. *Transfer of the immune state to lysozyme in Sprague-Dawley rats*

Transfer with	Skin reaction	
	at 3 hr	at 24 to 48 hr
PEC	±	+, N[a]
PEC+serum	+, P[b]	+, N
Serum	+, P	−

[a] N: typical delayed central necrosis with paleness.
[b] P: petechiae.

DISCUSSION

The results reported here show that our inbred strain of Donryu rats showed mainly an Arthus skin reaction with a very high homogeneity and that Sprague-Dawley rats after 14 litter-matings in our Research Institute showed a very homogenous delayed skin reaction. The high homogeneity of the latter may be because the first pair of Sprague-Dawley rats from a closed colony showed an unexpectedly high homogeneity. The coefficient of inbreeding F and the relationship R at the 14th generation of litter-mating are 95.1 and 98.5%, respectively (Kondo, 1970), so the real coefficients of our Sprague-Dawley rats seem to be much nearer to 100% than the calculated values. Preliminary studies on syngeneic skin transplantation were carried out in our Donryu rats

at the 20th generation and in the Sprague-Dawley rats at the 10th generation, and the syngrafts have survived up to the present.

In this paper, the degree of the immune response of these rats was examined by the simple method of skin testing. This method for detecting the cell-mediated immune state is a routine method and has been used especially in guinea pigs. The skin test was first successfully used in mice in 1959 to detect delayed type hypersensitivity to OVA (Crowle, 1959), but mouse skin is very thin and so skin-testing in mice requires much care and skill. In 1962, Flex and Waksman used the skin test to examine delayed type hypersensitivity to PPD in Sprague-Dawley rats, but the readings and degrees of inflammation of test sites were much more heterogeneous than in our Sprague-Dawley rats.

It is interesting that our results showed that the skin reactions in Donryu and Sprague-Dawley rats were opposite: the Donryu rats showed mainly an Arthus reaction whereas Sprague-Dawley rats showed mainly a delayed skin reaction. This difference may be due to differences in the gene(s) controlling the immune response, including its regulation mechanisms, or the amount of skin reacting factor produced or the levels of serum complement components.

The Arthus and delayed skin reactions could be passively transferred to syngeneic recipients with serum and PEC, respectively. The earlier appearance of these reactions in recipients than in the donors might be caused by the higher sensitization state of donors which received another booster injection 18 days before the time of transfer.

Our inbred rats showed very high homogeneity in skin tests. The Donryu rats should be useful in studies on the mechanisms of humoral immunity and the Sprague-Dawley rats in studies on mechanisms of cell-mediated immunity, including transplantation or tumor immunity.

We are currently performing pathological studies on skin reaction sites and also examining the immunological differences between our two strains of rats.

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REFERENCES

Arnason, B. G., C de Vaux st-Cyr, and E. H. Relyveld. 1964. Role of the thymus in immune reaction in rats. IV. Immunoglobulins and antibody formation. Int. Arch. Allergy 25: 206–224.

Banovitz, J., and K. Ishizaka. 1967. Detection of five components having antibody activity in rat antisera. Proc. Soc. Exp. Biol. Med. 125: 78–82.

Crowle, A. J. 1959. Delayed hypersensitivity in mice: its detection by skin tests and its passive transfer. Science 130: 159–160.

Dinsley, M. 1963. Inbreeding and selection, p. 235–259. *In* W. Lane-Petter [ed.] Animals for research. Academic Press, New York.

Fahey, J. L., J. Wunderlich, and R. Mishell. 1964a. The immunoglobulins of mice. I. Four major classes of immunoglobulins: 7Sγ2-, 7Sγ1-, γIA-(β2A)- and 18SγIM-globulins. J. Exp. Med. 120: 223–242.

Fahey, J. L., J. Wunderlich, and R. Mishell. 1964b. The immunoglobulins of mice. II. Two subclasses of mouse 7Sγ2-globulins: γ2a- and γ2b-globulins. J. Exp. Med. 120: 243–251.

Flex, M. L., and B. H. Waksman. 1962. Delayed cutaneous reactions in the rat. J. Immunol. 89: 496–504.

Kondo, K. 1970. On the genotype of the experimental animals, p. 31–105. *In* Y. Tajima [ed.] Jikken Dobutsugaku. Asakura-Shoten, Tokyo. [In Japanese].

Lane-Petter, W., and A. E. G. Person. 1971. The laboratory animal, principles and practice. Aca-

demic Press, New York.
Potter, M. 1972. Immunoglobulin-producing tumors and myeloma proteins of mice. Physiol. Rev. 52: 631–719.