Establishment and Characteristics of Five Analbuminemic Inbred Strains of Rats

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Five analbuminemic inbred strains of rats (AD/1, AD/2, AD/3, AD/4, AD/5) were established from Nagase analbuminemic rats (NAR). They showed no genetic differences in coat color, biochemical marker gene loci and skin grafting test. Their serum levels of total cholesterol, phospholipids, triglycerides, and β -lipoproteins were compared with normal inbred strains (L) derived from Sprague-Dawley rats. Their plasma apoproteins were also examined. All inbred strains of analbuminemic rats showed hyperlipidemia progressing with age although there were slight variations in their lipid and apoprotein levels. These analbuminemic inbred strains of rats may be multigenic models of lipid metabolism abnormality.

In 1977, Nagase *et al.* [12, 13] established an analbuminemic mutant strain of rats (NAR; Nagase Analbuminemic Rat) from outbred Sprague-Dawley rats. The analbuminemia is inherited by a single recessive autosomal gene (*alb*) [16] which was linked to the hooded locus belonging to the sixth linkage group [17]. Biochemically, the mutant is characterized by extraordinarily low levels of serum albumin and hyperlipidemia, but these characteristics are assumed to be modified by the genetic background and rearing environment [15].

Therefore, we have tried to establish analbuminemic strains of rats with unified genetic backgrounds and established the three analbuminemic congenic strains (ACI-alb, F344-alb, and SHR-alb) [18]. The present paper describes the establishment of five inbred strains of analbuminemic rats from NAR and several characteristics of these strains.

Materials and Methods

Original individuals for inbreeding were selected randomly from NAR stock. Attempts to establish analbuminemic inbred strains were made twice. The first started in 1977 [13] and the second in 1980.

Throughout the experiments, the rats were put in cages (265×425×200 mm) with wood shavings on the floor and kept in an Iso-Rack (Sanki Scientific Co. Ltd., Tokyo) into which a gentle flow of fresh air filtered by double microfilters was supplied horizontally from the back wall. They were maintained at the Sasaki Institute in the conventional animal room with air-conditioning (23±2°C and 55±5% relative humidity) and artificial lighting with 12 hours of light from 7 AM to 7 PM, and provided with a commercial diet (CE-2: CLEA Japan Inc., To-

kyo) and tap water *ad libitum*.

Pregnant females were removed to individual cages for delivery and rearing after three weeks of pairing with males.

Serum total cholesterol concentrations were measured in each generation at seven weeks of age with blood samples collected from the tip of the tail. The established albuminemic inbred strains of rats were monitored using more than five rats with regard to some coat color and biochemical marker gene loci as described previously [18]. The skin grafting test was carried out by the method of Festing and Grist [5]. Mature rats were anesthetized with sodium-pentobarbital, and a 7 mm square section of the tail skin of each rat was cut off and transplanted with the direction of hair growth reversed. Results of the skin graft were judged two weeks after the operation. Some biochemical analyses were carried out for characterization of the established strains. Serum total cholesterol (TC), phospholipids (PL), and triglycerides (TG) were analyzed by conventional enzyme methods with a photoelectric colorimeter (Klett Manufacturing Co. Inc., New York).

β -lipoproteins were examined by precipitation with heparin and calcium ions [10] at 7, 10, and 15 weeks of age in five rats of either sex. Serum lipoperoxide (LPO) was assayed by the fluorometric method [20] at 10 and 15 weeks of age in three rats of both sexes. In three females at 5 weeks of age the apolipoprotein composition of plasma lipoproteins ($d \leq 1.21$) isolated by ultracentrifugal flotation at 40,000 rpm for 24 hours were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 1) [8].

Results and Discussion

In the first experiment on inbreeding of NAR [13], two sibmating lines died out at the F7 and F6 generations. The breeding data of the two lines at the F6 generation are shown in Table 1. We could obtain three pairs in the A line and one pair in the B line. They delivered five and three litters, respectively. However, none of the three pairs set up in the former line at the F7 generation delivered pups, and no females were obtained in the latter line in three de-

Fig. 1. Analytical methods for plasma apoproteins.

Table 1. Breeding data of F6 generation in inbreeding lines of analbuminemic rats.

Delivery No.	Litter size	A line		B line		
		Weaned	pups	Litter size	Weaned	pups
		male	female		male	female
1	10	2	1	8	1	0
2	4	0	0	7	3	0
3	6	4	1	8	0	0
4	2	0	0			
5	6	3	3			

Table 2. Serum lipid concentrations of analbuminemic rats in the first inbreeding lines.

Generation	Total cholesterol (mg/dl)		Triglycerides (mg/dl)	
	male	female	male	female
F 3	128±12 (5)*	128±16 (5)	134±38 (5)	195±59 (5)
F 4	147±10 (11)	151±13 (20)	197±30 (11)	212±74 (20)
F 5	179±15 (8)	217±20 (13)	273±61 (8)	251±56 (13)

* mean ± SD (n)

Fig. 2. Pedigree maps of the analbuminemic rats.

liveries at the F6 generation. The infertile parents had atrophied testes and ovaries and hydro-uterus. One cause of this extinction appeared to be an infection such as mycoplasmosis. Another reason seemed to be unusual physiological conditions due to hyperlipidemia accelerated by the inbreeding (Table 2). We

again tried inbreeding six lines selected randomly from the NAR colony to study the cause of the extinction and to establish strains with high coefficients of inbreeding and relationship.

At the beginning, we intended to continue brother and sister matings as much as

possible but changed to the three-line rotation system when the animal stocks were about to become extinguished [19]. These inbred lines were continuously used to supply nuclear breeders of NAR stock which were propagated by random breeding to counterbalance genetic drift. We finally established five analbuminemic lines with over twenty generations of brother-sister matings. A pedigree map of each line is shown in Fig. 2. The A line died out at the F16 generation, apparently caused by a high incidence of infertile females from the early generations. The B line was weeded out at the F3 generation because of frequent cannibalism and infertility. After the F12 generation, the D line was divided into two lines with high (DH) and low (DL) levels of total cholesterol concentration at seven weeks of age. The established five inbred strains (C, DH, DL, E, R) were designated as AD/1, AD/2, AD/3, AD/4, and AD/5, respectively. At present, outbred NASS are produced by random matings among nuclear breeders taken from these five inbred strains.

The five analbuminemic lines and the normal inbred strain (L) derived from Sprague-Dawley rats had different cholesterol levels (Fig. 3). The five analbuminemic lines showed no increase in TC values with successive inbreeding generations. AD/1 had the highest mean TC values of 171 mg/dl in males and 189 mg/dl in females. AD/3 had the lowest values of 131 and 163 mg/dl in males and females, respectively. In contrast, the L line had 60 and 66 mg/dl in males and females, respectively. Thus, clear differences

Fig. 3. Total cholesterol concentrations in rat sera at 7 weeks of age in each generation.

appeared between the five analbuminemic lines and the L line.

Results of genetic monitoring of the five established analbuminemic inbred strains are shown in Table 3. The ACI, DONRYU and TM strains were used as reference strains for each marker gene. Among the five analbuminemic inbred strains, there were no differences in marker genes at coat color (*a*, *b*, *c*, *h*) and biochemical marker gene loci *Amy-1*, *Cs-1*, *Es-1*, *Es-2*, *Es-3*, *Es-4*, *Hbb*, *alb*, *Gc*). Results of inter- and intra-strain

Table 3. Coat color and biochemical marker genes of analbuminemic inbred strains of rats.

Strain	a	b	c	h	<i>Amy-1</i>	<i>Cs-1</i>	<i>Es-1</i>	<i>Es-2</i>	<i>Es-3</i>	<i>Es-4</i>	<i>Hbb</i>	<i>alb</i>	<i>Gc</i>
AD/1	a	B	c	h	a	b	a	a	b	b	b	—	a
AD/2	a	B	c	h	a	b	a	a	b	b	b	—	a
AD/3	a	B	c	h	a	b	a	a	b	b	b	—	a
AD/4	a	B	c	h	a	b	a	a	b	b	b	—	a
AD/5	a	B	c	h	a	b	a	a	b	b	b	—	a
ACI	A	B	C	h ⁱ	b	a	b	a	a	b	b	+	a
DONRYU	a	B	c	h	a	b	a	c	b	a	a	+	a
TM	a	B	C	h	b	a	b	a	c	a	b	+	b

1-5: analbuminemia; L: normal low lipidemia
Fig. 4. Serum lipid concentrations in each strain.

1-5: analbuminemia; L: normal low lipidemia
Fig. 5. Serum lipoperoxide value in each strain.

Table 4. Results of inter-and intra-strain skin grafting.

	AD/1	AD/2	AD/3	AD/4	AD/5	L
AD/1	+	+	+	+	+	—
AD/2		+	+	+	+	—
AD/3			+	+	+	—
AD/4				+	+	—
AD/5					+	—
L						+

+: reciprocal acceptance, —: reciprocal rejection
L: normal inbred strain originating from outbred Sprague-Dawley rats.

1-5: analbuminemia; L: normal low lipidemia
Fig. 6. Plasma apoprotein concentration and relative amount of its constituents in female rats at 15 weeks of age.

skin grafting are shown in Table 4. Garfts of the five analbuminemic strains were accepted reciprocally, but were rejected by the L strain. Original NARs are derived from the outbred stock of Sprague-Dawley rats. They were delivered to us at the F4 generation after divergence [13]. Several heterozygosities in the *Es-1*, *Es-3*, *Es-4*, and *Hbb* loci appear in the outbred stock of Sprague-Dawley rats (Kato, H. *et al.*: Personal communication). This and the above results suggest that the NAR stock was a population with a high level of homozygosity from an early stage.

Serum lipid concentrations (TC, PL, TG) at 7, 10 and 15 weeks of age are shown in Fig. 4. In the analbuminemic strains, serum lipid concentrations tended to increase with age although in cholesterol was small. Strain differences appeared in the lipid concentrations; AD/1 and AD/5 always showed higher levels. In contrast, the other three strains sometimes showed slightly lower levels compared with those two strains. Higher serum LPO appeared in the AD/2 and AD/3 strains, which showed relatively lower cholesterol levels (Fig. 5). The cause of this phenomenon is uncertain and further investigations are required to elucidate the underlying mechanism. Concentration of plasma apoproteins and the relative amount of its constituents in females at 15 weeks of age are shown in Fig. 6. A clear difference in the total apoprotein value appeared between the analbuminemic rats and the L strain. The AD/2 strain showed the lowest value among the analbuminemic strains. In the AD/3 strain, the apoA-I concentration was high and apoE was low. Among the minor components, apoB constituted 3.58% in strain AD/1, 2.54% in AD/2, 1.35% in AD/3, 2.13% in AD/4, 3.18% in AD/5 and 0.56% in the L strain.

Recently, genetic studies of human apolipoproteins have progressed rapidly and the structures of most apolipoprotein genes have been determined [4]. The complete amino acid sequence of human apoB has also been confirmed by two groups of researchers [9, 21]. The structure of rat apolipoproteins A-I, A-II, A-IV, C-III, and E genes has been analyzed [1-3, 6, 7, 11, 14], and the evolutionary

relationships with human apolipoprotein are apparent. Thus it appears that there are characteristics in each analbuminemic strain for the regulation of apoproteins. The five established analbuminemic inbred strains of rats may serve as multigenic models of lipid metabolism abnormality.

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5 系統の無アルブミン近交系ラットの育成とその特性

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無アルブミン血症ラット (NAR) をもとにして, 5 系統の無アルブミン近交系ラット (AD/1, AD/2, AD/3, AD/4, AD/5) を育成した。これらの 5 系統間には毛色, 生化的標識遺伝子座および皮膚移植試験による遺伝的差異は認められなかった。血清中の総コレステロール, 磷脂質, 中性脂質 および β -リポタンパク, 血漿アポタンパクについて, SD ラット由来の正常近交系ラット (L)

と比較した。その結果, すべての無アルブミン近交系ラットは著しい高脂血症を示し, 加齢とともに増加する傾向が認められた。また, 5 系統の近交系間には脂質とアポタンパクレベルにわずかな変異が認められた。今回育成した 5 系統の無アルブミン近交系ラットは脂質代謝異常に関する複合モデルとなる可能性がある。