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Molecular Biology for Genetic Deficiencies of Complement Components in Rabbits: C8α-γ Deficiency and C3 Hypocomplementemia

Masanori KOMATSU
Department of Animal Breeding and Genetics, National Institute of Animal Industry (Tsukuba, Ibaraki, 305 Japan)

Abstract
The complement system is composed of approximately 20 plasma proteins and constitutes a major humoral defense and clearance system in the bloodstream. New complement deficiencies, i.e. C8α-γ deficiency (C8α-γD) and C3 hypocomplementemia (C3-hypo), were identified in rabbits. The C8α-γD and C3-hypo were transmitted as a simple autosomal recessive and condominant trait, respectively. The physiological characteristics and molecular bases for C8α-γD and C3-hypo were identified as follows: C8α-γD is characterized by dwarfism (non-pituitary), small litter size, small thymus, a low survival rate, severely reduced serum bactericidal activity and enhanced delayed-type hypersensitivity (DTH), and normal expression of α and γ genes, but abnormal co-translational processing of C8α gene (a mutation of the exon/intron junction of the C8α gene). C3-hypo is characterized by approximately 10% of normal serum C3 levels, a low survival rate, reduced serum bactericidal activity, suppressed DTH, and low levels of liver C3 mRNA (pretranslational defect), and C3 phenotypes are dependent on RFLPs of the C3 gene,

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Introduction

The complement system is composed of approximately 20 complement components and regulator proteins, and constitutes a major humoral defense and clearance system in the bloodstream. Complement can be activated by two distinct routes, the classical (via immunoglobulins) and alternative pathways (not necessarily involving antibody) (Fig. 1). Components C5–C9 are designated as the terminal components that form the MAC (membrane attack complex), which is responsible for target cell damage and lysis. Other biologically important functions mediated by the complement system include: (1) the low molecular weight fragments (~9000 mol. wt) such as the anaphylatoxins C3a, C4a and C5a, which promote smooth muscle contraction and increase vascular permeability; (2) the large C4b and C3b fragments, which are involved in binding to the complement activator and can thereafter interact with specific receptors to allow efficient clearance of the activating cell or particle; and (3) an intact alternative pathway converts preformed insoluble immune complexes into soluble form to prevent immune complex-mediated diseases[13]. Since the loci controlling the synthesis of the three components of complement, C2, C4, and factor B, are coded within the major histocompatibility complex (MHC), they are referred to as the class III genes of the MHC[13]. In laboratory animals, several inherited deficiencies of complement components have also been identified: i.e. C5-deficient mouse[1,17], C6-deficient rabbit[1,17], C4-deficient guinea pig[1,17], C1-deficient chicken[1], C2-deficient guinea pig[1,17], C3-deficient dog[1,17], C8α-γ-deficient (C8D) rabbit[7], C8β-deficient

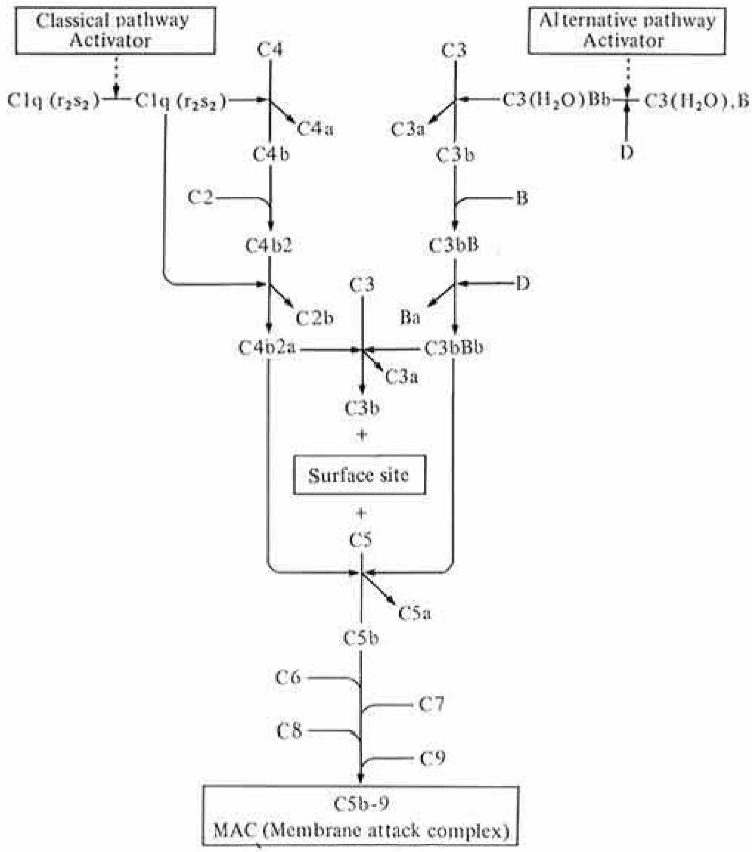

Fig. 1. The classical and alternative pathways of complement activation
Source: Law and Reid (1988)[13].

mouse[18], C3-deficient guinea pig[1,17], and C3 hypocomplementemic rabbit[8].

The purpose of this paper is to review the physiological characteristics and molecular bases for genetic C8α-γ deficiency and C3 hypocomplementemia in rabbits.

Rabbit C8α-γ deficiency

The eighth component of complement (C8), one of five constituents of the complement membrane attack complex (MAC), consists of three polypeptide chains, α, β, and γ; the α- and γ- chains are disulfide linked to each other, forming the α-γ-subunit, which is noncovalently associated with the β-subunit[4]. During the two-way selective experiment of breeding New Zealand White rabbits for high and low CH50, two rabbits defective in CH50 were found in the low line[5]. The serum of these complement-deficient rabbits lacked the α-γ-subunit of C8 immunochemically and functionally. The C8α-γ deficiency is transmitted as a simple autosomal recessive trait[7].

1) Physiological characteristics

The C8D rabbits were consistently smaller than normal littermates from birth (86% of normal size) to adulthood (68% of normal size) (Table 1)[9]. The actual and relative weights of the thymus in the C8D rabbits were consistently lower than those of normal rabbits throughout the ages under testing (Table 2)[9]. Histological examination of the thymus and lymphoid organs was performed; however, no marked abnormalities were observed. The C8D rabbit is fertile, but crosses of C8D females with C8D males led to a reduced delivery rate and small litter

Table 1. Changes in body weight for normal and C8α-γ-deficient rabbits after birth

(Unit: g)

Sex	Phenotype	Days after birth			
		0	35	120	> 360
Female	Normal	62[a]	585[a]	2429[a]	3573[a]
	C8D	55[c]	328[c]	1657[c]	2296[c]
Male	Normal	66[a]	568[a]	2236[a]	2909[a]
	C8D	55[c]	327[c]	1570[c]	2052[c]

a, c: $P<.01$.
Source: Komatsu, M. et al. (1990)[9].

Table 2. Changes in relative weight of thymus, testes, and ovaries for normal and C8α-γ-deficient (C8D) rabbits after birth[1)]

(Unit: %)

Organs	Phenotype	Age (days or months)				
		At birth (M & F)[2)]	81 days (M)	120 days (F)	8–9 months (F)	9–21 months (M)
Thymus	Normal	.225	.276	.359	.312[a]	.193[a]
	C8D	.205	.258	.306	.157[c]	.129[c]
Testes	Normal	.021	.125	—	—	.255
	C8D	.020	.041	—	—	.268
Ovaries	Normal	.017	—	.011	.010	—
	C8D	.022	—	.010	.012	—

1): Relative organ weight (%) = (organ weight/net body weight) × 100.
Net body weight is calculated by subtracting the weight of the gastrointestinal mass from the gross body weight.
2): M; Male, F; Female.
a, c: $P<.01$.
Source: Komatsu, M. et al. (1990)[9].

Table 3. Delivery rates and litter sizes of various crosses

Cross (Phenotype) F	× M[1)]	Delivery rate (%) (no. of deliveries/ no. of matings)	Litter size (mean ± SD)
N[2)]	D[2)]	79 (92/117)	5.6 ± 2.4
D	N	57 (4/7)	5.5 ± 0.5
D	D	5 (1/19)	2
N	N	91 (32/35)	6.1 ± 1.5

1): F; Female, M; Male.
2): N; C8α-γ-normal, D; C8α-γ-deficient.
Source: Komatsu, M. et al. (1990)[9].

Table 4. Immunologic characteristics of C8α-γ-deficient (C8D) rabbits or C3 hypocomplemententemic (C3-hypo) rabbits

Item	Rabbit	
	C8D	C3-hypo
WBC counts	Normal	Normal
Cellular immunity (Delayed type hypersensitivity)	Enhanced	Suppressed
Humoral immunity (Antibody response to BSA)	Normal	Normal
Production of antinuclear antibody	None	Few
Serum bactericidal activity	Very reduced	Reduced

Source: Komatsu, M. (1987)[6], Komatsu, M. et al. (1988)[8] and (1990)[9].

Table 5. Summary of endocrinological and biochemical features in C8α-γ-deficient (C8D) rabbits

Item	C8D	Normal	Age[1)]	P
Insulin (μU/m*l*)	6.3	15.5	a	<.01
Cortisol & Corticosterone (μg/d*l*)	3.7	2.6	a	
T4 (μg/m*l*)	4.2	5.0	a	
T3 (ηg/d*l*)	256	261	a	
IGF-1 (ηg/m*l*)	64	58	b	
Plasma glucose (mg/d*l*)	112	118	c	
Serum Ca (mg/d*l*)	15	15	a	
Serum phospholipids (mg/d*l*)	98	92	c	
Serum triglyceride (mg/d*l*)	39	41	c	
Serum acid phosphatase (K-A unit)	13	11	c	
Plasma total cholesterol (mg/d*l*)	70	92	c	<.01

1): a; 42~91-day-old, b; 19~27-day-old, c; 6~8-month-old.
Source: Komatsu, M. et al.[12)].

size (Table 3)[9)]. None of the eight C8D rabbits tested produced antinuclear antibodies (ANAs) (Table 4)[9)]. Since the relative thymus weight of C8D rabbits was consistently smaller than that of normal rabbits throughout the ages under testing (Table 2), it was considered that the deficient rabbits might have abnormal immune responses. The author has studied the immune response to Alum-BSA (1 mg/m*l*/rabbit, intraperitoneal injection) and delayed hypersensitivity reactions to tuberculin (DTH). The results show that the C8D rabbits can mount normal antibody response to BSA, but their DTH reactions are enhanced (Table 4)[6)].

Endocrinological and biochemical studies were undertaken to elucidate a mechanism of the dwarfism of C8D rabbits (Table 5, unpublished data)[12)]. Because the serum concentration of insulin-like growth factor (IGF-1), a mediator of growth hormone (GH), in C8D rabbits was within normal range, the C8D rabbit did not exhibit pituitary dwarfism. Since the serum insulin concentration in C8D rabbits (42-92 day-old) was lower than that of normal rabbits, the dwarfism of C8D rabbits might be attributed to a defect of carbohydrate metabolism in infancy (Table 5). In addition, dwarfism is not found in the C8β-deficient mouse[18)].

2) Molecular basis

To examine the possibility that C8α-γ deficiency was due to a pretranslational defect in α or γ gene expression, poly(A)$^+$RNA from normal and C8D rabbits was subjected to northern blot analysis, using the three human C8 cDNA probes[2,14,16)]. Importantly, the corresponding analysis of C8D rabbit poly(A)$^+$RNA identified messages for α and γ of the same size and amount as normal rabbits[10]. Thus, the α and γ genes are normally expressed in C8D rabbits. To further determine the molecular basis of C8α-γ deficiency, cDNA libraries were prepared from normal and C8D rabbit liver and screened with human C8α cDNA probes. Comparison of normal α to C8Dα revealed that the latter contained an additional insert of 31 amino acids (93 bp) within the leader sequence. Sequencing of genomic DNA after PCR amplification indicates that this 93 bp is intron sequence and suggests a mutation in an exon/intron junction of the C8D gene. Since the insert disrupts the leader sequence while maintaining an open reading frame, it is likely that the α-γ deficiency is caused by abnormal co-translational processing of C8α[3]. The cDNA sequencing of the C8γ gene of C8D rabbits is under way.

The function of C8α in cytolysis is well-understood, whereas the role of C8γ remains unknown. C8γ belongs to the alpha-2u-globulin or "lipocalin" family, including protein HC, serum retinol-binding protein, alpha$_1$-acid glycoprotein, beta-lactoglobulin, and others that have the ability to bind lipophilic ligands, i.e. vitamin A and steroid hormones[15]. The dwarfism may reflect that C8α or C8γ has some functional role *in vivo* other than cytolysis. In regard to other functions of C8α or C8γ, further studies of C8α-γ-deficient rabbits are required.

Rabbit C3 hypocomplementemia[8]

C3 is composed of two unequal polypeptide chains, α and β, linked by disulfide bonds, and is the most abundant among the complement proteins. C3 plays a central role in the complement system, which constitutes a major humoral effector (Fig. 1)[13]. C3 hypocomplementemia was found in the colony of rabbits, in which genetic C8α-γ deficiency was also observed when the rabbits were tested immunochemically and functionally for serum C3 levels in an individual member of this colony[8]. The serum C3 levels and total complement hemolytic activity (CH50) of these animals were 6–13% and 27–37% of the normal levels, respectively. The hemolytic complement activity in the C3 hypocomplementemic (C3-hypo) rabbit serum was restored in a dose-dependent manner by adding purified rabbit C3. The low level of serum C3 in C3-hypo rabbits was neither attributed to C3 conversion, partial C3 antigenicity, presence of a C3 inhibitor, nor hypercatabolism of normal C3. Mating tests showed that the C3 hypocomplementemia was transmitted as a simple autosomal codominant trait.

1) Physiological characteristics[6,8]

C3-hypo rabbits have a lower survival rate than normal rabbits. C3-hypo rabbit serum also has a lower bactericidal activity than normal rabbit serum. The C3-hypo, non-C8α-γ-deficient rabbits show normal antibody response to BSA; however, they have suppressed DTH reactions to tuberculin *in vivo*. There is a tendency to produce antinuclear antibodies in C3-hypo rabbits (Table 4). The C3-hypo gene, unlike the C8α-γ-deficient gene, has no adverse effect on body weight[8].

2) Molecular basis[11]

To determine the molecular basis for rabbit C3-hypo, a study on the C3 genes and the C3 proteins was undertaken, using rabbit C3 cDNA, and SDS-PAGE, and isoelectric focusing (IEF)[6]. The C3-hypo rabbits have low levels of liver C3 mRNA, correlating with the serum C3 concentrations. The molecular weights of C3 for C3-hypo were identical to those for the heterozygous and normal animals on SDS-PAGE under reducing conditions. The C3 bands on IEF consisted of one major band of pI 6.2 and one minor band of pI 6.5. Normal and heterozygous rabbits have both the major and the minor bands, whereas the C3-hypo rabbits have only the minor band (Plate 1)[6]. These results indicate that rabbit C3 molecules have heterogeneity (two distinct patterns of pI 6.2 and 6.5) due to different glycosylations of the C3 protein, and the major band is deficient in the C3-hypo rabbit.

Genomic DNA from C3-hypo, heterozygous and normal animals was subjected to Southern blot analysis after digestion with 16 different restriction endonucleases. With Bgl II, Stu I, and Sac I, RFLPs of the C3 gene, dependent on the C3 phenotypes were detected in the three types of rabbits (Plate 2)[11]. These results suggest that rabbit C3 hypocomplementemia be attributed to a pretranslational defect resulting from mutations within the C3 gene (i.e. promoter region). The molecular basis of C3 hypo-

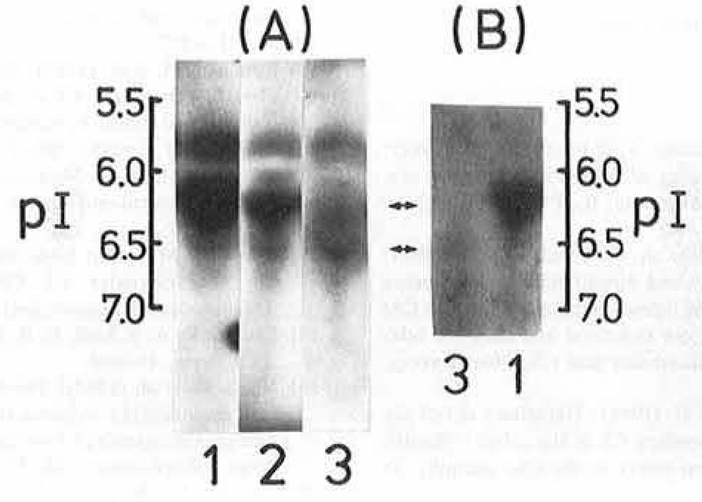

Plate 1. Isoelectric focusing (IEF) patterns of C3 in normal, heterozygous-C3-hypo and C3-hypo rabbits

(A) C3 protein was transferred from IEF agarose gel to a nitrocellulose sheet which was then treated with goat anti-rabbit C3 serum followed by ^{125}I-labeled rabbit anti-goat IgG.
1: normal, 4 μl of serum; 2: heterozygous-C3-hypo, 4 μl of serum; 3: C3-hypo, 4 μl of serum.

(B) C3 protein in the IEF agarose gel was demonstrated by immunofixation with 1.4% agarose gel containing 10% w/w goat anti-rabbit C3.
1: normal, 5 μl of serum; 3: C3-hypo, 10 μl of serum.

Source: Komatsu, M. (1987)[6].

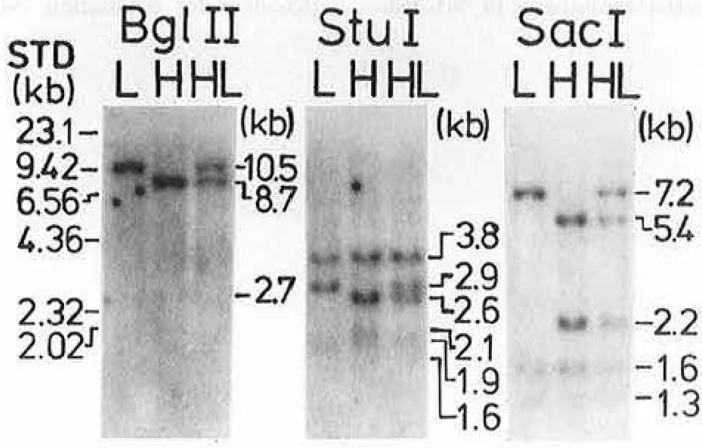

Plate 2. RFLPs detected after Bgl II, Stu I and Sac I digestion of genomic DNA in normal (H), heterozygous-C3-hypo (H/L) and C3-hypo (L) rabbits
The blot was hybridized with a 1.6 kb fragment of rabbit C3 cDNA.
Source: Komatsu, M. et al.[11].

complementemia will be undoubtedly clarified by C3 gene cloning in the near future.

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