Review

Studies on erythrocyte glycolipids

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(Contributed by Tamio YAMAKAWA, M. J. A.)

Abstract: The discovery and structural identification of glycolipids in erythrocyte membranes by the author's research group are reviewed. Differences among species and individuals were the characteristic features of glycolipids in erythrocytes. Individual differences were subjected to genetic analysis and led the discovery of genes involved in the genetic regulation of glycolipid expression in dogs and mice.

Key words: Hematoside; globoside; ABO blood group antigens; pedigree of dogs; glycolipids of mouse liver; origin of Japanese race; genetics of glycolipids.

Prior to 1950, when I started this series of studies, only four types of glycolipid isolated from brain had been mentioned in biochemistry textbooks. These were phrenosine, kerasine, nervone, and hydroxynervone, which are composed of sphingosine, galactose, and long-chain fatty acids, with or without a hydroxyl group at the α-position. Although Professor Klenk of the University of Cologne had already found ganglioside in the brain of a patient with Tay-Sachs disease (1936) and later in the gray matter of normal brain, and had isolated crystalline methoxyneuraminic acid from ganglioside for the first time (1942), only a few researchers were studying these substances at that time.

Discovery of hematoside and globoside. In the course of antiserum production, a large quantity of horse blood clots was discarded at the Institute for Infectious Diseases where I started biochemical research. I obtained the discarded clots and gently disrupted them to release erythrocytes. After hemolyzing the erythrocytes in an excess of 0.5% acetic acid in water, the solution was centrifuged and the precipitated erythrocyte membranes or ghosts were further washed with water. About 100 g of dried ghosts were obtained from 10 liters of packed erythrocytes. The dried ghosts were extracted three times with diethylether-methanol (1:1, v/v) at room temperature and were further extracted in a Soxhlet apparatus with chloroform-methanol (1:1, v/v). The resulting dark-gray extract was dissolved in chloroform and was then precipitated with acetone, which produced a fairly large amount of solid material. This material was a type of ganglioside, because it exhibited a positive reaction to neutral sugar and also exhibited a positive orcinol reaction to neuraminic acid. We named the glycolipid hematoside.[1)] After further purification, hematoside was demonstrated to contain one mole each of sphingosine, glucose, galactose, and *N*-glycolylneuraminic acid (Fig. 1). This was the first discovery of the presence of sialic acid at the surface of cell membranes. Hematoside is now also called GM3.

At the same time, Klenk found an entirely different glycolipid in human erythrocytes. The difference between Klenk's glycolipid and hematoside is the presence of *N*-acetylgalactosamine in place of neuraminic acid and an additional mole of galactose.[2)] We re-examined and confirmed these results, and named this glycolipid globoside to emphasize the difference between horse and human erythrocyte glycolipids (Fig. 2).[3)] The

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Abbreviations: Glc, glucose; Gal, galactose; GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; and Cer, ceramide.

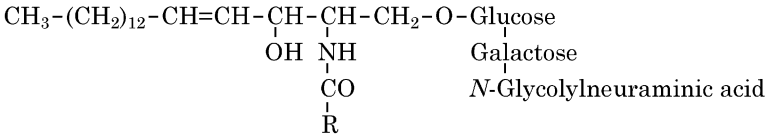

Fig. 1. Structure of hematoside, the major glycolipid isolated from horse erythrocytes. R, a fatty acid.

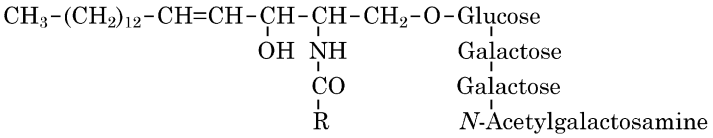

Fig. 2. Structure of globoside I, the major glycolipid isolated from human erythrocytes.

Table I. Nature of the hexosamine and sialic acids in the major glycolipids of erythrocytes from various animals

Animal	Glucosamine	Galactosamine	Sialic acids
Men	–	++	–
Hog	–	++	–
Guinea pig	–	++	–
Sheep	–	++	–
Goat	–	++	–
Rabbit	++	–	–
Cattle	++	–	++ or –
Horse	–	–	++
Dog	–	–	++
Cat	–	–	++

aforementioned difference in carbohydrate composition prompted us to study the glycolipids of other mammalian erythrocytes, because we thought that this could be a chemical basis for species-specific differences among mammalian erythrocytes. With respect to the major glycolipids, mammals have been classified into three groups (Table I). The first group is the globoside-type, which refers to glycolipids with hexosamine but no sialic acid, which are found in humans, hogs, guinea pigs, sheep, and goats. The second group is the hematoside-type, which refers to glycolipids that contain sialic acid but no hexosamine, which are found in dogs, cats, and horses. The third group is the ganglioside-type, which refers to glycolipids that contain both sialic acid and hexosamine, which are found in some individual cattle.[4] Characterization of Forssman antigen was an attractive research subject in the 1960s, and this molecule was purified from sheep erythrocytes, and subsequently classified as a globoside-type glycolipid, GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Ceramide.[5),6)]

The glycolipid content of human erythrocytes is relatively low, as compared to other lipids such as phospholipids and cholesterol (Fig. 3). Glycolipids comprise only 5% of total lipids in humans. Investigation of the minor glycolipids in a large amount of human erythrocytes led to the identification of more than twelve types of glycolipid (Table II).[7]

In 1953, we found ABO blood group activity in a crude globoside preparation.[8] This was the first discovery that glycolipids were involved in cell recognition, which is now an accepted biological activity of glycolipids. After using column chromatography to purify glycolipids, the active portions were separated from the major globoside molecule (globoside I) and were further divided into two fractions, namely globoside II and III.[9] Further studies indicated that these blood group-active glycolipids were ceramide-hexasaccharide and -octasaccharide, and the structures of these molecules were elucidated by Hakomori and coworkers.[10] The chemistry of blood group substances had already been

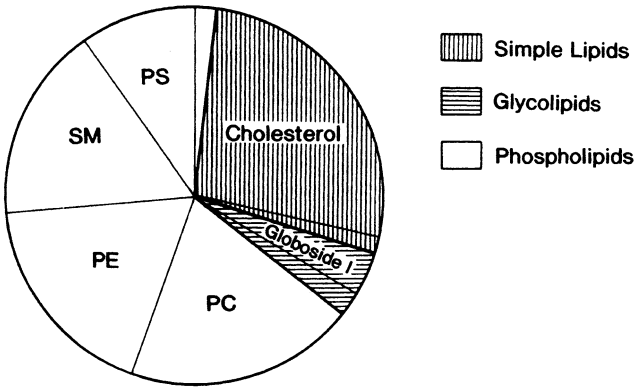

Fig. 3. Lipid composition of human erythrocyte membranes. PC, phosphatidylcholine; PE, phosphatidylethanolamine; SM, sphingomyelin; PS, phosphatidylserine.

Table II. Structures and amounts of various glycolipids in human erythrocytes

Structure	Name	Amount (μg/100 ml)
Glc-Cer	Ceramide monohexoside	279
Gal-Glc-Cer	Ceramide dihexoside	2,211
Gal-Gal-Glc-Cer	Ceramide trihexoside	1,021
GlcNAc-Gal-Glc-Cer	Amino-ceramide trihexoside	47
GalNAc-Gal-Gal-Glc-Cer	Globoside I	9,600
GalNAc-GalNAc-Gal-Gal-Glc-Cer	Para-Forssman antigen	7
Gal-GlcNAc-Gal-Glc-Cer	Paragloboside	553
Gal-Gal-GlcNAc-Gal-Glc-Cer	P_1 antigen	5-10
GalNAc-Gal(Fuc)-GlcNAc-Gal-Glc-Cer	A antigen	124
or Gal-Gal(Fuc)-GlcNAc-Gal-Glc-Cer	B antigen	120
NeuAc-Gal-Glc-Cer	Hematoside	355
NeuAc-Gal-GlcNAc-Gal-Glc-Cer	Sialosylparagloboside	426

elucidated by Morgan and coworkers in England, and by Kabat and associates in the U.S.A., using active material from body fluids or secretions. These blood group substances were polysaccharide-protein complexes, but we found that the antigenic material on the erythrocyte surface was glycolipid in nature with the same antigenic determinant groups. Nevertheless, some investigators still insisted that the blood group material on erythrocyte membranes was glycoprotein,[10),11)] whereas others believed that it must be a glycolipid with more than twenty carbohydrate chains (i.e., a macroglycolipid).[12),13)] However, I believe that the major activity is attributable to glycolipids with relatively short carbohydrate chains of 6 to 8.[15)] The number of different minor glycolipids will probably increase as further studies proceed, although the total amounts of these molecules are very small compared to the major glycolipid, globoside I. These glycolipids are believed to be located on the surface of the erythrocyte membrane, with the carbohydrate moiety projecting from the cells and this characteristic feature facilitates the importance of these glycolipids in cell-to-cell and cell-to-macromolecule recognition.

The precise chemical structures of glycolipids purified from various sources have been determined using chemical and biochemical techniques. For example, the structure of globoside I was elucidated by gas chromatography of partially methylated sugars after methanolysis of permethylated glycolipids, and by isolation of the oligosaccharides after hydrolysis with mild acid and specific hydrolases. Recently, direct mass spectrometry and high-resolution nuclear magnetic resonance spectroscopy have been useful in elucidating the structure of minute amounts of these compounds.[16),17)] Although the erythrocyte membranes of different animals have several glycolipids, the dominant gly-

Table III. Predominant glycolipids in the erythrocytes of various animals

Globoside type	
Man	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer
Hog	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer
Guinea pig	GalNAcβ1-4Galβ1-4Glcβ1-Cer
Sheep (Forssman antigen)	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer
Goat (Forssman antigen)	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer
Japanese serow	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-Cer
Rabbit	Galα1-3Galβ1-3 or 4GlcNAcβ1-3Galβ1-4Glcβ1-Cer
Cattle	Galα1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer
Ganglioside type	
Cattle	NeuGcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer
Mouse (C3H/He, DBA/2)	GalNAcβ1-4Gal(3-2αNeuGc)β1-4Glcβ1-Cer
Hematoside type	
Horse, Mouse (WHT/Ht)	NeuGcα2-3Galβ1-4Glcβ1-Cer
Horse	4-*O*-Ac-NeuGcα2-3Galβ1-4Glcβ1-Cer
Shika deer	NeuAc(and NeuGc)α2-3Galβ1-4Glcβ1-Cer
Dog(European), Jackal, Dingo(A-type)	NeuAcα2-3Galβ1-4Glcβ1-Cer
Dog(some oriental), (G-type)	NeuGcα2-3Galβ1-4Glcβ1-Cer
Racoon dog, Giant panda	NeuGcα2-3Galβ1-4Glcβ1-Cer
Cat, Lion, Hyena, Giant panda	NeuGcα2-8NeuGcα2-3Galβ1-4Glcβ1-Cer
Cat(some Persian cats)	NeuAcα2-8NeuAcα2-3Galβ1-4Glcβ1-Cer
Mouse(C3H/He)	NeuAcα2-3Galβ1-Cer

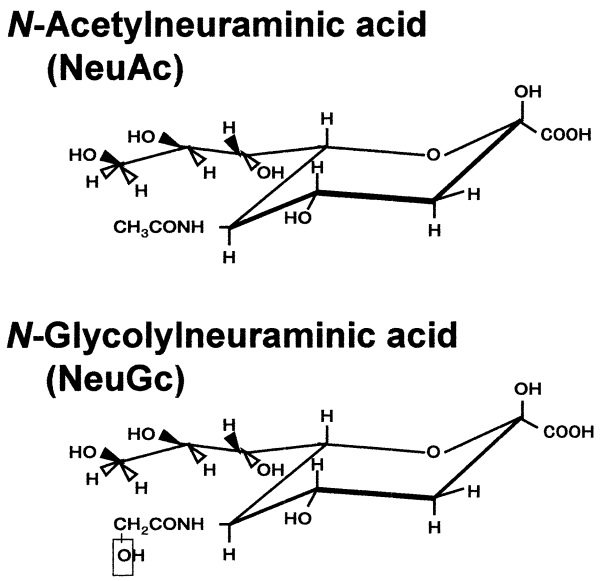

Fig. 4. The structural difference between *N*-acetylneuraminic acid (NeuAc) and *N*-glycolylneuraminic acid (NeuGc).

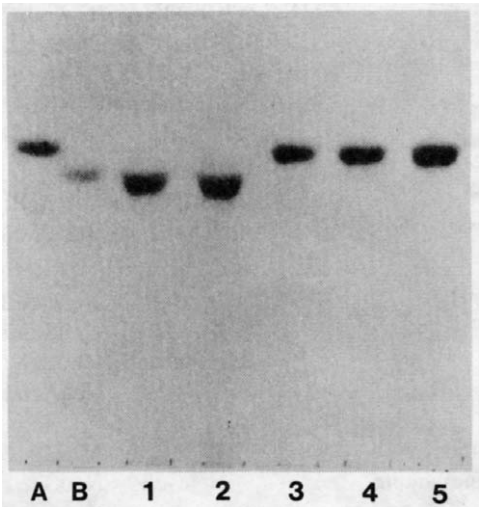

Fig. 5. Thin-layer chromatogram of erythrocyte glycolipids from individual dogs. A: hematoside with *N*-acetylneuraminic acid. B: hematoside with *N*-glycolylneuraminic acid. Numbers 1-5 correspond to erythrocyte glycolipids from 5 individual dogs.

colipids are species-specific. The chemical structures of these major glycolipids have been elucidated for different animal species (Table III), and different species of animal can be distinguished by the chemical structures of erythrocyte glycolipids; thus, glycolipids can be used to characterize erythrocytes and species.

Individual differences of erythrocyte glycolipids. One characteristic feature of erythrocyte glycolipids is that there are individual differences, like the ABO blood group antigens in human erythrocytes. In 1964, we reported that the erythrocyte glycolipid in dog is the hematoside-type glycolipid, sialyl-lactosyl ceramide, and that sialic acid comprises 73% of *N*-acetylneuraminic acid (NeuAc) and 27% of *N*-glycolyl-

Table IV. Types of hematosides in erythrocytes from European dogs

Breed	Phenotypes	
	NeuAc	NeuGc
Afghan Hound	6	0
American Cocker Spaniel	3	0
Beagle	371	0
Bulldog	3	0
Borzoi	4	0
Boxer	8	0
Cairn Terrier	2	0
Chihuahua	2	0
Collie	10	0
Dachshund	6	0
Dalmatian	5	0
Doberman Pinscher	8	0
Fox Terrier	3	0
French Poodle	1	0
Great Dane	4	0
Laika	1	0
Maltese	43	0
Mastiff	1	0
Miniature Poodle	1	0
Old English Sheepdog	2	0
Pointer	38	0
Pomeranian	11	0
Poodle	10	0
Setter	5	0
Shepherd	41	0
Shetland Sheepdog	9	0
Siberian Husky	5	0
St. Bernard	5	0
Yorkshire Terrier	22	0
Weimaraner	1	0
West Highland	1	0
Total	632	0

Table V. Types of hematosides in erythrocytes from oriental dogs

Breed	Phenotypes	
	NeuAc	NeuGc
Japanese dogs		
Hokkaido-dog	102	0
Akita-dog	160	35
Kai-dog	47	53
Kishu-dog	39	33
Shiba-dog		
(Shinshu)	103	51
(San'in)	23	8
(Mino)	42	28
Shikoku-dog		
(Okayama)	17	66
(Chyoshun)	0	51
Tosa-dog	8	1
Nihon Spitz	5	3
Subtotal	546	329
Chinese origin dogs(north)		
Chow Chow	5	0
Pug	2	0
Shih Tzu	6	0
Subtotal	13	0
Chinese origin dogs(south)		
Chin	5	5
Pekinese	0	3
Subtotal	5	8
Korean native dogs(Jindo)	50	151
Taiwanese native dogs	97	11
Eskimo dogs	18	1
Total	729	500

neuraminic acid (NeuGc) (Fig. 4).[18] By contrast, when Klenk and Heuer examined the structure of dog erythrocyte glycolipid in 1960, they found that the neuraminic acid was exclusively *N*-acetylated.[19] This apparent discrepancy puzzled us for a long time.

In 1978, a graduate student in my laboratory reexamined erythrocyte glycolipids from individual dogs and found that some dogs had the *N*-glycolyl type of hematoside (G type hematoside), while others had the *N*-acetyl type (A type); no mixed type existed (Fig. 5).[20] Whether a hematoside contains NeuAc or NeuGc can be determined readily by thin-layer chromatography, and the sialic acid species can be determined by gas chromatography with trimethylsilylation after mild acid hydrolysis. Because our previous study was carried out with a pool of blood from several dogs, the sialic acid might not have been structurally uniform. In addition, because the dogs that we examined in the aforementioned study were mongrels, we tried to determine which breeds of dog had the A or G type hematoside.

We found only the A type (not the G type) in the erythrocytes of all of the European dog breeds that we examined (Table IV).[20),21] On further investigation, we found the G type in some oriental dogs, namely the Shiba dog, Kai dog, Kishu dog, Chin, and Pekinese. Akita dogs and Hokkaido dogs had the A type, even though these dogs originated from Japan (Table V).[20),21] Shiba dogs originate from Japan and are rather small in size (~9 kg in weight). The population of Shiba dogs in Japan is decreasing, so the Society for Preservation of the Shiba dog was established to preserve this breed. By courtesy of this Society, we had an opportunity to examine the sialic acid species of hematoside in a family of Shiba dogs that had been guaranteed to be purebred. As indicated in the pedigree in Fig. 6, both the G and A types occurred in males and females in every generation. The female (★) indicated by an arrow in Fig. 6 had the A type, whereas both her parents (★) had the G type. The same

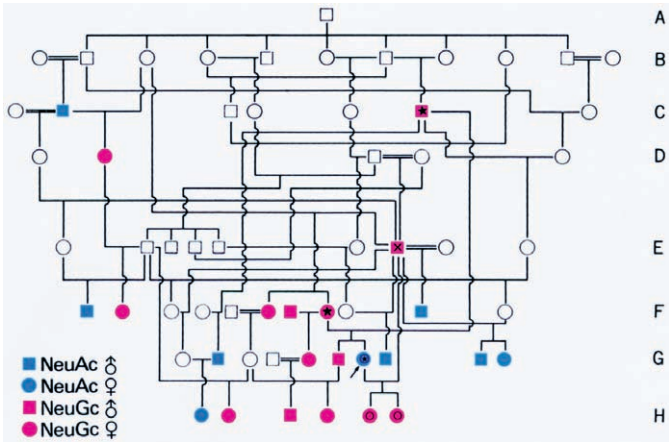

Fig. 6. Pedigree of pure-bred Shiba dogs. □, male not examined; ○, female not examined; =, consanguinity (when consanguinity is obvious, this symbol is omitted). Dogs in generation B had 7 different mothers that were all related to the same father (a dog in generation A; not indicated in the figure).

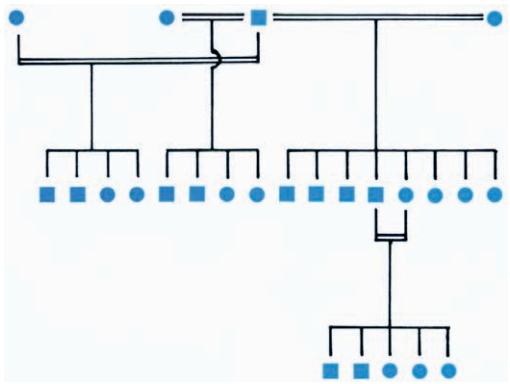

Fig. 7. Pedigree of the beagles. ■, male with NeuAc-hematoside; ●, female with NeuAc-hematoside; =, consanguinity.

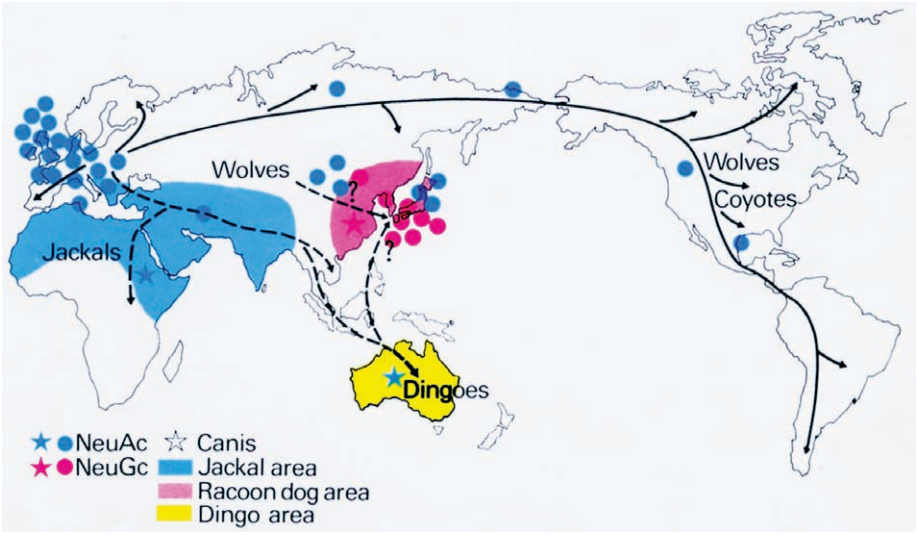

Fig. 8. Geographical distribution of dogs and related animals indicating the possible origins of Japanese breeds of dog.

female had two pups (○) with the G type by a dog (X) that had the G type. This indicates that the expression of the G type in erythrocytes is inherited as an autosomal dominant trait. Furthermore, in the case of the pedigree of a family of beagles (a European breed), the genotype is a recessive homozygote, and all dogs examined expressed only the A type hematoside (Fig. 7).

The difference between the A and G types of hematoside lies only in the existence of one additional oxygen atom per molecule in the latter (Fig. 4). As reported by Shaw and Schauer[22] and Bouhours and Bouhours,[23] the conversion of NeuAc to NeuGc is catalyzed by a monooxygenase that acts preferentially at the step of CMP-*N*-acetylneuraminic acid rather than at established glycoconjugates or free sialic acid. My colleagues determined that the expression of NeuGc is regulated by the expression of CMP-*N*-acetylneuraminic acid hydroxylase together with cytochrome b5 and cytochrome b5 reductase in the presence of nicotinamide adenine dinucleotide (NADH).[24)-27)] The molecular

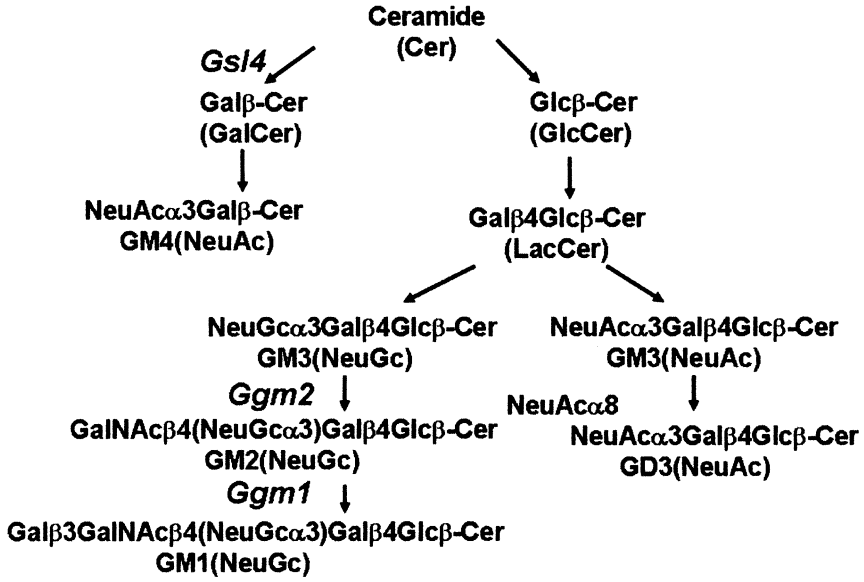

Fig. 9. Structures, biosynthetic pathways, and genes that regulate the expression of glycolipids.

mechanisms that are involved in the regulation of the expression of the G type hematoside are the subject of further studies, and might include a mutation of the gene that controls erythrocyte-specific expression of the hydroxylase.

The origin of Japanese breeds of dog. The origin of Japanese breeds of dog is controversial. Some claim that Japanese breeds arrived in Japan from Europe via a northern route through Siberia, whereas others are of the opinion that they migrated from South Asia via a southern route. We believe that Akita and Hokkaido dogs in Japan originated in Europe, because the erythrocytes of these breeds have the A type hematoside. Dogs that are indigenous to Japan and have the G type hematoside are thought to have come from another area, possibly from South Asia (Fig. 8).[20),21)]

In the course of this work, we examined blood from various animals obtained through the courtesy of many friends. We examined the erythrocytes of jackals and dingoes (of the genus *Canis*) and also examined raccoon dogs (a member of the dog family, *Canidae*). In addition, we had the opportunity to study the erythrocyte glycolipid of the giant panda. Raccoon dogs and giant pandas live in the Far East and the erythrocytes of these species were found to contain the G type hematoside. This is interesting in light of the fact that the presence of the G type is limited to the erythrocytes of some oriental dogs. Both dingoes and jackals have the A type hematoside. Dingoes are wild dogs that are indigenous to Australia; these animals have not been domesticated. Dingoes are thought to be the ancestor of modern domestic dogs (*Canis familiaris*). At first, we suspected that some Japanese breeds of dog might have originated from dingoes. If this was the case, the erythrocytes of dingoes would be expected to be the G type hematoside. However, erythrocytes from five dingoes were found to have the A type, as do most European dogs. Therefore, it is unlikely that dingoes are the ancestors of Japanese breeds, although this possibility cannot be excluded.

Historically, dogs are thought to have been accompanied by humans. Therefore, an investigation of the origin of Japanese breeds of dog requires a study of the origin of the Japanese race. From an archaeological aspect, there are two hypotheses concerning the origin of the Japanese. One holds that more than 2,300 years ago, an ancestral people migrated from the south of mainland China to the Kyushu district, where they developed an agricultural culture. The other hypothesis is that the ancestors arrived at the southern part of Japan via the Korean Peninsula. The distribution of the A and G type hematoside in dogs does not allow us to favor one of the aforementioned hypotheses over the other, but it does support the view that the development of the modern Japanese people has been influenced strongly by Chinese and Korean people (Fig. 8).

We also examined the erythrocytes of cats.[28)] Cat

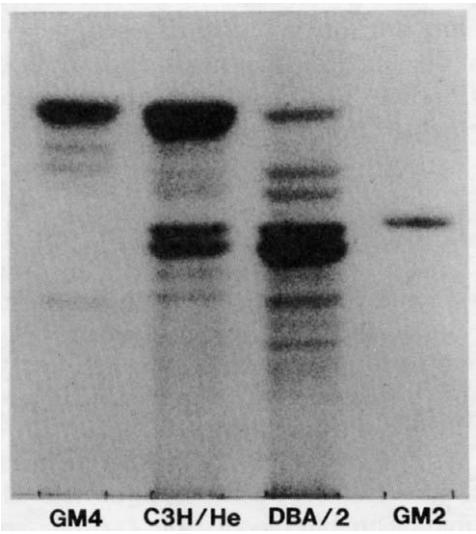

Fig. 10. Thin-layer chromatogram of gangliosides from erythrocytes of inbred mice. Sialic acid-containing glycolipids were visualized with resorcinol reagent.

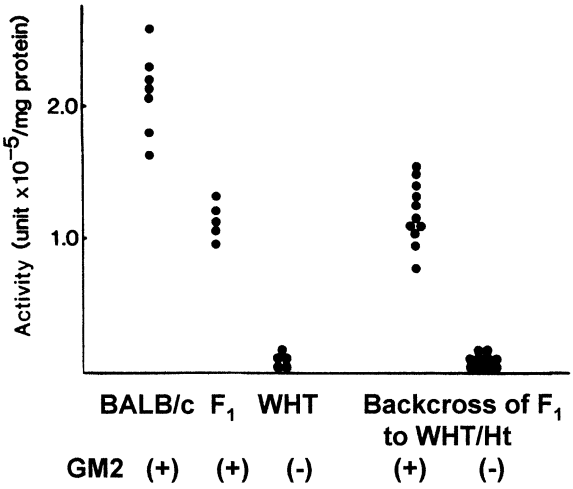

Fig. 11. The activity of UDP-GalNAc:GM3(NeuAc) *N*-acetylgalactosaminyltransferase in the liver of WHT/Ht mice, BALB/c mice, and their progeny. Each dot represents the transferase activity in the liver of individual mice. In the backcross of F_1 and WHT/Ht, GM2(+) represents a group of mice that expressed GM2(NeuGc), while GM2(-) indicates mice that lacked GM2(NeuGc).

erythrocyte glycolipid has two moles of NeuGc per molecule, which was characterized as GD3, NeuGcα2-8NeuGcα2-3Galβ1-4Glcβ1-Ceramide (Fig. 9). Of 41 samples of blood obtained from individual cats of various breeds, only two Persian cats were found to contain GD3 with two moles of NeuAc. GD3 with two moles of NeuGc occurred in the siblings of a family of Persian cats, but because relatively few of the family members were analyzed, no definitive conclusion about genetic regulation could be drawn.

The erythrocytes of lions (*Felidae*) and hyenas (a close relative of felids) were found to contain GD3 with two moles of NeuGc, which is similar to the majority of cats; GD3 with NeuAc, which occurred in some Persian cats, was not found in these species.

Polymorphism of mouse erythrocyte glycolipids. We found polymorphic expression of erythrocyte glycolipids among various strains of inbred mice.[29] As revealed by thin-layer chromatography (Fig. 10), C3H/He and BALB/c mice have mostly GM4 or sialyl galactosylceramide, as well as a small amount of GM2, GalNAcβ1-4(NeuGcα2-3)Galβ1-4Glcβ1-Ceramide (Fig. 9). Other strains, such as DBA/2, DDD, SS, NC, SII, C57BL/6, BDF_1 (C57BL/6 × DBA/2), FM, and SIII, have more GM2 than GM4. Strains NZB and 129/J have a nearly equal amount of GM4 and GM2. Only one strain (WHT/Ht) has only GM3 (NeuGcα2-3Galβ1-4Glcβ1-Ceramide). Structural characterization of GM4 and GM2 purified from erythrocytes of C3H/He and CDF_1 (C3H/He × DBA/2) mice indicated that the sialic acid of GM4 is exclusively NeuAc, whereas that of GM2 is NeuGc (Fig. 9).[30]

The ganglioside content of mouse erythrocytes is very low: lipid-bound sialic acid comprised only 1 µg/ml of packed erythrocytes. Therefore, mouse erythrocytes appeared to be an unsuitable model to study the control mechanisms that underlie genetic regulation of glycolipid expression, because erythrocyte gangliosides of individual mice must be analyzed. We happened subsequently to examine liver gangliosides of various inbred strains of mice. We found that the liver ganglioside composition resembled that of erythrocytes, but that the lipid-bound sialic acid content of the liver was almost 100 times that of erythrocytes. These results prompted us to analyze polymorphic variation in liver gangliosides.[31),32]

We carried out a genetic study of the expression of GM2 that contained NeuGc, GM2(NeuGc). By mating WHT/Ht mice (which lack GM2(NeuGc) but express GM3(NeuGc) in liver) with BALB/c mice (which express GM2(NeuGc) in liver), we were able to demonstrate that the expression of GM2(NeuGc) is a dominant phenotype over the expression of GM3(NeuGc). Backcrossed mice (F_1 × WHT/Ht) segregated into two phenotypes that were GM2(NeuGc)-positive and -nega-

tive at a ratio of the number of individuals (almost 1:1). These results revealed the presence of a single autosomal gene, which we named *Ggm2*.[31] *Ggm2* has been mapped to mouse chromosome 10 (unpublished data) and controls the expression of GM2(NeuGc) by regulating the activity of UDP-GalNAc:GM3 β-*N*-acetylgalactosaminyltransferase in liver[33] (Fig. 11). Subsequent to this discovery, we developed a method to analyze small amounts of erythrocyte glycolipids from individual mice[34] and then analyzed the relationship between the expression of GM2(NeuGc) in erythrocytes and liver. We found that 102 backcrossed mice were either GM2(NeuGc)-positive or -negative in both tissues, while none of the mice exhibited GM2(NeuGc) in only one of the two tissues (Fig. 12). This indicated that *Ggm2* controls the expression of GM2(NeuGc) in erythrocytes and liver (Fig. 12).[34] *Ggm2* seems to be involved in tissue-specific regulation of transcription, but the *Ggm2* gene has not been cloned and remains to be characterized in future.

We performed a genetic study of the expression of GM1 that contained NeuGc, GM1(NeuGc), in liver. The study was similar to that described above for GM2(NeuGc). By mating C57BL/10 mice (which lack GM1(NeuGc) and express GM2(NeuGc) in liver) and SWR/J mice (which express GM1(NeuGc) in liver), we identified a single autosomal gene that we called *Ggm1*.[35)-38)] *Ggm1* was demonstrated to link to the *H-2* complex by the analysis of *H-2* congenic mice[36] and was mapped 1 cM centromeric to *H-2K* using 758 backcrossed mice (Fig. 13).[35] We have not analyzed erythrocyte glycolipids from the 758 backcrossed mice, but the expression of GM2(NeuGc) and GM1(NeuGc) in the liver of the inbred strains of mice appears to be identical to that in erythrocytes,[39] which suggests that *Ggm1* also regulates GM1(NeuGc) expression in erythrocytes. As in the case of *Ggm2*, *Ggm1* is involved in the tissue-specific regulation of transcription, and the nature of *Ggm1* is unknown.

The expression of GM4(NeuAc) was analyzed by mating BALB/c mice (which express GM4(NeuAc) in erythrocytes) and WHT/Ht or C57BL/6 mice (which lack GM4(NeuAc) expression). The GM4(NeuAc) expression was a dominant phenotype, and 140 backcrossed mice segregated into 70 mice that expressed GM4(NeuAc) and 70 mice that did not, which indicated the existence of a single autosomal gene. We named this gene *Gsl4*. Further analysis of neutral erythrocyte glycolipids from 92 of 140 backcrossed mice indicated that the positive expression of galactosylceramide (Gal-Cer), which is the immediate biosynthetic precursor of GM4(NeuAc), paralleled the expression of GM4(NeuAc). This suggested that *Gsl4* directly regulates the expression of Gal-Cer rather than GM4(NeuAc) and that the transfer of NeuAc to Gal-Cer is not polymorphic (Fig. 12).[34] Unfortunately, further biochemical studies of the sialyltransferase that is responsible for GM4(NeuAc) biosynthesis and of the galactosyltransferase that regulates Gal-Cer biosynthesis were not

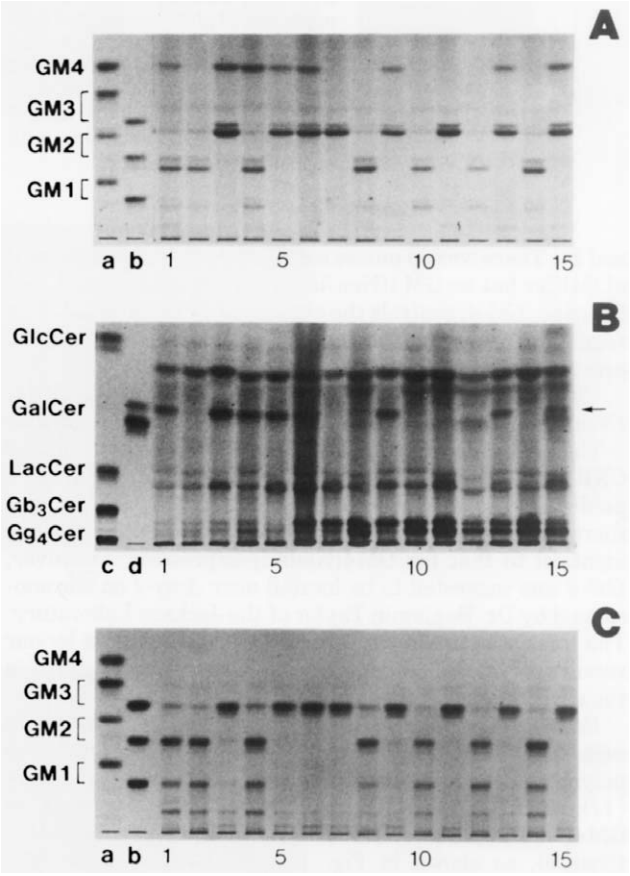

Fig. 12. *Ggm2* regulates the expression of GM2(NeuGc) in erythrocytes and liver. A: erythrocyte gangliosides, B: erythrocyte neutral glycolipids, and C: liver gangliosides were analyzed by thin-layer chromatography. Letters a-d indicate standard glycolipids and lanes 1-15 correspond to 15 individual backcrossed mice obtained by mating WHT/Ht and (BALB/c × WHT/Ht)F_1 mice. Comparison of A and C demonstrates that the expression of GM2(NeuGc) and GM3(NeuGc) is the same in erythrocytes and liver, indicating that *Ggm2* regulates the expression of GM2(NeuGc) in erythrocytes and liver. Comparison of A and B demonstrates that the expression of GM4 is the same as that of Gal-Cer in erythrocytes, indicating that *Gsl4* regulates the expression of GM4(NeuAc) as a consequence of direct control of Gal-Cer expression as the immediate biosynthetic precursor of GM4(NeuAc).

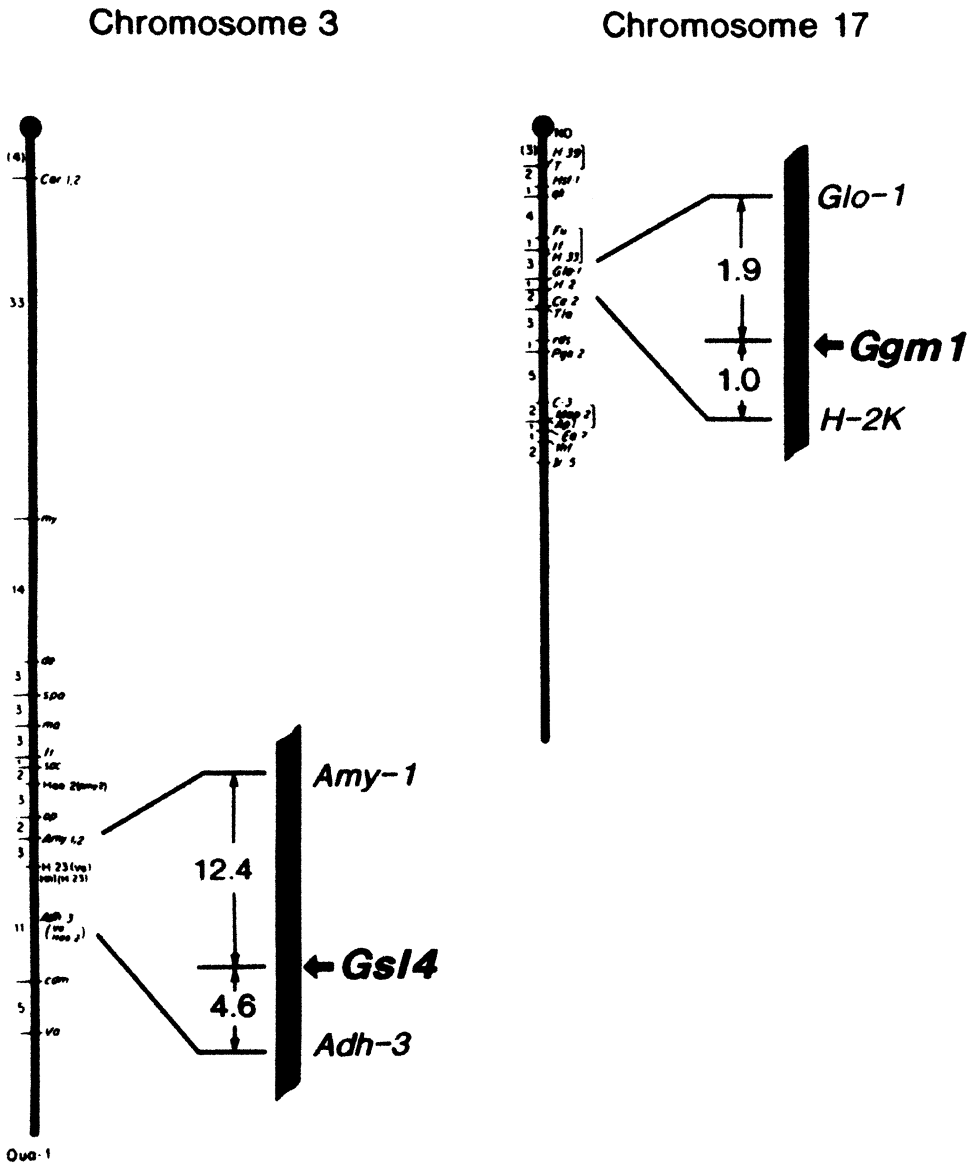

Fig. 13. Chromosomal maps for *Ggm1* and *Gsl4*.

possible, because erythrocytes do not contain the Golgi apparatus in which these enzymes are present. However, we were able to map the *Gsl4* gene 4.6 cM centromeric to *Adh-3* on mouse chromosome 3 (Fig. 13) (34; unpublished results), and it is now possible to characterize *Gsl4* using the genomic database.

Inbred mice are used widely as experimental animals, particularly in the fields of immunology and cancer research, and transgenic and gene-targeting experiments are indispensable. The polymorphic differences of glycolipids described above may contribute to the creation of unexpected phenotypes, which appeared when gene-targeted mice were mated with mice with different genetic background.

In conclusion, the structural differences among erythrocyte glycolipids are valuable markers for species characterization. Structural differences sometimes exist among different strains of a single species, and this is under genetic control.

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Profile

Tamio Yamakawa was born in 1921 and started his research career in 1945 with studies on the metabolic fate of branched-chain fatty acids in the animal body at the Institute for Infectious Diseases, after graduating from the Faculty of Medicine at the University of Tokyo. He performed pioneering work on the isolation and structural characterization of a ganglioside from the membrane of horse erythrocytes in 1950; this was the first demonstration of the occurrence of glycosphingolipids and sialic acid in the plasma membrane, and this study led to subsequent extensive studies that demonstrated species-specific carbohydrate structures of glycolipids in erythrocyte membranes. He was promoted to Professor at the University of Tokyo in 1959 and became Head of the Department of Chemistry at the Institute for Infectious Diseases. He moved to the Department of Biochemistry, Faculty of Medicine, at the University of Tokyo in 1966, where he educated many students in the field of biochemistry. He was awarded the Asahi Culture Award in 1975, the Japan Academy Prize in 1976, and the Sphinx Prize by the International Glycolipid Research Associates for his pioneering studies. Between 1982 and 1991, he was Director of the Tokyo Metropolitan Institute of Medical Science. He was elected a member of the Japan Academy in 1988 and is an honorary member of the American Society for Biochemistry and Molecular Biology, the Japanese Biochemical Society, and the Pharmaceutical Society of Japan. Between 1991 and 1994, he was President of the Tokyo College of Pharmacy.