

Serum Immunoglobulin Levels in Grey Collies (35314)

H. Y. REYNOLDS, D. C. DALE, S. M. WOLFF, AND J. S. JOHNSON

The Department of Health, Education and Welfare, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Clinical Investigation, Bethesda, Maryland 20014

The grey collie syndrome is characterized by an autosomal recessive inheritance, grey coat color, cyclic neutropenia, and frequent pyogenic infections (1). Amyloidosis (2) and malabsorption (3) have been observed. The severe neutropenia (less than 500 neutrophils/mm³) recurs at 12-day intervals and lasts for 3 to 4 days. Susceptibility to naturally acquired infections has coincided with the periods of neutropenia. Recently we reported observations on the hematologic features of this syndrome (4).

The present work was undertaken to determine if serum immunoglobulin levels showed cyclic changes which might contribute to the susceptibility in infections. Also, a preliminary finding in one grey collie (dog 1, Table I) that an IgG subclass, IgGd, was present in reduced amounts prompted us to screen these dogs for possible immunoglobulin abnormalities. We have found that these dogs have elevated immunoglobulin levels from very early in life, that they have no common immunoglobulin deficiency, that their immunoglobulin levels do not vary cyclically and that heterozygote carriers have normal immunoglobulin levels.

Materials and Methods. Blood specimens. Serum samples were obtained after centrifugation from clotted whole blood of grey collie dogs at 3-day intervals for one 12-day neutrophil cycle and at random other times. All sera were stored at -20° until used. The blood neutrophil counts corresponding to these serum samples varied from less than 100/mm³ to greater than 20,000/mm³. Serum specimens from 11 normal adult collies and a litter of 6 collie puppies with no history of grey collies in their lineages were used as age matched controls. Adult collies, normal in appearance but known to carry the

grey collie gene by having had grey collie offspring were also studied.

Assay method. Quantitative serum immunoglobulin levels were measured with a single radial diffusion immunoprecipitation technique, which has been previously described (5). Briefly, rabbit anticanine immunoglobulin antisera were purified with protein specific sepharose 2B immunoabsorbants. Antisera were then absorbed with IgG Fab fragments (6) to make them specific for canine immunoglobulin heavy chain determinants. One ml of antiserum was mixed with 7 ml of a 2.5% agarose medium and spread evenly on a 3 × 4-in. glass slide. Antigen wells, spaced 1 cm apart, contained about 1-μl volume of serum. A standard antigen curve was determined for each plate and several control sera were included in each. After the immunoprecipitation plates were photographed, precipitin ring diameters were measured and concentrations (mg/ml) were calculated from a calibration curve. All specimens were run in duplicate or triplicate and mean values were determined. Canine immunoglobulins, isolated from serum, were used as antigen standards (7).

Results. Serum samples were obtained at 3-day intervals from four grey collies (1, 2, 4, and 5 in Table I) during typical cycles. These sera were quantitated for immunoglobulin classes IgM, IgA, and IgG (subclasses a, b, and d). For each dog, all immunoglobulin levels remained relatively constant throughout the cycle with approximately 5 to 10% variation in the individual values.

Table I shows the immunoglobulin profiles of 9 grey collies. Dogs 1, 2, and 3 are the only dogs surviving greater than 6 months. When compared with normal adult collies

TABLE I. Serum Immunoglobulin Levels (mg/ml) in Grey Collie Dogs.

Grey collie	Age ^a (months)	Sex	Survived for (months)	IgM	IgA	IgG a,b	IgG d
1	9	F	10	6.0 ^b	2.8	6.0	0.4
2 A ^c	8	F	—	2.3	0.4	2.7	0.76
2 B ^c	17	—	Alive	3.7	0.9	3.5	1.6
3	6	M	Alive	3.0	0.3	4.8	0.3
4 ^d	2.5	F	3	0.7	0.15	1.0	0.23
5 ^d	2.5	M	3	1.0	0	1.2	0.3
6	2	F	3.5	1.4	0.8	2.7	0.3
7	2	M	5	2.5	0.2	1.9	0.3
8	2	F	4	1.3	0.46	4.4	0.45
9	2	M	3	1.3	0.22	1.2	0.3

^a Age at bleeding for immunoglobulin determinations.

^b All values are the mean of determinations done in triplicate.

^c Same dog bled at two intervals 9 months apart.

^d Litter mates.

(Table II) these three dogs have elevated IgM levels and somewhat low IgGd levels. The increase in IgA in dog 1 is striking.

The remaining grey collies in Table I (dogs 4-9) lived less than six months. In this group at age 2 to 2.5 months immunoglobulin levels were higher than age matched controls (Table III). The immunoglobulin pattern found for normal collie pups at 2 weeks and 2 months (Table II) is similar to that found in other litters of normal noncollie puppies (unpublished data).

Immunoglobulin levels for a group of normal appearing adult collies known to carry the grey collie gene were not different from normal adults (Table II).

Discussion. At present the cyclic neutropenia of grey collies is best related to regular variations in the production of neutrophils (4). The neutrophil disorder is only the most obvious cyclic phenomenon of these dogs since more extensive hematologic observations show that the number of monocytes, reticulocytes, and platelets also vary cyclical-

TABLE II. Serum Immunoglobulin Levels (mg/ml) in Normal and Grey Collie Dogs.

	No. and sex	IgM ^a	IgA ^a	IgG a,b ^a	IgG d ^a
Normal collie pups	6 (3F, 3M)				
Age: 2 weeks		0.73 (0.6-1.0)	0	0.56 (0.36-0.7)	0
2 months		0.99 (0.68-1.3)	0	0.79 (0.5-1.7)	0
Normal adult collies	11 (5F, 6M)				
Age: 2-5 years		1.99 (0.98-3.1)	0.60 (0.42-1.2)	4.61 (2.8-6.8)	2.90 (0.9-6.2)
Adult collies with grey gene ^b	12 (7F, 5M)				
Age: 2-8 years		1.80 (1.0-4.0)	0.45 (0.3-1.0)	3.50 (2.5-6.5)	2.20 (0.6-4.8)

^a Mean and concentration range.

^b Known to have produced grey collie pups.

TABLE III. Comparison of Mean Immunoglobulin Levels (mg/ml) in Grey Collie Pups and Normal Collie Pups at 2 Months.

	IgM	IgA	IgG a,b	IgG d
Normal collie pups	0.99	0	0.79	0
Grey collie pups ^a	1.37	0.31	2.07	0.32
<i>p</i> value ^b	>.10	<.025	<.05	<.001

^a Grey collies nos. 4-9, Table I.

^b *t* Test comparing respective groups of pups.

ly (8).

In these studies, sera from several grey collies were examined for variations in immunoglobulin levels during typical neutrophil cycles. No significant changes were demonstrable that could not be explained by variation in the immunoprecipitation assay or slight changes in hydration of the animal during the neutropenic phase of the cycle when fever and anorexia are common.

There are few data available relevant to the metabolism of canine immunoglobulins. Two published studies of canine IgG metabolism indicate a survival half time of 8.0 days (9) and a fractional catabolic rate of approximately 21% of the intravascular pool/day (10).

If these values are correct, then a 12-day cyclic disappearance of immunoblasts might result in an alteration of serum IgG concentration. There are no published data concerning the metabolism of canine IgA and IgM. However, since numerous studies in other animal species have demonstrated a shorter plasma survival half time of IgA and IgM than of IgG (11), one might assume that this is also the case in dogs. Therefore, it is even more likely that IgA and IgM serum concentrations would reflect a cyclic disappearance of immunoblasts. The data in this report provide no evidence for cyclic fluctuation of immunoglobulin levels.

When immunoglobulin levels from grey collies approximately 2 months of age were compared with 2-month-old collie pups, significant increases in all immunoglobulin classes were found (Table III). It is probable that the impetus of repeated infections results in the acquisition of serum immunoglobulins, particularly IgA class and IgGd subclass in an accelerated manner. Most dogs

begin to have measurable IgA and IgGd at 3 to 5 months of age (5). Certainly grey collies have no quantitative defect in their ability to synthesize immunoglobulins and would presumably have no abnormality in humoral antibody production. It is probable that the development of amyloidosis is related to their repeated infections.

Adult collies who carry the grey collie gene have immunoglobulin levels comparable to normal adult collies and other purebred dogs (5). The preliminary finding of a decreased IgGd level in a grey collie suggested the possibility that an immunoglobulin abnormality might be present in the heterozygous carriers. Such a marker of the heterozygous state would be very useful to collie breeders. However, in this group of carriers as in other groups of mongrel and pure bred dogs the concentration of IgGd was quite variable. Therefore no conclusion could be made from this small group of carrier dogs about a possible inherited immunoglobulin deficiency except that an absolute deficiency was not a marker of the carrier state.

Summary. Sera from a group of grey collies with cyclic bone marrow maturation arrest and sera from a group of adult collies who carry the autosomal recessive grey gene were quantitated for immunoglobulin levels. These levels were compared with a group of normal adult collies and a litter of age-matched normal collie pups. No cyclic variation in immunoglobulin levels were found in the grey collies. However, the appearance of significantly increased levels of serum immunoglobulins in the young grey collies, suggested that the impetus of repeated infections accelerated the development of serum immunoglobulins as a compensatory host defense mechanism. In the group of adult collie carriers of the grey gene no abnormal immunoglobulin pattern was found which might identify the heterozygote.

1. Lund, J. E., Padgett, G. A., and Ott, R. L., *Blood* **29**, 452 (1967).

2. Cheville, N. F., *Blood* **31**, 111 (1968).

3. Windhorst, D. B., Lund, J. E., Decker, J., and Swatez, I., *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **26**, 260 (1967).

4. Dale, D. C., Kimball, H. R., and Wolff, S. M., *Clin. Res.* **18**, 402 (1970).

5. Reynolds, H. Y., and Johnson, J. S., *J. Immunol.* **105**, 698 (1970).
6. Reynolds, H. Y., and Johnson, J. S., *J. Immunol.* **104**, 1000 (1970).
7. Reynolds, H. Y., and Johnson, J. S., *Fed. Proc., Fed. Amer. Soc. Exp. Biol.* **29**, 2227 (1970).
8. Dale, D. C., Ward, S. B., and Wolff, S. M., unpublished data.
9. Dixon, F. J., Talmage, D. W., Mauer, P. H., and Deichmiller, M., *J. Exp. Med.* **96**, 313 (1952).
10. Anderson, S. B., Glenert, J., and Wallevik, K., *J. Clin. Invest.* **42**, 1873 (1963).
11. Waldmann, T. A., and Strober, W., *Progr. Allergy* **13**, 1 (1969).

Received Sept. 25, 1970. P.S.E.B.M., 1971, Vol. 136.