Elevated mononuclear leukocyte phosphodiesterase in allergic dogs with and without airway hyperresponsiveness

C. A. Hirshman, M.D., D. B. Leu, D.V.M., D. R. Austin, M.S., S. C. Chan, M.D., L. Veith, D.V.M., and J. M. Hanifin, M.D. *Portland, Ore.*

To investigate if mononuclear leukocyte β-adrenergic hyporesponsiveness of Basenji greyhound (BG) dogs is associated with atopy or nonspecific airway hyperresponsiveness, we examined the relationship between mononuclear leukocyte cAMP phosphodiesterase levels, airway responsiveness to methacholine, and intradermal allergen responses in 17 BG dogs, five unrelated purebred Basenjis, and five greyhounds. BG dogs were hyperresponsive to aerosols of methacholine compared to Basenjis and greyhounds. Both BG dogs and Basenjis were allergic and had increased leukocyte cAMP phosphodiesterase activity compared to greyhounds. We concluded that the leukocyte abnormality is not associated with airway hyperresponsiveness. The leukocyte abnormality is either associated with the allergic state, with some hereditary trait that BG dogs acquired from the Basenji ancestry, or the leukocyte abnormality is necessary but not sufficient for the development of airway hyperresponsiveness. (J ALLERGY CLIN IMMUNOL 1987;79:46-53.)

The BG dog model of asthma manifests the two distinct disease processes of allergy and nonspecific airway reactivity.[1, 2] In 1968 Szentivanyi[3] proposed that these abnormalities resulted from decreased β-adrenergic responsiveness. Subsequent studies of patients with asthma or atopic dermatitis demonstrated subnormal intracellular cAMP levels after β-adrenergic stimulation of leukocytes.[4, 5] Later, the finding of similarly reduced cAMP responses after histamine and prostaglandin E_1 stimulation suggested a broader defect of cyclic nucleotide metabolism.[6-8] Those studies led to the realization that reduced cAMP levels were due to increased hydrolysis by elevated cAMP PDE activity in atopic leukocytes.[9]

We then questioned whether a similar abnormality was present in the BG dog model. Because direct investigation of cellular mechanisms in lung tissues from valuable dogs are impractical on a large scale, the peripheral blood leukocyte has been studied in this model with the considerations that β-adrenergic hyporesponsiveness may be a generalized phenomenon or that leukocytes and other bone marrow–derived cells may mediate the inflammatory lesions of asthma and allergy. We found that peripheral blood MNL from BG dogs failed to increase their cAMP levels when they were challenged with isoproterenol, whereas MNL from mongrels doubled their cAMP concentrations under similar conditions.[10] Moreover, the depressed cAMP responses in MNL from BG dogs are due to high PDE activity rather than to a defect in the β-adrenergic receptor adenylate cyclase system.[10]

It is unclear, however, whether this defect in leukocyte adrenergic responsiveness is related solely to

Abbreviations used

BG:	Basenji greyhound
MNL:	Mononuclear leukocytes
cAMP:	3,'5'-cyclic adenosine monophosphate
PDE:	Phosphodiesterase
R_L:	Pulmonary resistance
C_{dyn}:	Dynamic compliance
CMF-HBSS:	Calcium- and magnesium-free Hank's balanced salt solution
PBS:	Phosphate-buffered saline
GBSS:	Gey's balanced salt solution
K_m:	Michealis constant
V_{max}:	Maximum flow calculated at 50% of vital capacity

From the Departments of Anesthesiology and Dermatology, Oregon Health Sciences University, Portland, and the Animal Dermatology Clinic, Portland, Ore.

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Reprint requests: Carol A. Hirshman, M.D., Johns Hopkins Hospital, 600 N. Wolfe St., Baltimore, MD 21205.

atopy, to processes affecting airway hyperresponsiveness, or to the combination, since both aspects of asthma are found in the BG dog model. The possession of a group of Basenji dogs that are atopic like BG dogs, but do not have airway hyperresponsiveness, allows us the unique opportunity to understand the relationship between the three parameters. We therefore examined the relationship between airway responsiveness to methacholine, MNL cAMP PDE levels, and intradermal allergen responses in BG dogs and atopic and nonatopic control dogs.

METHODS

Study subjects

Studies were performed in 17 BG dogs, five purebred Basenjis, and five greyhounds. Dogs were of both sexes and ranged in age from 1 to 3 years. None of these animals had intestinal helminthiasis or microfilaria. The BG dogs and Basenjis displayed signs of atopic dermatitis characterized by pruritus and facial and digital involvement. The greyhounds displayed no signs of atopic dermatitis.

Inhalational challenge testing

For all inhalation challenge testing procedures, the dogs were not premedicated and were anesthetized standing, supported by a sling. After induction of anesthesia with intravenous thiamylal (12 to 15 mg/kg), the dogs were paralyzed with succinylcholine (0.5 mg/kg), intubated with a 7.5 to 9.0 mm cuffed endotracheal tube, and mechanically ventilated (Harvard Apparatus Co., Millis, Mass.) with 100% O_2 at a tidal volume of 20 ml/kg and a frequency of 15 min^{-1}. Additional increments of thiamylal (2 mg/kg) and succinylcholine (0.2 mg/kg) were administered as needed at approximately 20-minute intervals. An esophageal balloon (Dynasciences, Blue Bell, Pa.) was placed in the esophagus and positioned at the point where recorded end expiratory pressure was lowest. The balloon contained 0.8 to 1.5 ml of air. A separate catheter connected to suction was placed in the esophagus to keep it empty of air and liquid. Transpulmonary pressure was measured with a differential transducer (Hewlett-Packard 270, Waltham, Mass.) connected to the esophageal balloon and to a needle inserted into the endotracheal tube. Airflow was measured with a pneumotachograph head (Hewlett-Packard 2100) and a differential flow transducer (Hewlett-Packard 4730A). Pressure and flow signals were recorded with a Hewlett-Packard 47601 polygraph. C_{dyn} was calculated by dividing tidal volume by the difference in pressure between point of zero flow. R_L was calculated by the method of Von Neergaard and Wirz[11] by dividing transpulmonary pressure minus elastic pressure by airflow at midtidal volume. Apparatus resistance, determined by ventilating a mechanical lung analog with known parameters, was subtracted from the resulting value to give R_L. R_L and C_{dyn} were calculated from a mean of seven consecutive breaths.

Aerosols were delivered by a 3000 jet nebulizer (Hudson Co., Temelcula, Calif.) driven by compressed O_2 via an Ayre's T tube. The expiratory port was occluded until an inflation pressure of 15 cm of H_2O had been obtained for each breath. The nebulizer delivered aerosol particles with a mass median diameter of 5.7 μm. Challenge solutions were made up in distilled water. Methacholine bromide was administered as a series of challenges with increasing drug concentration (0.03 to 10.0 mg/ml) for five consecutive breaths at each concentration with a 10-minute rest period between doses. R_L and C_{dyn} were measured immediately before and after each dose.

A response was defined as an increase in R_L to 200% and as a decrease in C_{dyn} to 65% of the prechallenge value.[12] A response at a methacholine dose <0.10 mg/ml was considered 3+. A response at a dose >0.10 mg/ml but <0.75 mg/ml was considered 2+. A dose >0.75 mg/ml was considered a 1+ response.

Intradermal testing

All dogs were skin tested. During skin testing, the dogs were awake and sedated with xylazine (0.05 mg/kg, intravenously) and atropine (0.5 mg, intravenously). The test was performed on the right lateral wall after gentle clipping with a No. 40 blade. No glucorticoids, antihistamines, tranquilizers, sedatives, or other medications had been administered for at least 2 weeks before testing. The injection sites were marked with 12 mm diameter circles and 0.05 ml of each allergen (aqueous, nonglycerinated, at a concentration of 1:1000 w/v for most allergens and a concentration of 1:2000 w/v for irritant allergens) was injected intradermally with a 1 ml tuberculin syringe and a 26-gauge needle. Isotonic PBS solution was injected as the diluent control and histamine diphosphate (1:100,000) as the positive control. The results were interpreted at 5, 15, and 20 minutes after the injection. The reactions were graded subjectively and objectively with the negative control assigned a value of 0 (3 mm) and the positive control a value of 4 (12 mm). An objective positive reaction was considered to be at least 3 mm larger than the negative control reaction. A subjective positive reaction was considered to be equal to or greater than two times the negative control reaction with erythema and height of the wheal given positive considerations. A subjective grading of 2 or more was considered positive.

All dogs were tested to a battery of canine allergens common to this area of the country: late fall mixture, 7-grass mix, mold mixes 1, 2, and 3, western ragweed mix, house, mattress, rug, and upholstery, and dust mix (Greer Laboratory, Lenoir, N. C.). Five dogs (two BGs, two greyhounds, and one Basenji) were tested with allergens (red alder, Oregon ash, white birch, box elder, black cottonwood, western juniper, coast maple, Oregon oak, English walnut, yellow willow, Kentucky bluegrass, brome, meadow fescue, orchard grass, redtop, rye grass, timothy grass, velvet grass, vernal grass, cocklebur, yellow dock, lamb's-quarters, redroot pigweed, English plaintain, western ragweed, Russian thistle, sagebrush, sheep sorrel, *Alternaria*, *Aspergillus* mix, *Fusarium*, *Helminthosporium*, *Cladosporium (Hormodendrum)*, *Mucor*, *Penicillium* mix,

Rhizopus, cat dander, cotton linters, feathers, house dust mix, house dust mite, kapok, pyrethrum, tobacco, and sheep wool) (Hollister-Stier, Spokane, Wash).

The Prausnitz-Küstner test was used to confirm the presence of IgE in the atopic dogs used in this study.[13] In performing the passive transfer test, sterile technique was used in collecting serum samples. Two dogs were used to perform this test: (1) a recipient greyhound with no positive skin test reactions and no clinical signs of atopy, and (2) a donor BG with clinical signs of atopy and 11 positive skin test reactions. Intradermal skin tests were performed on both dogs as outlined above. Blood was withdrawn from the donor dog, and the serum was removed. One half of the serum was refrigerated, and one half of the serum was heat treated at 56° C for 4 hours to destroy the heat labile IgE. The shaved lateral thoracic wall of the greyhound donor was marked with 15 mm circles in a manner that would provide for the following controls: (1) an intradermal injection of serum without challenge to serve as a serum control, (2) an intradermal injection of diluent and histamine controls at a serum site and a site without serum, and (3) an intradermal injection of allergens in an area without serum.

Paired sites for allergen injections for heated and nonheated serum were injected with 0.1 ml of serum. Twenty-four hours later the test sites were injected with 0.1 ml of the four most positive test allergens. The test sites were observed for reactions at 5, 10, 15, and 20 minutes.

An atopic dog was defined as one demonstrating at least three of the four features of atopic dermatitis[14, 15] and immediate skin test reactivity to commonly occurring allergens.[16]

Isolation of canine MNL

Blood was drawn from the femoral vein into syringes containing preservative-free heparin (10 U/ml). MNL was isolated by centrifugation on Hypaque-Ficoll (Pharmacia Fine Chemicals, Piscataway, N. J.) at 450 × g for 40 minutes. The cells at the interface were collected and washed with GBSS. The cells were then counted in a Coulter counter (Coulter Electronics Inc., Hialeah, Fla.) and suspended in GBSS at appropriate concentrations. Differential cell counts were performed with Giemsa staining, and viability was determined by trypan blue exclusion.

Identification of lymphocyte subpopulations in cells of BG, Basenji, and greyhound dog

Mononuclear cells harvested from Hypaque-Ficoll gradients were washed twice with CMF-HBSS and resuspended at 10^7 cells per milliliter. One to 2 ml of the MNL suspension was layered on preformed Percoll gradients generated by spinning a mixture of 7 ml of Percoll to 6 ml CMF-HBSS at 21,000 × g for 40 minutes. The Percoll gradient was then spun at 100 × g for 20 minutes. Monocytes and lymphocytes were harvested from the first two layers of the gradient and washed with CMF-HBSS for the subsequent experiments. This method achieved populations of monocytes and lymphocytes of 80% and 95% purity, respectively.

Erythrocytes were prepared by previously described methods.[17] A 0.2 ml aliquot of MNL (3×10^6/ml) was combined with 0.2 ml of a suspension of human erythrocytes (6×10^7/ml) in PBS, pH 7.4, and incubated in duplicate tubes at 25° C for 30 or 60 minutes. The cell mixture was then centrifuged at 200 × g at 25° C for 5 minutes. The supernatant was decanted, and the cell pellet gently resuspended with 0.1 ml of a crystal violet solution (0.01%) in PBS.

A drop of the rosette suspension was added to a hemocytometer, and the percentage of lymphocytes binding three or more erythrocytes was determined microscopically. A total of 200 MNL from each dog were examined.

Immunoglobulin-bearing cells were quantitated with fluorescein-conjugated rabbit antiserum prepared against the 7S fraction of canine immunoglobulin (Miles Laboratories, Elkhart, Ind.).[17] An aliquot (0.2 ml) of canine leukocyte suspension (3×10^6/ml) was combined with 0.2 ml of a 1:4 or 1:8 dilution of fluorescein conjugate.

After incubation at 37° C for 30 minutes, cells were washed twice in iced PBS centrifuged at 4° C and resuspended in three drops of glycerol-PBS (1:1) mounting medium.[17] A total of 200 MNL from each dog were counted on a Zeiss fluorescene microscope (Carl Zeiss Inc., New York, N. Y.) equipped with a BG-3 primary filter and a 500 mm secondary filter and HBO 200 mercury arc lamp. Cells were examined under both incandescent and ultraviolet light, and the number of immunoglobulin-bearing cells was expressed as a percentage of total cells.

Isolation of canine MNL subpopulations

Mononuclear cells harvested from Hypaque-Ficoll gradients were washed twice in RPMI 1640 (Gibco, Grand Island, N. Y.). Cells were resuspended at 1.5×10^7 cells per milliliter in monocyte-adhering medium consisting of 10% (v/v) fetal calf serum (Gibco), 1% (v/v) glutamate (Gibco), 1% (v/v) antibiotic-antimycotic solution (Gibco), and 88% (v/v) RPMI 1640 placed in 100 by 15 mm Petri dishes (Falcon Labware, Oxnard, Calif.).[18] The cells were incubated 3 hours at 37° C with 5% CO_2 in a Shel Lab incubator (Sheldon Mfg. Co., Portland, Ore.).

After incubation, the lymphocyte-rich supernatant was decanted into a 50 ml conical centrifuge tube (Corning Glass, Corning, N. Y.), and the Petri dish was washed twice with 2 ml of RPMI 1640 at 37° C. Two milliliters of RPMI 1640 at 37° C was added to the Petri dish. The monocytes were gently dislodged with a rubber policeman and decanted into a 50 ml conical tube, and the Petri dish was washed twice with 2 ml of RPMI 1640 at 37° C.

The lymphocyte- and monocyte-rich fractions were washed twice in CMF-HBSS at 400 × g and 4° C for 10 minutes each and resuspended in GBSS at 10^7 cells per milliliter. Fraction purity was determined by cell counts of Geimsa-stained slides and viability by trypan blue exclusion.

PDE assay

Mixed MNL, monocyte-rich, and lymphocyte-rich subpopulations were washed and resuspended in GBSS at a

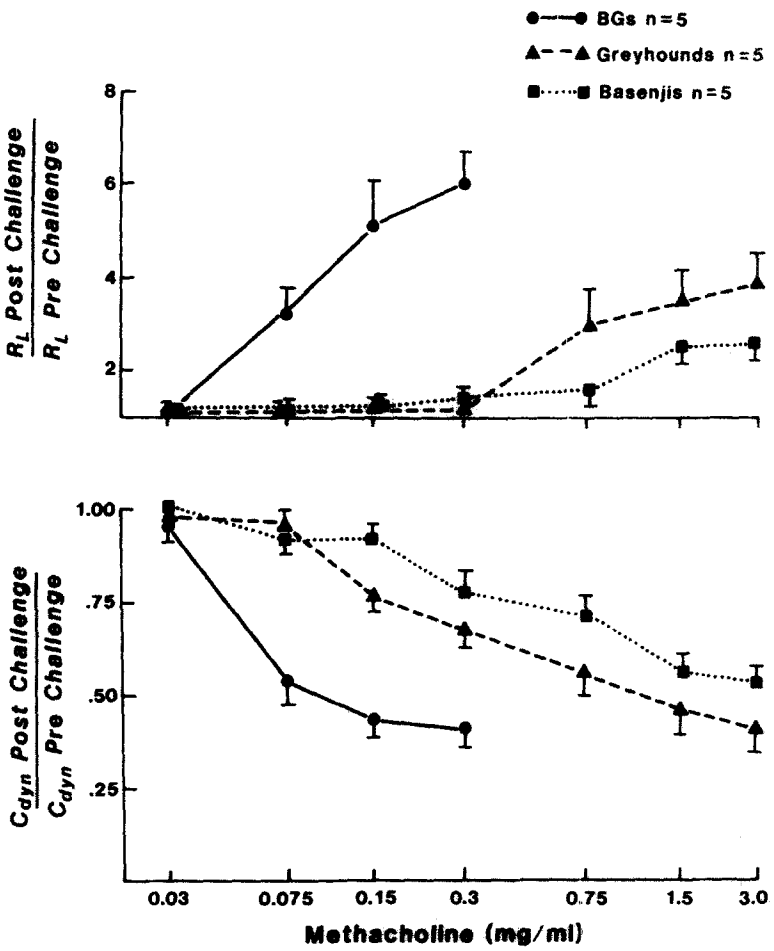

FIG. 1. Increase in R_L and decrease in C_{dyn} to increasing doses of methacholine in BG, Basenji, and greyhound dogs.

concentration of 10^7/ml. A 1 ml aliquot was homogenized with the use of a polytron PP 10-32 (Brinkman, Westbury, N. Y.). The cAMP PDE activity was assayed with the use of a modification of the procedure of Thompson and Appleman[19] in which ^{3}H-cAMP hydrolyzed by PDE to ^{3}H-5′-AMP is enzymatically converted by snake venom 5′-nucleotidase to ^{3}H-adenosine, which is quantitated in a liquid scintillation counter after absorption of unreacted ^{3}H-nucleotides by anion exchange resin (AG1 × 2, Bio-Rad Laboratories, Richmond, Calif.). The reaction mixture (0.4 ml) contained 1.36 μm of cAMP, 20,000 cpm of ^{3}H-cAMP, and 0.2 ml sample in 40 mmol/L Tris-chloride buffer (pH 8.0) with 3.74 mmol/L of β-mercaptoethanol and 5 mmol/L $MgCl_2$. For monocyte-rich or lymphocyte-rich subpopulations cAMP PDE enzyme kinetics, the reaction mixture contained from 0.05 μmol/L to 100 μmol/L of cAMP. After incubation (30° C for 10 minutes), the reaction was terminated by snap freezing in ethanol dry ice, and the mixture was boiled for 1 minute. Purified 5′-nucleotidase (0.45 units, Sigma Chemical Co., St. Louis, Mo.) was added to mixtures that were incubated at 30° C for 10 minutes and then transferred to Pasteur pipette columns containing AG1 by 2 resin (Bio Rad) and assayed for ^{3}H-cAMP as previously described.[6] Enzyme activity was expressed as picomoles of cAMP hydrolyzed per minute per 10^8 cells. Duplicate determinations varied by <5%. The cAMP PDE levels >0.05 pM per minute per 10^8 cells were considered elevated.[9]

Statistical analysis

One-way analysis of variance Scheffé's multiple comparison method, and multiple t tests with Bonferroni's correction[20] were used to test the difference between the three dog breeds. The level of statistical significance was $p < 0.05$.

RESULTS

BG dogs ranged in weight from 18 to 22 kg, whereas Basenjis and greyhounds ranged from 10 to 14 kg and 27 to 37 kg, respectively.

Baseline R_L averaged 2.0 ± 0.29 (mean ± SEM) cm of H_2O/L/sec, 3.4 ± 0.48 ($p < 0.05$) and 2.0 ± 0.30 in BGs, Basenjis, and greyhounds, respectively,

TABLE I. Summary of methacholine responsiveness, skin testing, and leukocyte cAMP PDE in the three dog populations

	No. of dogs	Reactivity to methacholine	Positive skin test	cAMP PDE
BG	17	3+ (16/17)	17/17	Markedly to moderately elevated
Basenji	5	1+ (5/5)	5/5	Moderately elevated
Greyhound	5	1+ (4/5)	0/5	Low

TABLE II. Passive cutaneous transfer of reactivity

	Brome	Orchard grass	Lamb's-quarter	House dust	Histamine	Diluent
Control	0	0	0	0	4+	0
Test						
Nonheated serum	2+	3+	2+	4+	4+	0
Heated serum	0	0	0	0	4+	0

TABLE III. Apparent K_m and V_{max} for the high and low affinity forms of cAMP PDE in lymphocyte- and monocyte-rich subpopulations of dog MNLs

	High affinity				Low affinity			
	Monocyte-rich		Lymphocyte-rich		Monocyte-rich		Lymphocyte-rich	
	K_m*	V_{max}†	K_m*	V_{max}†	K_m*	V_{max}†	K_m*	V_{max}†
BG	1.52	142.85	2.64	15.32	55.89	580.86	71.26	357.69
Basenji	2.92	41.67	2.37	14.13	68.57	811.91	67.76	138.72
Greyhound	1.76	5.12	2.30	3.57	72.74	170.43	58.23	103.81

*Expressed as micrometers of cAMP.
†Expressed as picomoles per minute per 10^8 cells.

consistent with differences in body size. C_{dyn} averaged 75 ± 7, 50 ± 1, and 119 ± 17 ($p < 0.05$) ml/cm of H_2O in BGs, Basenjis, and greyhounds, respectively. R_L was significantly higher in Basenjis than in BGs and greyhounds, whereas C_{dyn} was significantly different in all three groups.

BG dogs were significantly more responsive to methacholine than either Basenjis or greyhounds (Fig. 1), as measured by increase in R_L or decrease in C_{dyn}. In 16 of 17 BG dogs, a response to methacholine was elicited at or below 0.1 mg/ml of methacholine (a 3+ response). In the remaining BG dog, a response occurred at 0.15 mg/ml. In Basenjis a response did not occur until the methacholine dose exceeded 0.75 mg/ml in two and 1.5 mg/ml in the remaining three Basenjis (a 1+ response). In four of five greyhounds, a response did not occur until the methacholine dose exceeded 0.75 mg/ml (a 1+ response). In one greyhound a response was elicited at 0.15 mg/ml of methacholine (Table I).

The five BG dogs represented in Fig. 1 were of similar age to the greyhounds (2 years) and were slightly more responsive to methacholine than the total number of 17 greyhounds studied.

All 17 BGs and all five Basenjis had at least two positive immediate wheal skin test responses equal to or greater than the positive control, histamine. All 22 dogs had at least one negative skin wheal response to antigen, indicating that the skin responses in these dogs was not "a nonspecific response." There were no significant differences in numbers of positive tests in BG and Basenji dogs. No greyhound demonstrated immediate wheal skin test responses to these same antigens, but all greyhounds exhibited positive skin test reactions to histamine.

A BG dog with positive 3+ skin reactions to

brome, orchard grass, lamb's-quarter, and pigweed and a 4+ positive reaction to house dust donated blood for passive cutaneous transfer of reactivity. The greyhound used in these studies failed to react to these antigens. Twenty-four hours after injection of serum from the BG dog in each test site, positive reactions occurred in the nonheated test site and did not occur in the test site into which heated serum had been injected (Table II).

The proportion of lymphocytes in the Ficoll-Hypaque preparations ranged from 80% to 89% and was similar in all three dog groups. Trypan blue exclusion indicated that MNL viability was >94% in all cell preparations. No differences in E-rosette forming cells in MNL from the three groups were found. There were also no differences in immunoglobulin-bearing cells in MNL preparations from the three groups.

The distribution of cAMP PDE activity for BG, Basenji, and greyhound dogs mixed MNLs is illustrated in Fig. 2. The mean cAMP PDE activity was significantly higher in MNL from both BG and Basenji dogs compared to greyhounds ($p < 0.05$) with no significant differences in PDE between BG and Basenji dogs. Leukocyte cAMP PDE activity per 10^8 cells per minute averaged 21.1 ± 1.9 (mean ± SEM), 12.2 ± 2.3, and 2.7 ± 0.29 in BG, Basenjis, and greyhounds (N = 17, 5, and 5), respectively.

The lymphocyte-rich subpopulation in the supernatant after adherence contained 92% to 96% lymphocytes, whereas the monocyte-rich adhered cells were 97% to 98% monocytes. Trypan blue exclusion indicated that viability was >93% in all cell preparations.

No differences in the K_ms of both the high and low affinity cAMP PDEs were noted in the lymphocyte-rich subpopulations between the three dog groups (Table III). The K_ms of the high and low affinity cAMP PDEs in the monocyte-rich subpopulations were similar in BG, Basenji, and greyhound dogs.

In both subpopulations, the V_{max} for the high and low affinity forms of cAMP PDE were considerably lower in greyhounds than in Basenji or BG dogs (Table III).

No relationship was found between airway responsiveness to methacholine and the number of positive skin wheal responses or MNL cAMP PDE levels in the 27 dogs studied.

DISCUSSION

This study demonstrates that elevated MNL PDE more closely relates to allergy than to airway hyperresponsiveness to methacholine, since the allergic Basenji dogs, whose airways were not hyperresponsive to methacholine, as well as the BG dogs, had MNL PDE in the range producing subnormal β-adrenergically stimulated cAMP levels, whereas nonallergic nonhyperresponsive greyhounds did not.

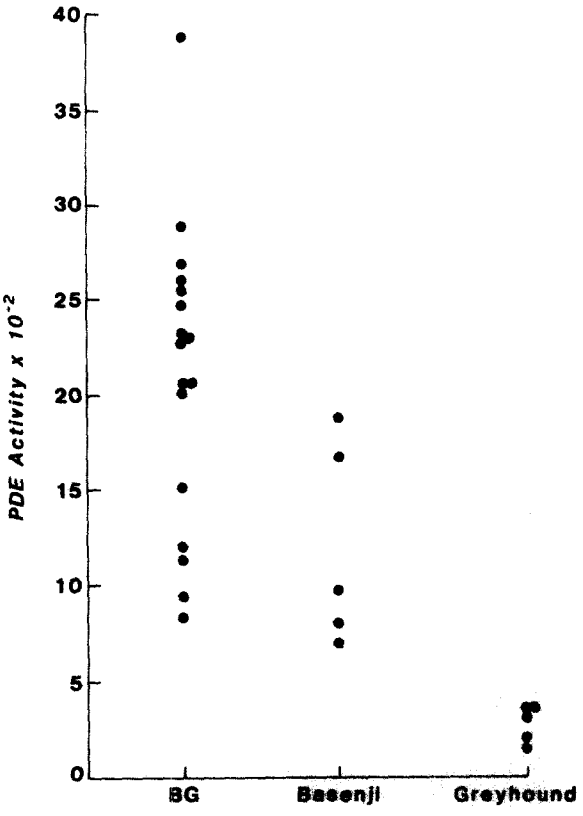

Fig. 2. Scattergram of cAMP PDE activity in 17 BG dogs, five Basenjis, and five greyhounds.

The atopic state can be defined as a genetically determined IgE-mediated hypersensitivity in which there is a predisposition to develop IgE antibodies to environmental allergens that are inhaled.[21] In dogs the primary target tissue is the skin, and signs related to seasonal or nonseasonal pruritic dermatitis are the most common manifestations. Atopic dermatitis in the dog presents clinically as a persistent, pruritic dermatitis, which may be papular, pustular, exudative, lichenified, excoriated, or alopecic and most commonly involves the head, neck, and flexor surfaces of the trunk and extremities (e.g., the antecubital fossae, popliteal fossae, interdigital spaces, external ear canals, and medial pinnae, periorbital, perioral, and peroneal integument, axillae, ventral abdomen, flexor surfaces of the carpal and tarsal joints, and the tail base).[16] Both BG[22] and Basenjis but not greyhounds had clinical signs of atopic dermatitis as well as positive intradermal skin test. Moreover, the skin test reactions were confirmed to be IgE mediated by pas-

sive transfer of serum containing IgE as demonstrated by the Prausnitz-Küstner test.

Airway resistance varies inversely with the fourth power of the radius when flow is laminar, and any decrease in the radius of a narrower airway causes a much greater increase in the airway resistance than does the same increase in the radius of a more dilated airway.[23] It is unlikely that the airway hyperresponsiveness of the BG dog could be due to a narrower airway in the control state, since the Basenji dogs had significantly higher resistance and lower compliance than did the BG dogs, and yet the Basenjis were far less responsive to methacholine than were the BGs.

It is unlikely that the difference in MNL PDE between the greyhounds and other dogs reflected morphologically differing cell populations, since the percentages of lymphocytes and monocytes were similar in all three groups. Moreover, we found no significant differences in T and B cell percentages and cell viability in all three dog groups.

Szentivanyi hypothesized[3] that bronchial hyperresponsiveness in allergic asthma is related to an inherited or acquired alteration in adrenergic responsiveness, and evidence to date suggests an association between cyclic nucleotide responses in leukocytes and allergic asthma in BG dogs[10] as well as in humans.[4, 24-26] However, in previous studies of patients with asthma, the allergic abnormality and the airway hyperresponsiveness were not separated out,[4, 25, 26] and it is conceivable that many patients with asthma were allergic.

Previous studies clearly demonstrate that in canine leukocytes, β-adrenergic hyporesponsiveness is associated with increased cAMP PDE activity.[10] The present study demonstrates that leukocytes from allergic Basenji dogs, which lack airway hyperresponsiveness, have cAMP PDE activity in the lower end of the same range as BG dogs. Calculations from previous studies in our laboratory demonstrated that the level of enzyme activity found in the MNL from BG and Basenjis but not from the MNL from greyhounds was sufficient to degrade the cAMP formed during a 15-minute period of isoproterenol stimulation.[27, 28]

Human monocytes and lymphocytes have three distinct kinetic forms of PDE demonstrated by differences in K_m and V_{max} characteristics.[9] In the three dog groups we find two forms of PDE that correspond to the medium and high K_m forms observed in human monocytes and lymphocytes. The similar K_ms in BGs, Basenjis, and greyhounds demonstrate that we are dealing with the same enzyme type in the three dog populations. The differences in V_{max} reflect the differences in amounts of enzyme present. The data are consistent with previously published data in BG and mongrel dogs.[10]

Even though the V_{max} of the low affinity form of cAMP PDE is much greater than that of the high affinity cAMP PDE, it is probably not as important for the degradation of cAMP in MNLs as the high affinity form of the enzyme. The affinity for cAMP of the high affinity form of cAMP PDE is approximately 35 times higher than that of the low affinity cAMP PDE.

There are at least four possibilities for these results. The leukocyte abnormality could be associated (1) with the allergic state, (2) with some hereditary trait that BG dogs acquired from the Basenji line, (3) with both atopy and airway hyperresponsiveness each having an additive effect, or (4) the leukocyte abnormality is necessary but not sufficient for the development of airway hyperresponsiveness. It is unlikely that atopy and nonspecific airway hyperresponsiveness have additive effects on MNL cAMP PDE, since there were no differences in airway responses to methacholine in BG dogs with markedly and moderately elevated levels of cAMP PDE. To rule out possibility 4 requires the presence of a dog population that is nonatopic but that have airway hyperresponsiveness. In our screening of >60 non-BG dogs, we have been unable to find dogs with these traits.

The first possibility is consistent with studies in humans. Kaliner et al.[29] and Shelhamer et al.[30] have demonstrated that patients with allergic rhinitis as well as patients with asthma demonstrated β-adrenergic hyporesponsiveness. Grewe et al.[9] reported elevated PDE in patients with atopic dermatitis or with allergic respiratory disease. In these studies,[9, 29, 30] as in our dogs, none of the subjects were receiving medications known to induce β-adrenergic hyporesponsiveness at the time of testing.

In summary, the present study clearly demonstrates that MNL PDE activity in a range sufficient to cause β-adrenergic hyporesponsiveness is not necessarily associated with airway hyperresponsiveness. Rather this leukocyte abnormality is either associated with the allergic state, with some hereditary trait that BG dogs acquired from their Basenji ancestry, or the leukocyte abnormality is necessary but not sufficient for the development of airway hyperresponsiveness.

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REFERENCES

1. Hirshman CA, Downes H, Veith L. Airway responses in offspring of dogs with and without airway hyperreactivity. J Appl Physiol 1984;56:1272.
2. Hirshman CA, Downes H. Antigen sensitization and metha-

choline responses in dogs with hyperreactive airways. J Appl Physiol 1985;58:485.
3. Szentivanyi A. The beta adrenergic theory of the atopic abnormality in bronchial asthma. J ALLERGY 1968;42:203.
4. Parker CW, Smith JW. Alterations in cyclic adenosine monophosphate metabolism in human bronchial asthma. I. Leukocyte responsiveness to β-adrenergic agents. J Clin Invest 1973;52:48-59.
5. Reed CE, Busse WW, Lee TP. Adrenergic mechanisms and the adenylate cyclase system in atopic dermatitis. J Invest Dermatol 1976;67:333.
6. Safko MJ, Chan SC, Cooper KD, Hanifin JM. Heterologous desensitization of leukocytes: a possible mechanism of beta adrenergic blockade in atopic dermatitis. J ALLERGY CLIN IMMUNOL 1981;68:218.
7. Parker CW, Kennedy S, Eisen AZ. Leukocyte and lymphocyte cyclic AMP responses in atopic eczema. J Invest Dermatol 1977;68:302.
8. Busse WW, Lantis SOH. Decreased H_2 histamine granulocyte response in active atopic eczema. J Invest Dermatol 1979; 73:184.
9. Grewe SR, Chan SC, Hanifin JM. Elevated leukocyte cyclic AMP phosphodiesterase in atopic disease: a possible mechanism for cyclic AMP-agonist hyporesponsiveness. J ALLERGY CLIN IMMUNOL 1982;70:452.
10. Chan SC, Hanifin JM, Holden CA, Thompson WJ, Hirshman CA. Elevated leukocyte phosphodiesterase as a basis for depressed cyclic adenosine monophosphate responses in the Basenji greyhound dog model of asthma. J ALLERGY CLIN IMMUNOL 1985;76:148.
11. Von Neergaard K, Wirz K. Die messung der stromungswiderstande in des atemwegen der menschen, insbesondere bei asthma und emphysem. Z Klin Med 1927;105:51.
12. Snapper JR, Drazen JM, Loring SH, Schneider W, Ingram RH. Distribution of pulmonary responsiveness to aerosol histamine in dogs. J Appl Physiol 1978;44:738.
13. Halliwell REW. The localization of IgE in canine skin: an immunofluorescent study. J Immunol 1973;110:422.
14. Hanifin JM, Lobitz WC. Newer concepts of atopic dermatitis. Arch Dermatol 1977;113:663.
15. Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) 1980;92:44.
16. Willemse A. Investigations on canine atopic dermatitis. Utrecht: Drukkerij Elinkwijk B.V. 1984.
17. Bowles CA, White GS, Lucas D. Rosette formation by canine peripheral blood lymphocytes. J Immunol 1975;114:399.
18. Nicholas A, Paulowski, Kaplan G, Hamill AL, Cohn ZA, Scott WA. Arachidonic acid metabolism by human monocytes: studies with platelet-depleted cultures. J Exp Med 1983;158:393.
19. Thompson WJ, Appleman MM. Multiple cyclic nucleotide phosphodiesterase activities in rat brain. Biochemistry 1971; 10:311.
20. Glantz BA. Primer of biostatistics. New York: McGraw Hill, 1981:63-90.
21. Spector SL, Farr RS. Atopy reconsidered. Clin Allergy 1976; 6:83.
22. Butler JM, Peters JE, Hirshman CA, White CR, Margolin LB, Hanifin JM. Pruritic dermatitis in asthmatic basenji greyhound dogs: a model for human atopic dermatitis. J Am Acad Dermatol 1983;8:33.
23. Rubinfeld AR, Pain MCF. Relationship between bronchial reactivity, airway caliber, and severity of asthma. Am Rev Respir Dis 1977;115:381.
24. Parker CW. Adrenergic responsiveness in asthma. In: Lichtenstein LM, Austen KF, eds: Asthma: physiology, immunology, and treatment. New York: Academic Press, 1973:185-206.
25. Tashkin DP, Conolly ME, Deutsch RI, Hui KK, Littner M, Scarpace P, Abrass I. Subsensitization of beta-adrenoceptors in airways and lymphocytes of healthy and asthmatic subjects. Am Rev Respir Dis 1982;125:185.
26. Neijens HJ, Raatgeep RE, Degenhart HJ, Duiverman EJ, Kerrebijn KF. Altered leukocyte response in relation to the basic abnormality in children with asthma and bronchial hyperresponsiveness. Am Rev Respir Dis 1984;130:744.
27. Butler JM, Chan SC, Stevens SR, Hanifin JM. Increased leukocyte histamine release with elevated cyclic AMP–phosphodiesterase activity in atopic dermatitis. J ALLERGY CLIN IMMUNOL 1983;71:490.
28. Chan SC, Grewe SR, Stevens SR, Hanifin JM. Functional desensitization due to stimulation of cyclic AMP-phosphodiesterase in human mononuclear leukocytes. J Cyclic Nucleotide Res 1982;8:211.
29. Kaliner M, Shelhamer JH, Davis PB, Smith LJ, Venter JC. Autonomic nervous system abnormalities and allergy. Ann Intern Med 1982;96:349.
30. Shelhamer JH, Marom Z, Kaliner M. Abnormal beta-adrenergic responsiveness in allergic subjects. II. The role of selective beta$_2$ adrenergic hyporeactivity. J ALLERGY CLIN IMMUNOL 1983;71:57.