
Morphologic characterization of specific granules in Greyhound eosinophils

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Background: "Vacuolated" eosinophils (ie, eosinophils with empty, nonstaining granules) have been described previously in normal Greyhounds. However, to our knowledge, detailed studies of granules in vacuolated and normal eosinophils in this breed have not been performed. **Objective:** The objective of this prospective study was to characterize some of the morphologic, ultrastructural, and cytochemical staining features of eosinophils (primary) granules in both normal and vacuolated eosinophils in Greyhound blood. **Methods:** Morphologic features of eosinophils in Wright's- and Diff-Quik-stained peripheral blood smears from 49 Greyhounds were compared with 200 blood smears from non-Greyhound dogs. Transmission electron microscopy was done on blood from 3 Greyhounds with vacuolated eosinophils and 3 with normal eosinophil granules. Blood smears from 4 of these dogs also were stained cytochemically with alkaline phosphatase (AP), chloracetate esterase (CAE), and alpha naphthyl butyrate esterase (ANBE). The morphologic features and tinctorial properties of vacuolated and normal eosinophils were compared. **Results:** Twenty-six Greyhounds (53%) had vacuolated eosinophils and 23 (47%) had normal granulated eosinophils in smears stained with Wright's stain. Only 1% of eosinophils were vacuolated in non-Greyhound dogs. Twenty of the 23 (85%) Greyhounds with normal granulated eosinophils on Wright's-stained smears had vacuolated eosinophils in smears stained with Diff-Quik. Ultrastructurally, no morphologic differences were observed between granules of vacuolated and normal eosinophils. Both vacuolated and normal eosinophils in Greyhounds were positive for AP and negative for CAE and ANBE, as expected for normal dogs. **Conclusion:** Vacuolated eosinophils in Greyhounds likely reflect, at least in part, differential staining properties of the specific granules with different hematologic stains. Ultrastructural and cytochemical features of eosinophil granules were similar in normal and vacuolated eosinophils from Greyhounds. (*Vet Clin Pathol.* 2005;34:140-143)

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Abnormal eosinophil granules have been described in Greyhounds since the 1960s. Jones and Paris¹ reported that some Greyhounds had "vacuolated" eosinophils, which they referred to as "grey" eosinophils. Furthermore, they reported that eosinophils in Greyhound puppies tended to have normal granules but that eosinophils in adults were typically vacuolated. Recognizing this abnormal granulation is important because vacuolated eosinophils resemble toxic neutrophils or vacuolated monocytes and can make accurate identification difficult for veterinarians and technicians who are not familiar with the hematologic idiosyncrasies of the breed. Moreover, because total WBC and neutrophil counts in Greyhounds are typically below the reference interval for the species,^{2,3} the presence of vacuolated polymorphonuclear cells in a dog with a low WBC or neutrophil count may falsely suggest an infectious process to the clinician.

We initially recognized vacuolated eosinophils in a 3-year-old female spayed Greyhound that presented for evaluation as a potential blood donor. Despite being healthy and having normal total and differential WBC counts (based on reference intervals for the breed), laboratory personnel reported toxic neutrophils. Further evaluation of the smear revealed the cells in question were likely vacuolated eosinophils. The purpose of this prospective study was to character-

ize some of the morphologic, ultrastructural, and cytochemical staining features of eosinophil granules in Greyhounds.

Materials and Methods

Blood smear evaluation

Blood smears from 49 adult Greyhounds (42 blood donors and 7 patients evaluated between December 2001 and December 2003) were compared with blood smears from 200 dogs of 47 different breeds. All dogs were handled according to approved Institutional Animal Use guidelines. Three milliliters of whole blood were collected from the jugular vein in EDTA-containing Vacutainer tubes (Monoject, Sherwood, St Louis, MO, USA). For each dog, 2 blood smears were made directly from the syringe and 2 were made from the tube within 20 minutes of sample collection. One slide from each group (1 from the syringe and 1 from the tube) was stained with Diff-Quik (Protocol, Fisher Diagnostics, Middletown, VA, USA), and the other slide was stained with Wright's stain (Wright's Blood Stain 0.2%, The Ohio State University, Lab Stores Reagent, Columbus, OH, USA) in a standard automated stainer.

Blood samples from 3 Greyhounds with vacuolated eosinophils were collected in EDTA-containing tubes, and 3

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smears were made from each dog. One slide from each dog was stained with Wright's stain, and the other 2 were stained with Diff-Quik. One slide from each dog was fixed for 5 minutes in standard fixative (solution 1), and the third slide was fixed for 10 minutes before staining with Diff-Quik. All the slides were then stained for 10 seconds in solution 2 and for 20 seconds in solution 3. Blood smears from 6 Greyhounds were evaluated every 6–8 weeks over a 2-year period. All blood smears were examined by the same person (MCI), with 200–250 leukocytes counted and eosinophils classified as either vacuolated or granular.

Blood samples from non-Greyhound dogs were selected at random from the Hematology Laboratory of the Veterinary Teaching Hospital (VTH) between February and March 2003. The dogs were presented for routine health evaluation or elective surgical procedures, such as spays and neuters, and had normal CBC results. Only EDTA-preserved samples were available from these dogs.

All blood donor Greyhounds were serologically negative for *Babesia canis*, *B gibsoni*, *Ehrlichia canis*, *Bartonella vinsoni*, *Rickettsia rickettsi*, and *Dirofilaria immitis*; the results of CBCs and serum biochemical profiles were within the reference interval for the breed.² The blood donor Greyhounds were current on all vaccines and were receiving flea, tick, and heartworm-preventive medication. The 7 Greyhound patients were presented to the VTH for wellness exams, minor surgery, or dental prophylaxis.

Transmission electron microscopy

Blood samples were collected in EDTA-containing tubes as described above. Three samples from Greyhounds with granular eosinophils and 3 from Greyhounds with vacuolated eosinophils were randomly selected and routinely processed for transmission electron microscopy (TEM). Briefly, 2 macrohematocrit tubes (Becton Dickinson Vacutainer Systems, NJ, USA) per dog were filled to capacity and centrifuged at 3000g for 10 minutes. The buffy coat was collected and fixed in 3% glutaraldehyde (8 hours) and osmium tetroxide (1 hour). After dehydration, the samples were embedded in Eponate (Ted Pella Inc and PELCO International, Redding, CA, USA). After 8 hours in an oven at 60°C, the samples were cut with an LKB Ultratome (LKB instruments Inc, Rockville, MD, USA) and stained with 1% uranyl acetate and 1% lead citrate.

The samples were evaluated in a Philips 300 (Eindhoven, Holland) with accelerating voltage of 60 kV at ×11,000 magnification. The TEM images were obtained on Kodak (Eastman Kodak Co, Rochester, NY, USA) electron image film. Eosinophils were identified based on the size, shape, and homogeneity of the granules.⁴ The densities of normal and abnormal granules were visually compared.

Cytochemical stains

Blood samples that were randomly selected from 4 of the 6 Greyhounds used for TEM, 2 with normal eosinophil granules and 2 with vacuolated eosinophils, were collected from the jugular vein and centrifuged as described above. Buffy coat

smears were made. Cytochemical stains for alkaline phosphatase (AP), chloracetate esterase (CAE), and alpha naphthyl butyrate esterase (ANBE) (Sigma Diagnostics, St Louis, MO, USA) were performed as previously described.⁵ Even though we expected the AP enzymatic activity to be found in the cytoplasm (ie, between the granules), we wanted to know if there were any differences between vacuolated and granular eosinophils. A non-Greyhound dog with 600 granular eosinophils per microliter was used as a positive control for cytoplasmic AP. Blood smears from the same dog were used as positive controls for CAE (neutrophils) and ANBE (monocytes) to verify the staining techniques were working properly.

Results

Fifty-three percent (26 of 49) of Greyhounds had vacuolated eosinophils and 47% (23 of 49) had typical pink-orange granules in blood films stained with Wright's stain (both in samples collected with and without EDTA) (Figure 1). In Greyhounds with vacuolated eosinophils, 100% of eosinophils observed had the same granule morphology. Occasionally, a vacuolated granule would have a very small pink-orange inclusion in the center. Six Greyhounds with vacuolated eosinophils consistently had the same morphologic abnormality when evaluated at different times during a 2-year period. One percent (2 of 200) of non-Greyhound dogs had vacuolated eosinophils; both dogs were Golden Retrievers.

Eighty-five percent (11 of 13) of blood smears from Greyhounds with normal eosinophil granules on Wright's-stained smears had vacuolated granules when the slides were stained with Diff-Quik, regardless of whether the smears were made directly from the syringe or from the sample in EDTA. All dogs with vacuolated eosinophils in Wright's-stained smears also had vacuolated eosinophils in Diff-Quik-stained smears. In samples from the 3 Greyhounds with vacuolated eosinophil granules that were stained with Diff-Quik after different fixation times, there was no appreciable eosin staining of the granules in any of the smears.

On TEM, no appreciable difference was noted in granule density between Greyhounds with normal and vacuolated eosinophils (Figure 2). Both vacuolated (Figure 3) and normal (not shown) eosinophils in Greyhounds and normal eosinophils in the control dog stained positive for AP. The cells were negative for CAE and ANBE, as expected for eosinophils from normal dogs.⁶

Discussion

In the present study, we described the morphologic, ultrastructural, and cytochemical features of specific granules of eosinophils in a large population of Greyhounds. We found vacuolated or "grey" eosinophils¹ in >50% of the dogs based on Wright's-stained smears; 85% of eosinophils with orange granules in Wright's-stained smears had vacuolated eosinophils on Diff-Quik-stained smears. Some authors believe that mast cell granules stain poorly (or not at all) with Diff-Quik, perhaps because of poor fixation and subsequent dissolution of granules by this largely aqueous stain.⁷ It is possible that

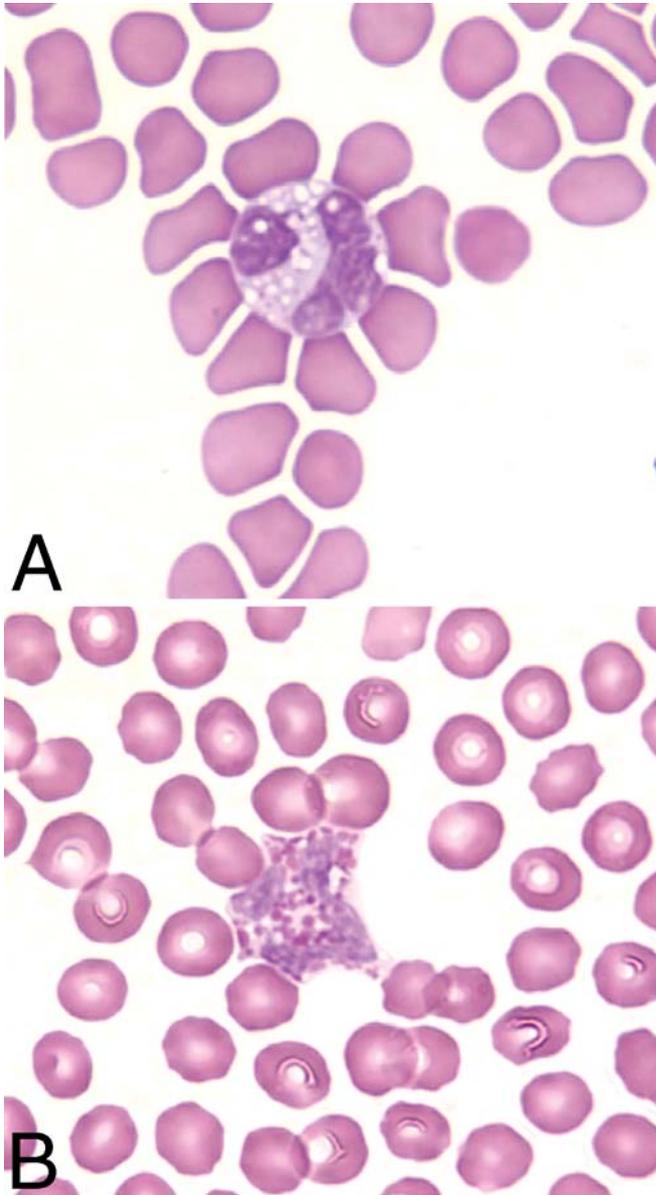


Figure 1. Blood smear with (A) vacuolated and (B) normal-appearing eosinophils in a Greyhound. Wright's, $\times 100$ objective.

fixation of granules with Diff-Quik may also contribute to the differential staining of eosinophils in Greyhounds. It is likely that the eosinophil basic proteins that confer the pink-orange hue to eosinophil granules are abnormal in Greyhounds or have a higher pH than do normal granules, so that the eosin in the Romanowsky-type stains does not bind to the components of the granules. We suspected that a longer fixation period may have allowed for better penetration of eosin into the granules, but no staining of the vacuolated granules was apparent after either 5 or 10 minutes of fixation.

Granules of both normal and vacuolated eosinophils were ultrastructurally similar to granules described by Hudson⁸ in normal canine eosinophils. Moreover, vacuolated eosinophils

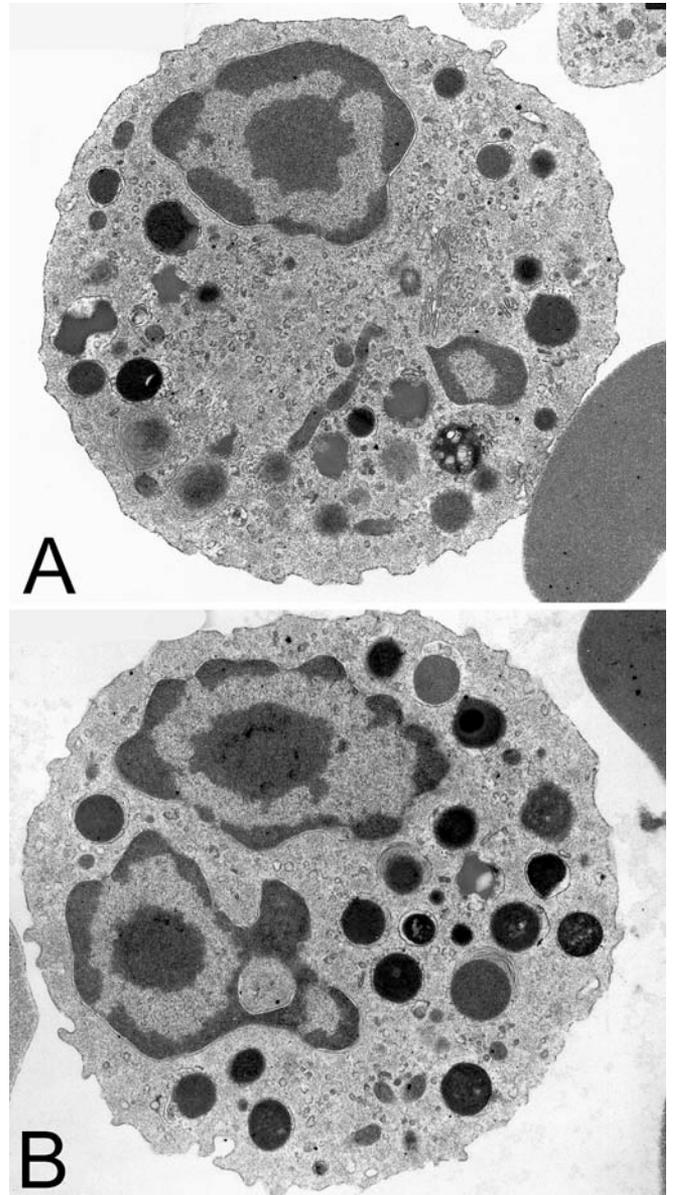


Figure 2. Transmission electron micrograph of Greyhound eosinophils. A vacuolated eosinophil (A) has fewer granules than the normal-appearing eosinophil (B) but individual granule density and the average number of granules were similar. $\times 11,000$ magnification.

had the same cytochemical-staining pattern with AP as that of normal eosinophils. We postulate that vacuolated granules in Greyhound eosinophils represent a change in tinctorial properties, not a functional abnormality, because affected Greyhounds did not appear to have any clinical signs of disease associated with this morphologic change.

The high prevalence of vacuolated eosinophils in Greyhounds indicates a breed predisposition. Moreover, individual Greyhounds had either normal or vacuolated eosinophils consistently throughout the study period (2 years), suggesting a genetic trait in these individuals. We also found vacuolated

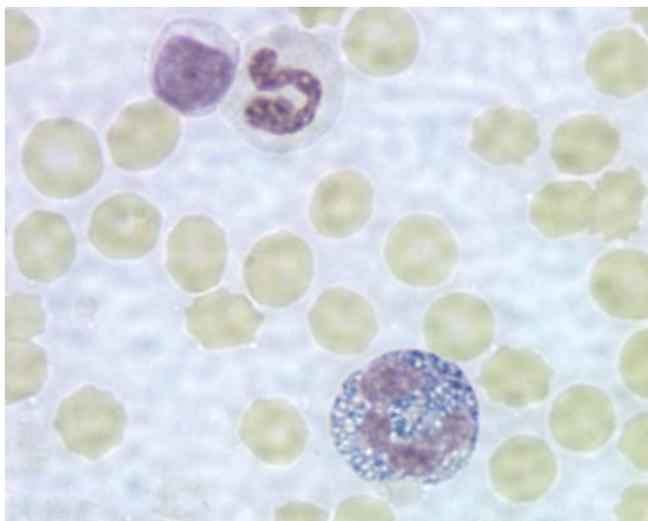


Figure 3. Positive cytoplasmic staining reaction with alkaline phosphatase in a vacuolated eosinophil in a Greyhound. $\times 100$ objective.

eosinophils in 2 Golden Retrievers, and since the time of this study have observed vacuolated eosinophils in 8 additional Golden Retrievers, further suggesting that genetics may play a role in this morphologic abnormality.

Further characterization of abnormal specific eosinophil granules or granule staining could include chemical analysis of the eosinophil granules in affected versus normal dogs, as performed by Piller and Portmann⁹ in horses with liquid chromatography. However, given the small number of circulating eosinophils in the dog, it would be extremely difficult to obtain an eosinophil-enriched suspension by conventional methods. For example, 40 L of horse blood were required to obtain 1 g of eosinophils in Piller and Portmann's study. Alternatively, eosinophil-enriched cell populations could be obtained by flow cytometry and cell sorting; however, to our knowledge, no eosinophil-specific monoclonal antibodies are available for the dog. In a previous study,

antihuman CD18 monoclonal antibodies recognized both eosinophils and neutrophils in the dog.¹⁰

Recognizing vacuolated eosinophils during routine blood smear examination is of practical importance because they may be inaccurately identified as toxic neutrophils, prompting the clinician to erroneously search for a source of infection. Laboratories that use Diff-Quik should be aware of the greater likelihood of finding vacuolated eosinophils in Greyhounds.

Acknowledgments

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