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Eosinophil levels in mice are significantly higher in small blood vessels than in large blood vessels

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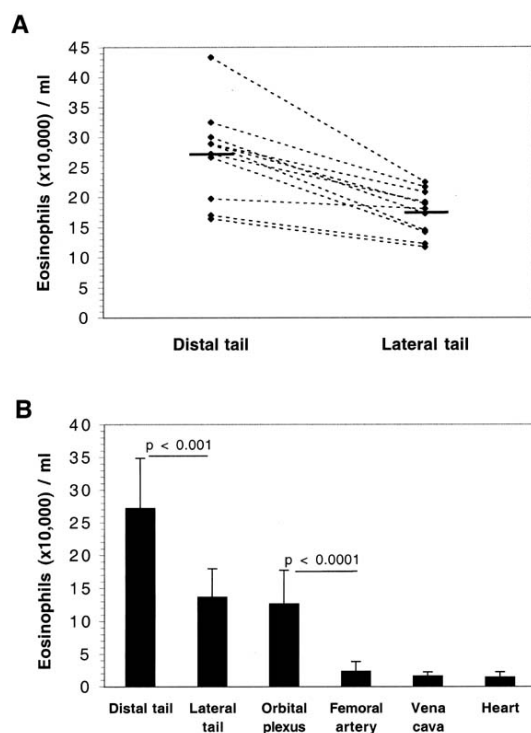
To the Editor:

The mechanisms regulating eosinophilia, a hallmark feature of allergic diseases, have been investigated largely in laboratory mice, a species that allows for unique scientific investigation. Numerous methods of collecting blood samples from rodents have been described.¹ Some of these techniques involve the collection of large amounts of blood (~1 mL) and either surgery with the laboratory animal under anesthesia or euthanasia of the animal. These methods include cardiac puncture and severing of various blood vessels (aorta, vena cava, carotid, and femoral blood vessels). Techniques involving collection of smaller sample volumes better suited for serial blood collection have also been developed.

The most widely used methods for repetitive blood sampling involve puncture of the ophthalmic venous plexus, amputation of the distal tail, and incision of the lateral caudal tail vein.¹ However, detailed counts of leukocytes, including eosinophils, have not been characterized in mice. We have therefore compared eosinophil levels in blood samples obtained from distinct vascular locations; in addition, as a control, we sought to determine the effect of blood retrieval techniques on eosinophil levels.

In our initial experiments, we found that eosinophil levels in capillary blood collected from the distal tail were significantly different from the levels in capillary blood collected from the larger lateral tail vein (Fig 1, A). Importantly, eosinophil levels were routinely lower in blood collected from the lateral tail vein than in blood collected from the distal microvessels ($17.35 \pm 3.75 \times 10^4$ and $27.22 \pm 7.63 \times 10^4$ cells/mL, respectively) when the same mouse was examined ($P < .001$). To ensure that the order of collection was not responsible for these observed differences, we collected blood from both sites on the same day and then repeated the experiment the following week with the order of blood collection reversed. No significant differences between the 2 experiments were observed for either the distal tail or the lateral tail (data not shown).

Fig. 1. A, Baseline blood eosinophilia in 2 different locations of the tail. Before blood collection, the mice were warmed under a heating lamp to allow for the free flow of blood. Through use of both paired and unpaired Student *t* tests, the distal tail was found to yield significantly more eosinophils than the lateral tail vein ($P < .001$). The results are representative of 2 different experiments with Balb/c mice ($n = 11$); each line represents a single mouse. **B,** Blood eosinophil levels in small and large blood vessels and the heart. Blood from the lateral tail vein ($n = 23$) and the distal tail ($n = 11$) was collected after individual mice were mildly heated. For blood collection from the orbital venous plexus ($n = 24$), femoral artery ($n = 15$), and heart ($n = 15$), the mice were lightly anesthetized with methoxyflurane. Blood from the vena cava ($n = 7$) was collected post mortem. Through use of both paired and unpaired Student *t* tests, small vessels were found to yield significantly more eosinophils than larger vessels or the heart ($P < .0001$).



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We were next interested in determining whether eosinophil levels were different when blood was collected from the lateral tail vein and when it was collected from the ophthalmic venous plexus, the latter being another site rich in venous and capillary blood (Fig 1, *B*). For 3 different experiments ($n = 24$ mice), comparisons of eosinophil levels in the ophthalmic venous plexus and the lateral tail vein revealed similar eosinophil levels ($12.58 \pm 5.11 \times 10^4$ and $13.61 \pm 4.33 \times 10^4$ cells/mL, respectively). To ensure that neither the use of body heating nor the use of anesthesia was affecting eosinophil blood levels, blood was collected under similar heating conditions (without anesthesia) from the ophthalmic venous plexus and lateral tail veins of the same mice ($n = 8$); no significant difference was observed ($10.55 \pm 2.69 \times 10^4$ and $10.14 \pm 2.31 \times 10^4$ cells/mL for the ophthalmic venous plexus and lateral tail vein, respectively). These data indicate that eosinophil levels are comparable for blood in the lateral tail vein and blood in the orbital venous plexus, 2 sites that have eosinophil levels significantly lower than the level in blood in the capillary vessels of the distal tail.

We next hypothesized that eosinophil levels in small vessels would be different from levels in larger vessels (eg, the femoral artery, vena cava, and the vessels of the cardiac circulation; Fig 1, *B*). Interestingly, eosinophil levels in the ophthalmic venous plexus were found to be 5-fold higher than those in the femoral vessels ($12.58 \pm 5.11 \times 10^4$ and $2.30 \pm 1.53 \times 10^4$ cells/mL, respectively). Furthermore, eosinophil levels were modestly lower in the vena cava and the heart than in the femoral circulation; however, this difference did not reach significance ($1.59 \pm 0.60 \times 10^4$, $1.39 \pm 0.84 \times 10^4$, and $2.30 \pm 1.53 \times 10^4$ cells/mL, respectively).

Taken together, these results indicate that circulating eosinophil levels are substantially higher in small blood vessels than in large blood vessels. These results are in accord with findings of higher polymorphonuclear leukocyte levels (3- to 6-fold) in venule blood than in

systemic blood.² Differences in eosinophil levels in distinct blood vessels might be related in part to distinct adhesion molecule expression.^{3 4} In addition, decreased cellular deformability of activated neutrophils has been proposed as a mechanism to explain neutrophil retention in pulmonary capillaries.⁵ It has also been suggested that leukocyte entrapment in alveolar capillaries is not solely attributable to the mechanical hindrance associated with leukocyte shape changes; it might also be attributable to interactions with the capillary endothelium.⁶ Both of these mechanisms could explain the observed increase in eosinophil blood levels in the distal tail microcirculation compared with the lateral tail veins.

In conclusion, our study is the first to demonstrate the effect of sampling site on eosinophil blood levels. We report increased eosinophil levels in small blood vessels in comparison with large blood vessels and the heart. These results have important implications in the design, interpretation, and comparison of experimental studies of eosinophilic responses in mice. In addition, because blood samples are frequently collected from microvascular locations in patients (eg, heel punctures), these results have potential implications when eosinophil levels in clinical samples are being monitored.

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