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Morphological Characterization of Peripheral Blood Leukocytes in Blacktip Sharks (*Carcharhinus limbatus*)

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Introduction

- The morphology and function of leukocytes is not well studied in elasmobranchs
- Sharks and other cartilaginous fishes are the most primitive type of vertebrates to have both innate and adaptive immunity comparable to that observed in mammals[1].
- Understanding the role of leukocytes in a greater variety of elasmobranchs furthers research in comparative immunology
- Understanding the makeup of blacktip shark's blood facilitates comparisons to other organisms and determination of conserved traits between species.
- The findings of this study will further research in husbandry, and aid in the care of captive species and in clinical assessments of wild sharks.
- This study focuses on the morphological characteristics of blacktip shark leukocytes using light, SEM, and TEM microscopy
- The goal of this study was to determine a baseline set of reference intervals for healthy wild blacktip shark peripheral blood leukocytes, and to determine the range of the granulocyte-lymphocyte ratio in blacktip sharks

Methods

- 22 blacktip sharks were caught off of Biscayne Bay in Miami, Florida, using circle-hook drumline system[21].
- Whole blood was collected for fixation in light, SEM and TEM microscopy
- Counts of 100 were conducted for WBC in light microscopy
- Cell morphology was characterized in light, SEM and TEM
- Measurements were taken in light microscopy using ZEN
- Minitab and Excel were used for statistical analysis

Cell Counts

Table 1: Average count with standard deviation and minimum and maximum count for each cell type.

Cell Types	*Minimum*	*Maximum*	*Mean*	*Standard Deviation*
FEG	0	4	0.545	0.707
CEG1	4	31	14.66	8.49
CEG2	1	27	14.99	9.89
Neutrophil	0	6	1.693	0.00
Monocyte	0	2	0.250	0.00
Lymphocyte	48	87	68.08	19.1
Thrombocyte	5	36	17.31	9.89
Immature	3	38	13.97	6.36

Results

Lymphocyte and Thrombocyte Morphology in SEM*

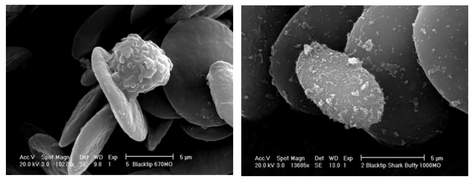

Figure 1: Image of a lymphocyte in SEM. Lymphocytes were distinguishable by their spherical shape, textured surface and microvilli protruding outward.
Figure 2: Image of a thrombocyte in SEM. The thrombocytes were distinguishable by their elongated shape and rough surface with small light-colored particles covering it.

Lymphocyte Morphology in TEM and Light Microscopy

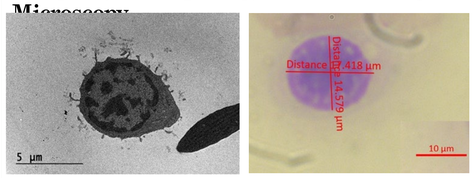

Figure 3: A lymphocyte in TEM. Lymphocytes present with a large nucleus with a small amount of cytoplasm surrounding it. Microvilli are seen extending from the cell.
Figure 4: A lymphocyte in light microscopy. Lymphocytes present with a large nucleus. The microvilli is indistinguishable in light microscopy.

Monocyte Morphology in TEM and Light Microscopy

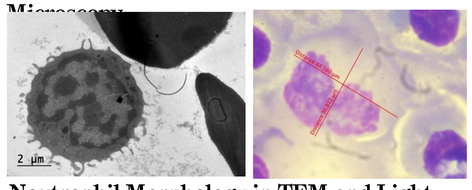

Figure 5: A monocyte imaged in TEM. Monocytes presented with a large kidney bean shaped nucleus surrounded by cytoplasm. Microvilli were present on the cell. The cell was not granulated.
Figure 6: A monocyte imaged in light microscopy. The nucleus presents in a more lobed shape but takes up a similar area of the cell as is presented in TEM. The cytoplasm stains very lightly in light microscopy and is more difficult to see than in TEM.

Neutrophil Morphology in TEM and Light Microscopy

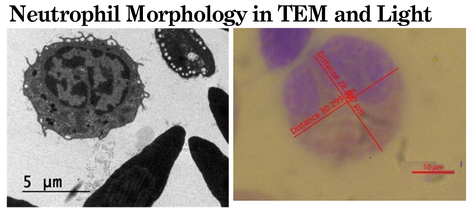

Figure 7: A neutrophil imaged in TEM. The nucleus has a small distinct kidney bean shape with cytoplasm surrounding it. There were some microvilli and granulation was present in the cytoplasm.
Figure 8: A neutrophil imaged in light microscopy. The nucleus presented more in lobes than in a kidney bean shape. Granulation was present in light microscopy.

Thrombocyte Morphology in TEM and Light Microscopy

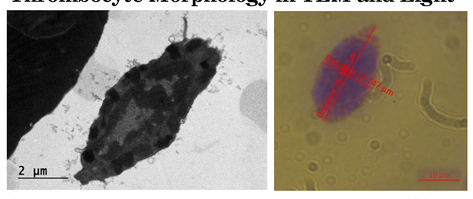

Figure 9: A thrombocyte imaged in TEM. Thrombocytes have an elongated nucleus. There is a thin layer of cytoplasm surrounding the nucleus. Darkly colored granules are present in the cytoplasm
Figure 10: A thrombocyte in light microscopy. Thrombocytes present with elongated nuclei and granulated cytoplasm

*Lymphocytes and thrombocytes were visualized using SEM, but other leukocyte types proved too similar in morphology to erythrocytes to be characterized using this technique.

Eosinophilic (CEG1) Morphology in TEM and Light Microscopy

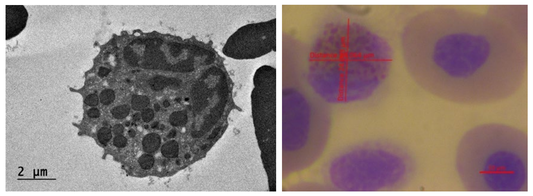

Figure 11: A TEM image of an eosinophilic (CEG1) cell. CEG1s present with lobed nuclei, sometimes looking as if there are multiple nuclei present. The cell presents with a large amount of granulation throughout the cytoplasm and the granulation varies in size and shape.
Figure 12: A CEG1 in light microscopy. The nuclei are lobed and there is large quantity of small granules present throughout the cytoplasm.

Eosinophilic (CEG2) Morphology in TEM and Light Microscopy

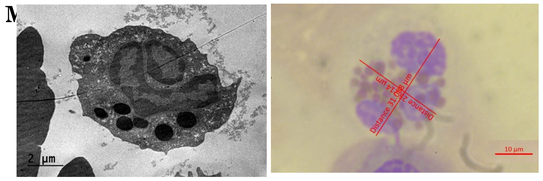

Figure 13: A TEM image of an eosinophilic (CEG2) cell. CEG2s present with lobed nuclei and a much smaller amount of granulation. The granulation is uniform in size
Figure 14: A CEG2 in light microscopy. The nuclei are lobed and there is a small quantity of large granules present throughout the cytoplasm.

Eosinophilic (FEG) Morphology in TEM and Light Microscopy

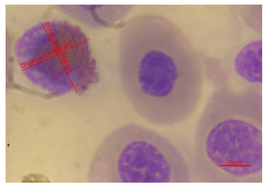

***Figure 15**: Image of an FEG in light microscopy. FEG's have dark purple staining. The granulation on an FEG has crystalline, rod-shaped structures. Granules fill the entire area of the cell's cytoplasm. The granules never appear to overlap, they uniformly fill in the space. FEG's have large nuclei that always present along the side of the cell.

*Due to the small sample size available for TEM, no FEGs were found for characterization

Granulocyte-Lymphocyte Ratio

Table 2: Average ratio with standard deviation and the minimum and maximum values for the granulocyte-lymphocyte ratio (GLR).

	GLR
Minimum	0.1918
Maximum	0.8115
Mean	0.4717
St.deviation	0.15156

Comparison of Gender and Age

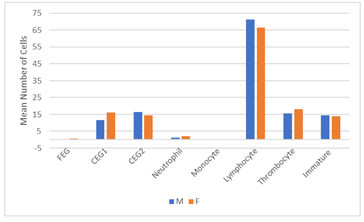

Figure 16: Mean leukocyte counts for males (n= 7) and females (n= 15). The average cell counts of CEG1s, neutrophils, and lymphocytes differed significantly between males and females (P<0.05). CEG2s, FEGs, monocytes, thrombocytes and immature cells were significantly different (P>0.05).

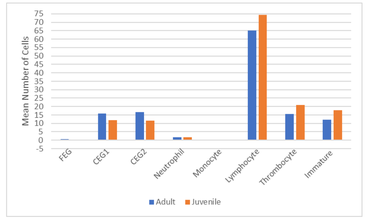

Figure 17: Figure 11: Mean leukocyte counts for adults (n = 15) and juveniles (n = 7). A comparison of each cell types average cell count between adult and juvenile blacktip sharks. The mean number of CEG1s, CEG2s, Monocytes, Lymphocytes, Thrombocytes and Immature cells differed significantly between adults and juveniles (P<0.05). All other observed cell types were not found to be significantly different (P>0.05).

References

1. Smith et al. 2015
2. Tavarea, 2008
3. Dikić et al. 2013
4. Harper & Wolf, 2009

Discussion

- Average blood cell type percentages per 100 cells include lymphocytes (68.08%), thrombocytes (17.31%), CEG type 2 (14.99%), CEG type 1 (14.66%), immature leukocytes (13.97%), neutrophils (1.69%) , FEG (0.55%), and monocytes (0.25%).
- Lymphocytes were the second most abundant cell type after erythrocytes.
- Vertebrate lymphocytes are highly conserved across species[25].
- Four granulocytic cell types were found. A neutrophil and three morphologically distinct eosinophilic granulocytes: (1) fine eosinophilic granulocyte (FEG), (2) coarse eosinophilic granulocyte (CEG) type 1, and (3) coarse eosinophilic granulocyte (CEG) type 2.
- Classification of specialized granulocyte subtypes is commonly observed in many shark species[25].
- The GLR is an indicator of stress in fish. Acute stress can cause a shift in peripheral blood leukocyte distributions and increase the GLR[35].
- Larger sample sizes of wild blacktip sharks should be used.

Acknowledgments

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