Structure and Function of Leukocytes in the Family Macropodidae

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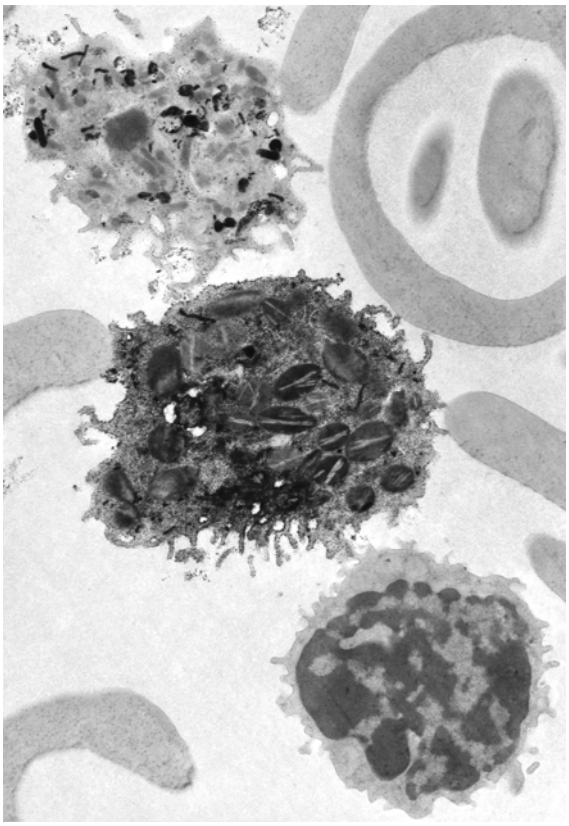

This thesis is presented for the degree of Doctor of Philosophy at Murdoch University,

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I declare that this thesis is my own account of my research and contains as its main content work, which has not been submitted for a degree at any tertiary education institution.

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Karen Lisa Hulme-Moir

This thesis is dedicated to the two great men of my life.

My past and my future,

my inspiration and my undying love.

Do not follow where the path may lead. Go instead where there is no path and leave a trail.

Muriel Strode

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Abstract

Leukocytes play a central role in protecting the body against infectious organisms and their research is essential for understanding the mechanisms of immunity. By studying leukocytes across a range of species, insights are provided into differing strategies employed to ensure resistance to disease. Surprisingly, the structure and function of marsupial leukocytes has received very limited study. Marsupials represent a major evolutionary pathway with distinct differences in reproduction and development from placental mammals. These differences in the life history of marsupials place unique challenges on the immune system, and differences in leukocyte structure and function could be reasonably expected. In this thesis, studies were undertaken to examine the cytochemical, ultrastructural and functional features of leukocytes from species of marsupials, belonging to the family Macropodidae (kangaroos and wallabies). The aim of these studies was to elucidate the characteristics of macropodid leukocytes and to compare and contrast these features with the known characteristics of other mammalian leukocytes.

Leukocytes from two species of macropodid, the tammar wallaby (*Macropus eugenii*) and the western grey kangaroo (*Macropus fuliginosis*), formed the basis of this study with additional material provided from quokka (*Setonix brachyurus*), woylie (*Bettongia pencillata*) and red kangaroo (*Macropus rufus*). Staining characteristics of cells were examined following reaction with Sudan black B, peroxidase, chloroacetate esterase, naphthyl butyrate esterase, alkaline phosphatase and periodic acid-Schiff. Peroxidase and Sudan Black B reactions were similar to domestic animal species but chloroacetate esterase and naphthyl butyrate esterase were unreliable as markers for macropodid neutrophils and monocytes, respectively. Significant variation in staining for alkaline phosphatase was seen between species of macropodid. Tammar wallabies and quokka demonstrated strong neutrophil alkaline phosphatase activity whereas western grey kangaroos, red kangaroos and woylies contained no activity within their leukocytes.

Peroxidase and alkaline phosphatase cytochemistry were also assessed at the ultrastructural level with transmission electron microscopy. This allowed the identification of distinct granule populations within macropodid neutrophils. Two subcellular compartments

containing alkaline phosphatase activity were identified within tammar wallaby neutrophils. These were considered equivalent to secretory vesicles and a subpopulation of specific granules. Tubular vesicles containing alkaline phosphatase were also identified within the eosinophils of tammar wallabies. These structures were a novel finding having not been reported previously in the eosinophils of other animal species.

In addition to cytochemistry, the general ultrastructure of leukocytes from tammar wallabies and western grey kangaroos were reported. Results were similar to previous reports for other marsupial species. The current body of knowledge was extended by the first detailed description of the ultrastructure of basophils in a marsupial.

To assess functional aspects of macropdid neutrophils, flow cytometric assays were performed examining oxidative burst responses and phagocytosis. Reactive oxygen species were generated by neutrophils from tammar wallabies and western grey kangaroos in response to phorbol 12-myristate 13-acetate but not N-formyl-Met-Leu-Phe or opsonised bacteria. Phagocytosis of opsonised bacteria was also measured in neutrophils from tammar wallabies, which was poor in contrast to ovine neutrophils. However, flow cytometric studies were limited by sample preparation. Further optimisation of isolation methods for tammar wallaby leukocytes should be undertaken before dogmatic conclusions are drawn.

Overall, the results of this thesis demonstrate that, in the areas examined, the general characteristics of leukocyte structure and function of mammals are present in macropodids. However differences were identified both within and outside of the macropodid group. These differences have important ramifications for the use of ‘model’ species in the study of leukocyte biology in marsupials. The results also provide potentially useful tools for the clinical diagnosis of haematological disease in macropodids and may be of interest to those studying comparative and evolutionary aspects of leukocyte structure and function.

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List of Units

ºC	degrees celsius
”	inch
dpi	dots per inch
g	gauge
x *g*	times gravity
Ha	hectare
kV	kilovolts
mOsm	milliosmole
nm	nanometre
µm	micrometre
mm^3	millimetre cubed
ng	nanogram
µg	microgram
mg	milligram
kg	kilogram
µL	microlitre
mL	millilitre
L	litre
µM	micromolar
mM	millimolar
M	molar
s	second
min	minute
hr	hour
wk	week
mth	month
yr	year
w/v	weight per volume

Abbreviations

ALP	alkaline phosphatase
AML	acute myeloid leukaemia
BSA	bovine serum albumin
CAE	chloroacetate esterase
CD	cluster of differentiation
CMCA	Centre for Microscopy, Characterisation and Analysis; The University of Western Australia
CML	chronic myelogenous leukaemia
CYTO B	cytochalasin B
DAB	diaminobenzidine
DCF	dichlorofluorescein
DCFH	dichlorofluorescin
DCFH-DA	dichlorofluorescin diacetate
DHR	dihydrorhodamine 123
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPBS	Dulbeccos phosphate buffered saline
EM	electron microscopy
EDTA	ethylenediaminetetraacetic acid
EPO	eosinophil peroxidase
FITC	fluorescein isocyanate
fMLP	N-formyl-Met-Leu-Phe
FSC	forward light scatter
GPI	glycosyl-phosphatidyl inositol
HBSS	Hank's balanced salt solution
HE	hydroethidine
HLA	human leukocyte antigen
HPO	horseradish peroxidase

ICE	samples incubated with opsonised bacteria on ice
Ig	immunoglobulin
LAMPs	lysosome-associated membrane proteins
LAP	leukocyte alkaline phosphatase
MFI	mean fluorescence intensity
MHC	major histocompatibility complex
MPO	myeloperoxidase
MVBs	multi-vesicular bodies
NADPH	nicotinamide adenine dinucleotide phosphate
NBE	naphthyl butyrate esterase
NSEs	non-specific esterases
PAS	periodic acid-Schiff
PBS	phosphate buffered saline
PBSgel	phosphate buffered saline containing 0.02M EDTA, 0.05M dextrose and 1% gelatin
PER	peroxidase
PI	propidium iodide
PMA	phorbol 12-myristate 13-acetate
PRPs	primary reaction products
RBC	erythrocyte concentration
RER	rough endoplasmic reticulum
SBB	Sudan black B
SD	standard deviation
SSC	side light scatter
UNOPS	samples incubated with unopsonised bacteria