Defining the role of myeloid cells in the regulation of developmental, tumour and inflammation-stimulated lymphangiogenesis

A thesis submitted for the degree of

Doctor of Philosophy

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Abstract

Lymphatic vessels are an integral component of the cardiovascular system. These specialised vessels are essential for the return of interstitial fluid to the bloodstream, immune cell trafficking and the absorption of fats from the digestive tract. Despite the crucial role played by lymphatic vessels in homeostasis and human disease, little is known about the signals that regulate lymphatic vascular growth and development (lymphangiogenesis). The aim of this project was to investigate the role of two lineages of immune cells, macrophages and mast cells, in embryonic, tumour and inflammation-stimulated lymphangiogenesis.

The first aim of this study was to determine the expression pattern of LYVE-1, a marker expressed on lymphatic vessels and a sub-population of macrophages, during early embryonic development. LYVE-1 expression was documented for the first time in the yolk sac blood vasculature and in early embryonic arteries, inter-somitic veins and endothelial cells of the lung and endocardium. These results have important implications for the use of LYVE-1 as a specific marker of lymphatic vascular endothelium.

The major aim of this project was to investigate the role of cells of the macrophage lineage in lymphangiogenesis. Macrophages expressing LYVE-1 were intimately associated with developing lymphatic vessels in the mouse embryo. Characterisation of this sub-population of LYVE-1-positive macrophages revealed that they shared a gene expression profile with Tie2-expressing monocytes. Lineage tracing studies illustrated that, while localised in close association with lymphatic vessels, macrophages did not trans-differentiate to lymphatic endothelial cells during embryonic or tumour-stimulated lymphangiogenesis. These data provide strong support to exclude myeloid cells as a source of lymphatic endothelial progenitor cells. Characterisation of lymphatic vascular development in macrophage-

deficient mice revealed that macrophages play a key role in shaping the dermal lymphatic vasculature during development by regulating lymphatic endothelial cell proliferation.

The final aim of this study was to investigate the role of mast cells during embryonic and inflammation-stimulated lymphangiogenesis. While mast cells appeared to be dispensable for the construction of the lymphatic vasculature, they were found to play a key role in UVB irradiation-induced lymphangiogenesis. Hyperplastic lymphatic vessels were a striking feature of UVB irradiated tissue in mast cell-deficient mice, revealing a novel role for mast cells in restraining the magnitude of lymphangiogenesis stimulated by this inflammatory insult.

In conclusion, these studies provide strong evidence to exclude cells of the myeloid/macrophage lineage as a pool of lymphatic endothelial progenitor cells during development and in the tumour microenvironment. This work has discovered that macrophages define the calibre of dermal lymphatic vessels during development by restraining lymphatic endothelial cell proliferation. This is in contrast to previous studies which reported macrophages stimulate lymphangiogenesis in settings of inflammation. In addition, a previously undescribed role for mast cells in the regulation of inflammation-stimulated lymphangiogenesis was identified. Taken together, these data illustrate that the sources of signals driving embryonic and disease-stimulated lymphangiogenesis are likely to be distinct. As myeloid cells regulate lymphangiogenesis in disease states, the results of this project have important implications when considering the targeting of myeloid cells for lymphangiogenic therapies.

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Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Emma Gordon and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Emma Gordon

Publications

The following publications have resulted from work performed by the candidate during the period of this candidature:

Gordon, E., Gale, N., Harvey ,N. Expression of the hyaluronan receptor LYVE-1 is not restricted to the lymphatic vasculature; LYVE-1 is also expressed on embryonic blood vessels. *Developmental Dynamics* 2008 Jul:237(7):1901-1909.

Gordon, E., Rao, S., Pollard, J., Nutt, S., Lang, R., Harvey, N. Macrophages define lymphatic vessel calibre during development by regulating lymphatic endothelial cell proliferation. *Development* 2010 Nov 15:137(22):3899-3910.

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Abbreviations

%:	percentage
°C:	degrees Celsius
adj:	adjusted
AP:	alkaline phosphatase
APC:	antigen presenting cell
BEC:	blood endothelial cell
BMCMC:	bone marrow-derived cultured mast cell
BSA:	bovine serum albumin
CAM:	chorioallantoic membrane
cDNA:	complimentary DNA
cm:	centimeter
CO_2:	carbon dioxide
CV:	cardinal vein
DA:	dorsal aorta
DAB:	3,3-diaminobenzidine
DAPI:	4',6-diamidino-2-phenylindole
DLV:	dermal lymphatic vessel
DMEM:	Dulbecco's Modified Eagles Medium
DNA:	deoxyribonucleic acid
dNTP:	deoxynucleoside triphosphate
DTT:	dithiothreitol
E:	embryonic day
EBM-2:	Endothelial Basal Medium-2
EDTA:	ethylenediaminetetraacetic acid
EGM-2:	EBM-2 supplemented with Endothelial Growth Media-2 MV SingleQuots
FBS:	foetal bovine serum

Fig: figure

h: hour

H_2O: water

HA: hyaluronic acid

HBBS: Hanks balanced salt solution

HCl: hydrochloric acid

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HHF: HBBS with 10 mM HEPES and 5% FBS

JLS: jugular lymph sac

$K_3Fe(CN)_{6:}$ potassium ferricyanide

$K_4Fe(CN)_6$: potassium ferrocyanide

kb: kilobase

kJ/m^2: kilojoules per metre squared

LEC: lymphatic endothelial cell

LLC: Lewis lung carcinoma

LS: lymph sac

LVAP: Lymphatic Vessel Analysis Protocol

M: molar

MAC: macrophage

MACS: magnetic activated cell sorting

mAmp: milliampere

M-CSF: macrophage colony-stimulating factor

mg: milligrams

$MgCl_2$: magnesium chloride

min: minute

ml: millilitre

mm: millimetre

mM: millimolar

MMP: matrix metalloproteinase

MQ: milliQ

mRNA: messenger RNA

n: number of replicates

NaCl: sodium chloride

NaH_2PO_4: sodium dihydrogen phosphate

ng: nanogram

NIH: National Institute of Health

nm: nanometers

nM: nanomolar

NT: neural tube

O_2,: oxygen

OD: optical density

PAGE: polyacrylamide gel electrophoresis

PBS: phosphate buffered saline

PBS-T: 0.1% Triton X-100/PBS

PBS-Tw: 0.1% Tween20/PBS

PCR: polymerase chain reaction

pen/strep: penicillin/streptomycin

PFA: paraformaldehyde

pH: hydrogen ion concentration

PLB: protein loading buffer

pmol: picomol

PVDF: polyvinylidine difluoride

RNA: ribonucleic acid

RPMI: Roswell Park Memorial Institute Media

RT: room temperature

RT-PCR: reverse transcription polymerase chain reaction

SDS: sodium dodecyl sulphate

sec: second

TAM:	tumour-associated macrophage
TBE:	Tris/boric acid/EDTA buffer
TBS-T:	Tris buffered saline/0.1% Tween 20
TEM:	Tie2-expressing monocyte
Tris:	Tris (hydroxymethyl) aminomethane
UVB:	ultraviolet B
v/v:	volume per volume
V:	volts
w/v:	weight per volume
WEHI:	Walter and Eliza Hall Institute
WT:	wild-type
x*g*:	x gravity
X-gal:	5-bromo-4-chloro-3-indolyl-β-D-galactosidase
α:	alpha
β-gal:	β-galactosidase
λ:	lambda
µg:	microgram
µl:	microliter
µm:	micrometre
µM:	micromolar