The Effect of Macrophages on Fibroblast Activity and Lesion Development in Mouse Models of Endometriosis

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Table of Abbreviations

Abbreviation	Description
αSMA	Alpha smooth muscle actin
β2m	Beta-2-microglobulin
µL	Microlitre
µm	Micrometer
AcLDL	Acetyl low density lipoprotein
BAX	BCL-2 associated X protein
BCL-2	B-cell lymphoma 2
bp	Base pair
BrdU	5-bromo-2'-deoxyuridine
BSA	Bovine serum albumin
CAM	Chorioallantoic membrane
CCL (e.g. CCL17)	Chemokine (C-C motif) ligand
CCR1	Chemokine (C-C motif) receptor 1
COCs/COC	Combined oral contraceptives
CSF-1/Csf-1	Colony stimulating factor-1
CSF-1R	Colony stimulating factor-1 receptor
CXCL (e.g. CXCL13)	Chemokine (C-X-C motif) ligand
CYP	Cytochrome
CYR61	Cysteine-rich, angiogenic inducer, 61
DAB	3,3'-diaminobenzidine
DAPI	4',6-diaminido-2-phenylindole dihydrochloride
dNTPs	Deoxynucleotide triphosphates
DT	Diphtheria toxin
ECM	Extracellular matrix
eGFP	Enhanced green fluorescent protein
EMMPRIN	Extracellular matrix metalloproteinase inducer
EMT	Epithelial-mesenchymal transition
eNOS	Endothelial nitric oxide synthase
FACS	Fluorescence-activated cell sorting

FAK	Focal adhesion kinase
FdU	2’-fluoro-2’-deoxyuridine
Fizz1	Resistin-like molecule alpha 1
Flt1	Fms-related tyrosine kinase 1
FSH	Follicle-stimulating hormone
GFP	Green fluorescent protein
GM-CSF	Granulocyte macrophage colony-stimulating factor
GnRH	Gonadotropin-releasing hormone
H&E	Haematoxylin and eosin
HIF-1α	Hypoxia inducible factor-1α
HLA (e.g. HLA-DR)	Human leukocyte antigen (MHC)
HRP	Horseradish peroxidase
HSV	Herpes simplex virus
ICAM-1	Intercellular adhesion molecule-1
IFN (e.g. IFNγ)	Interferon
Ig	Immunoglobulin
IL (e.g. IL-1)	Interleukin
iNOS	Inducible nitric oxide synthase
IVF	*In vitro* fertilisation
KC	Keratinocyte chemoattractant
LAP	Latency-associated peptide
LH	Luteinising hormone
LPS	Lipopolysaccharide
Ly6C	Lymphocyte antigen 6C
M0	Unpolarised macrophage
M1	Classically activated macrophage
M2	Alternatively activated macrophage
MCP-1	Monocyte chemoattractant protein -1
M-CSF	Macrophage-colony stimulating factor
MDSC	Myeloid derived suppressor cell
MHC (e.g. MHC class II)	Major histocompatibility complex
MIF	Macrophage inhibitory factor
MIP (e.g. MIP-1α)	Macrophage-inflammatory protein

mL	Millilitre
MMP (e.g. MMP-1)	Matrix metalloproteinase
Msr1	Macrophage scavenger receptor 1
MUC1	Mucin 1, Cell surface associated
NC	Negative control
Neo	Neomycin
Neo^r	Neomycin resistance gene
NO	Nitric oxide
NOS (e.g. Nos2)	Nitric oxide synthase
OCT	Optimum cutting temperature compound
OSE	Ovarian surface epithelium
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PE	Phycoerythrin
PFA	Paraformaldehyde
PPARγ	Peroxisome proliferator-activated receptor γ
Prkdc	Protein kinase, DNA-activated, catalytic polypeptide
RANTES	Regulated on activation, normal T cell expressed and secreted (CCL-5)
rASRM	Revised American Fertility Society (AFS) score
SCID	Severe combined immunodeficiency
SR-A	Class A scavenger receptor
TAE buffer	Tris-acetate-EDTA buffer
TAM	Tumor-associated macrophage
TCDD	2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (dioxin)
TCR	T cell receptor
TEM	Tie2-expressing monocytes/macrophage
TGFB/Tgfb (e.g. TGFB1)	Transforming growth factor beta
TNFα	Tumor necrosis factor α
TUNEL	Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling
uPA	Urokinase-type plasminogen activator
VEGF	Vascular endothelial growth factor
vWF	von Willebrand Factor

Abstract

Endometriosis is a gynaecological disease characterised by the growth of endometrial tissues at ectopic sites. Although this disease affects 10-15% of women worldwide, its pathogenesis is still poorly understood.

Human eutopic endometrial tissues were xenografted into two strains of immunodeficient (SCID) mice with 1) a null mutation for *Tgfb1* gene (*Tgfb1*-/-) and 2) macrophage-restricted expression of GFP (CSF-1R-eGFP/MacGreen). The resulting xenografts were collected at day 10 post-implantation for *Tgfb1*-/- mice and at days 4, 7, 10 and 14 in a time course study using MacGreen mice. Five xenografts collected from *Tgfb1*-/- mice were embedded in paraffin and were compared to *Tgfb1*+/+ tissues for macrophage number, myofibroblast staining (αSMA), proliferating cell number and blood vessel density. Another five xenografts from *Tgfb1*-/- mice and all xenografts from MacGreen mice were frozen in OCT to assess macrophage markers, MHC class II, iNOS, arginase 1 and scavenger receptor A, and collagen type 1.

Using *Tgfb1*-/-/SCID mice, we demonstrated that in the absence of host TGFB1, development of endometriosis-like lesions was suppressed and their glandular area was reduced. We also observed lower numbers of macrophages and a reduced density of myofibroblasts in the lesions from *Tgfb1*-/- mice.

Using MacGreen/SCID mice, we followed the changes in macrophage phenotypes during endometrial xenograft development. Macrophages were phenotypically diverse and pre-dominantly expressed the inflammatory markers MHC class II and iNOS at the early stage of disease development (days 4 and 7). The tissue repair marker, arginase

1, appeared later in lesion development at day 7. Meanwhile, another macrophage marker for tissue healing, scavenger receptor A was higher at day 14 than at the earlier time point. In addition, collagen type 1 staining increased throughout lesion development with its highest intensity evident at day 14.

In the absence of host TGFB1, the number of cells expressing MHC class II was significantly reduced at day 10 compared to the lesions from the wildtype controls. Similarly, iNOS-positive cells were decreased in lesions from *Tgfb1*-/- mice. The number of arginase 1-positive cells was not altered in the lesions from *Tgfb1*-/- mice, suggesting TGFB1 was not critical for arginase 1 expression in these tissues. The abundance of cells expressing scavenger receptor A was significantly reduced in lesions from *Tgfb1*-/- mice. A reduction in the collagen type 1 density was detected in the lesions which developed in a TGFB1-deficient environment.

These studies show that host-derived TGFB1 is critical during endometriosis-like lesion development. The presence of this cytokine altered the abundance of infiltrating macrophages and myofibroblasts. In a time course study, macrophages shifted from inflammatory phenotypes at the early stage to an alternatively activated phenotype associated with tissue healing at later stages. The secretion of collagen type 1 fibres was increased and this was associated with the transition to a remodelling macrophage population. Lesion development appeared to be interrupted when lesions were grown in a TGB1-deficient environment resulting in diminished lesion weight. The understanding behind macrophage activation in endometriotic lesions may provide new information on how endometriosis may be interrupted by moderating macrophage behaviours.

Declaration

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Publications and conference presentations

Publications and conference presentation arising from this thesis

1. Hull, M.L., Johan, M.Z., Hodge, W.L., Robertson, S.A., Ingman, W.V., *Host-Derived TGFB1 Deficiency Suppresses Lesion Development in a Mouse Model of Endometriosis*, The American Journal of Pathology, 2012, vol. 180 (3), p.880-887
2. Johan, M.Z., Ingman, W.V., Robertson, S.A., Hull, M.L., *Lesion Weight and Glandular Development are Suppressed in a TGFB1 Deficient Mouse Model of Endometriosis*, 41st Society of Reproductive Biology Annual Conference, 29th August-1st September 2010, Sydney, Australia (Abstract 133) – Oral presentation
3. Johan, M.Z., Ingman, W.V., Robertson, S.A., Hull, M.L., *Suppression of Endometriosis-like Lesion Development in a TGFB1-/- SCID Mouse Model of Endometriosis*, 11th World Congress on Endometriosis, 4th -7th September 2011, Montpellier, France (Abstract FC5-3) – Poster presentation
4. Johan, M.Z., Ingman, W.V., Robertson, S.A., Hull, M.L., *Activations Status of Macrophages in Lesions from a MacGreen/SCID Mouse Model of Endometriosis*, 60th Annual Meeting Society for Gynecologic Investigation, 20th-23rd March 2013, Orlando, Florida, USA (Abstract T-109) – Poster presentation
5. Johan, M.Z., Ingman, W.V., Robertson, S.A., Hull, M.L., *Altered macrophage phenotypes and collagen level in Tgfb1$^{-/-}$/SCID mouse model of endometriosis*, Society of Reproductive Biology Annual Conference, 25th -28th August 2013, Sydney, Australia (Abstract) – Oral presentation

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